Original Contribution

Thrombus Histology Suggests Cardioembolic Cause in Cryptogenic Stroke

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Background and Purpose—Ischemic stroke of undetermined cause is a major health issue because of its high frequency and clinical relevance. Histopathologic analysis of human thrombi, retrieved from stroke patients with large-vessel occlusion during mechanical thrombectomy, may provide information about underlying pathologies. This study examines the relationship between stroke causes and histological clot composition to identify specific patterns that might help to distinguish causes of cryptogenic stroke.

Methods—Thrombi of 145 consecutive stroke patients with large-vessel occlusion were collected during intracranial mechanical recanalization. The hematoxylin and eosin–stained specimens were quantitatively analyzed in terms of the relative fractions of the main constituents (red and white blood cells and fibrin/platelets). These data, along with additional clinical and interventional parameters, were compared for different stroke subtypes, as defined by the international Trial of Org 10172 in Acute Stroke Treatment criteria.

Results—The composition of thrombi from cardioembolic and noncardioembolic stroke patients differed significantly for all main thrombus components. Cardioembolic thrombi had higher proportions of fibrin/platelets (P=0.009), less erythrocytes (P=0.003), and more leucocytes (P=0.035) than noncardioembolic thrombi. Cryptogenic strokes showed strong overlap with cardioembolic strokes but not with noncardioembolic strokes, in terms of both thrombus histology and interventional and clinical outcome parameters.

Conclusions—Quantitative evaluation of thrombus composition may help to distinguish between different stroke causes. Our findings support the notion that the majority of cryptogenic strokes are cardioembolic. **(*Stroke*. 2016;47:00-00. DOI: 10.1161/STROKEAHA.116.013105.)**

Key Words: blood cells ■ histology ■ pathology ■ stroke ■ thrombosis

Up to 39% of acute ischemic strokes cannot be assigned to a definite cause despite intensive diagnostic workup[1,2] and are, therefore, classified as cryptogenic. To the clinician, cryptogenic strokes pose multiple unresolved problems, most of all in regard to secondary stroke prevention. Several studies show high rates of recurrent stroke episodes of ≤30% in the first year after cryptogenic stroke.[3,4] Accordingly, the so-called cryptogenic stroke is a major health issue and an important research focus,[5,6] calling for multidisciplinary approaches.

With the introduction of thrombus-extracting devices into acute stroke therapy in the mid-2000s, human thrombus material became available for histopathologic analysis. The subsequent development of highly efficient stent retrievers and the increasing implementation of mechanical thrombectomy in clinical practice led to much higher numbers of analyzable thrombus samples. Recent preliminary studies evaluated basic thrombus morphology and categorized main thrombus components, such as fibrin/platelet (F/P) conglomerates and red and white blood cells (RBCs and WBCs, respectively).[7–13] In preceding studies, we, and others, have observed differences in thrombus composition between different stroke subtypes, with higher F/P and leukocyte counts but smaller RBC fraction in cardioembolic stroke compared with other stroke causes.[10,11] The present study assesses the hypothesis that histopathologic clot composition might provide relevant information about stroke cause in the so-called cryptogenic strokes.

Patients and Methods

The study was approved by the Institutional Review Board, and informed consent of patients was obtained. A total of 145 intracranial

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thrombi were collected between October 2010 and September 2012 during endovascular recanalization therapy in patients with acute stroke (including all patients of our first published series [n=34][10]).

As primary end points, we investigated the relationship between stroke cause and clot composition, expressed as percentage of the main components (F/P, RBCs, and WBCs).

Quantitative analysis of these components was done using semi-automated color-based segmentation (Adobe Photoshop CS4, Adobe Systems, San Jose, CA), defining their relative fractions as previously described.[10] As hematoxylin and eosin staining does not allow to adequately distinguish fibrin and platelets, F/P aggregations are labeled as F/P. Thrombus size was estimated by using the totalized pixel count per specimen.

Stroke cause according to the international Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification[14] was determined based on all diagnostic and clinical information available for each patient, including cerebral computed tomography (CT), CT angiography and magnetic resonance imaging, transcranial and extracranial duplex sonography, coagulation tests, long-term electrocardiography recording, and transthoracic or transesophageal echocardiography.[10] Complete diagnostic workup was defined as at least long-term electrocardiography, any imaging of the brain (CT or magnetic resonance imaging) and the extracranial vessels (angiography, CT angiography, or duplex sonography), and any form of echocardiography (transthoracic or transesophageal echocardiography). All TOAST assignments were additionally verified by an experienced senior neurologist (H. Poppert).

In a second step, we prespecified interventional and outcome variables that are potentially associated with stroke cause: time to reperfusion (time from symptom onset to recanalization), recanalization time (time from first angiographic series in the target vessel to final run), number of retraction maneuvers, preinterventional National Institutes of Health Stroke Scale (NIHSS) scores, NIHSS scores at discharge, and modified Rankin Scale (mRS) scores within 90 days. NIHSS scores on admission and at discharge were determined by the respective responsible neurologist, and mRS scores were assessed by personal clinical examination, by phone interview, or by evaluating the medical reports of the rehabilitation centers.

Besides age and sex, basic clinical and interventional data assessed included relevant vascular risk factors, site of occlusion, time to treatment (time from symptom onset to groin puncture), and application of intravenous alteplase, which are summarized in Table 1 and Table I in the online-only Data Supplement.

Between-group differences, especially on the different TOAST groups, were assessed by nonparametric Kruskal–Wallis tests. Boxplot diagrams were generated to illustrate group differences and similarities. All statistical analyses were performed or verified by an experienced statistician (F. Scheipl) using R (http://www.r-project.org) and SPSS Statistics version 23.0 (SPSS Inc, IBM, Ehningen, Germany).

Further information on interventional procedures, thrombus processing, and analysis can be found in the Methods section in the online-only Data Supplement.

Results

A total of 145 thrombi were collected, and of these, 137 clot samples of sufficient quality were used for histological processing. The clinical characteristics of the remaining 137 patients are summarized in Table 1. The main occlusion site was the middle cerebral artery in >50% of all patients. Vertebrobasilar occlusions accounted for 11% of patients. Successful recanalization, defined as Thrombolysis in Cerebral Infarction 2b or 3, was reached in 89.7% of cases.

Workup was incomplete in 12 of the 36 patients with strokes classified as cryptogenic. In 7 of these cases, this was because of early death, and in 4 cases, it was because of early transfer to other hospitals. In 1 case, echocardiography was not performed for unknown reason. To exclude possible bias related to patients with incomplete workup, additional subgroup analyses were performed restricted to patients with complete workup. In these, transesophageal echocardiography was performed in 58.3% of cases and additional coagulation tests in 29.2%.

Table 1. Clinical Characteristics

Characteristic	n=137
Age, y, median, range	73, 18–92
Sex, n (%)	
Female	70 (51)
Vascular risk factors	
Hypertension	68% (90/133)
Coronary artery disease	17% (22/133)
Atrial fibrillation	47% (63/134)
Diabetes mellitus	18% (24/133)
History of smoking	33% (35/106)
IV tPA, n (%)	85 (62)
Occlusion site, n (%)	
MCA	74 (54)
ICA including carotid-T	27 (20)
Combined ICA and MCA/ACA	19 (14)
ACA	2 (1)
Basilar artery/PCA	15 (11)
Baseline NIHSS (range, median±SD), n=125 (91.2%)	15, 2–33
mRS (90 d), n=69 (50.4%)	
0–2	27 (39.1%)
>2	42 (60.9%)
Stroke cause (TOAST), n=136, n (%)	
1=arterioembolic	22 (16.2)
2=cardioembolic	67 (49.3)
4=other determined cause	11 (8.1)
5=cryptogenic	36 (26.5)

ACA indicates anterior cerebral artery; ICA, internal carotid artery; IV tPA, intravenous tissue-type plasminogen activator; MCA, middle cerebral artery; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; PCA, posterior cerebral artery; and TOAST, Trial of Org 10172 in Acute Stroke Treatment.

Of the 11 patients classified as TOAST 4 (other determined cause), 8 patients were with dissections, 1 with an inflammatory process infiltrating the internal carotid artery at the skull base, 1 with a radiogenic stenosis, and 1 with a local thrombus after clipping of an middle cerebral artery aneurysm.

Overall, thrombus composition showed comparable amounts of F/P and RBCs with a slightly higher amount of F/P. There were no significant differences in thrombus composition between anterior and posterior circulation stroke and between patients with or without thrombolysis. As expected, thrombus size decreased with the size of the occluded vessel (internal carotid artery>M1/BA>M2/A2).

Table 2. Differences Between Noncardioembolic and Cardioembolic Stroke Patients

	Noncardioembolic, Mean (±SD)	Cardioembolic, Mean (±SD)	*P* Value
F/P, %*	40.9 (±23.3) n=33	52.6 (±18.6) n=67	0.009
RBC, %*	52.7 (±25.2) n=33	38.3 (±20.0) n=67	0.003
WBC, %*	6.5 (±3.8) n=33	9.1 (±6.4) n=67	0.035
NIHSS (pre)	12.0 (±5.0) n=30	15.2 (±6.2) n=63	0.016
NIHSS (post)	4.8 (±9.2) n=31	11.9 (±13.4) n=59	0.001
Difference NIHSS	8.38 (±4.5) n=29	3.8 (±10.8) n=58	0.026
mRS (90 d)	1.4 (±1.8) n=12	3.6 (±1.9) n=37	0.002
No. of maneuvers	2.1 (±1.7) n=31	3.6 (±2.7) n=62	0.001
Recanalization time, min	47.7 (±41.1) n=31	64.1 (±51.7) n=63	0.035
Time to reperfusion, min	248.3 (±138.1) n=26	280.5 (±113.7) n=58	0.753

F/P indicates fibrin/platelet; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; RBC, red blood cell; and WBC, white blood cells.
*Primary target variables.

There were no significant differences between TOAST categories concerning thrombus size. All interventional and histological data are summarized in Table I in the online-only Data Supplement.

Primary Target Variables

Cardioembolic (TOAST 2) Versus Dichotomized Noncardioembolic (TOAST 1 and 4) Strokes

All main thrombus components showed significant differences in their percentages between cardioembolic and noncardioembolic stroke causes. Cardioembolic thrombi contained higher mean proportions of F/P (52.6% versus 40.9%; *P*=0.009), less RBCs (38.3% versus 52.7%; *P*=0.003), and more WBCs (9.1% versus 6.5%; *P*=0.035) than did noncardioembolic thrombi.

In addition, all predefined interventional and clinical parameters, except time to reperfusion, showed significant differences between these groups as summarized in Table 2.

Receiver operating characteristic analysis for F/P indicated that this parameter—as the dominant clot component—was a significant indicator of cardioembolic stroke cause (area under the curve, 0.661 [95% confidence interval, 0.537–0.784]; *P*=0.009; Figure I in the online-only Data Supplement). According to the receiver operating characteristic analysis, a specificity of >80%, potentially adequate to allow impact on treatment decisions (eg, about oral anticoagulation) also in individual patients, would be reached for a cutoff value of ≥60% for the F/P fraction (specificity, 81.8%, at a sensitivity of 35.8%). With an a priori probability (prevalence) of cardioembolic cause of 67%, this corresponds to a positive predictive value of 80.7%.

Cryptogenic (TOAST 5) Versus Dichotomized Noncardioembolic (TOAST 1 and 4) Strokes

In patients with cryptogenic strokes, the mean proportion of F/P was almost identical to that in cardioembolic stroke patients (50.8% versus 52.6%; *P*=0.592) but significantly larger than in noncardioembolic strokes (50.8% versus 40.9%; *P*=0.049). Likewise, the RBC proportion was about as high in cryptogenic strokes as in cardioembolic strokes (42.0% versus 38.3%; *P*=0.395), but substantially, though nonsignificantly, lower than in noncardioembolic strokes (42.0% versus 52.7%; *P*=0.069). The WBC proportion, in contrast, was similar in thrombi of cryptogenic and noncardioembolic stroke patients (7.1% versus 6.5%; *P*=0.487).

With the exception of time to reperfusion, difference NIHSS, and recanalization time, all predefined interventional and clinical parameters also showed significant differences between the groups as summarized in Table 3.

When analyses were restricted to those cryptogenic stroke patients with complete workup, the differences in thrombus composition compared with noncardioembolic clots persisted at even higher significance levels despite the lower number of cases (n=24; *P*=0.028 for F/P content [53.9%] and *P*=0.044 for RBC content [39.5%]). This subgroup contained no patients with competing causes and thus meets the embolic strokes of undetermined source (ESUS) criteria[15] of cryptogenic stroke.

Table 3. Differences Between Cryptogenic and Noncardioembolic Stroke Patients

	Noncardioembolic, Mean (±SD)	Cryptogenic, Mean (±SD)	*P* Value
F/P, %*	40.9 (±23.3) n=33	50.8 (±20.8) n=36	0.049
RBC, %*	52.7 (±25.2) n=33	42.0 (±21.4) n=36	0.069
WBC, %*	6.5 (±3.8) n=33	7.1 (±4.5) n=36	0.487
NIHSS (pre)	12.0 (±5.0) n=30	16.3 (±6.9) n=32	0.010
NIHSS (post)	4.8 (±9.2) n=31	14.6 (±16.9) n=32	0.006
Difference NIHSS	8.38 (±4.5) n=29	3.1 (±13.2) n=31	0.189
mRS (90 d)	1.4 (±1.8) n=12	3.9 (±2.2) n=21	0.004
No. of maneuvers	2.1 (±1.7) n=31	3.7 (±2.4) n=34	0.003
Recanalization time, min	47.7 (±41.1) n=31	57.7 (±39.5) n=34	0.096
Time to reperfusion, min	248.3 (±138.1) n=26	271.9 (±87.1) n=27	0.323

F/P indicates fibrin/platelet; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; RBC, red blood cell; and WBC, white blood cells.
*Primary target variables.

Pattern Analysis

A schematic illustration of the overall thrombus composition as a function of all 4 TOAST groups represented is shown in Figure 1 (top). Groups 1 and 4 (arterioembolic and other determined cause) show similar distributions of the different components with higher amounts of RBCs and groups 2 and 5 (cardioembolic and other undetermined cause/cryptogenic) with higher amounts of F/P. Significant differences between all groups are evident for the amount of RBCs (P=0.038; Figure 2A); the group differences of F/P content (P=0.056) and of WBCs do not reach significance (P=0.127). No differences in clot composition are detectable between groups 1 and 4 and between groups 2 and 5.

These findings are illustrated by typical thrombus samples for each TOAST group (Figure 1, middle and bottom). Basically, arterioembolic and TOAST 4 strokes show larger amounts of broadly distributed RBCs with smaller islets of F/P and fewer WBCs than the more highly organized appearing F/P-rich cardioembolic and cryptogenic stroke thrombi with predominantly centrally embedded RBC entrapments.

In addition to histological appearance, clinical and interventional parameters exhibit the described pattern of similarities between TOAST 1 and 4 groups and TOAST 2 and 5 groups, respectively. Figure 2 illustrates this similarity of patterns by combining histological component distribution (Figure 2A), necessary retraction maneuvers as interventional parameter (Figure 2B), and mRS as outcome parameter (Figure 2C): there were significantly higher numbers of necessary retraction maneuvers and significantly worse mRS scores in cardioembolic and cryptogenic strokes compared with the other 2 pathogenic groups.

All other prespecified secondary parameters, that is, recanalization time (P=0.168), preinterventional NIHSS (P=0.115), NIHSS at discharge (P=0.013), and difference NIHSS (P=0.091) exhibited analogous patterns.

There were also significant differences between TOAST groups with regard to age of patients but with a distinct pattern: patients with cardioembolic strokes were significantly older in comparison with all other causes, including cryptogenic stroke (P=0.000, graph not shown).

Discussion

These data show (1) that cardioembolic thrombi have histopathologic characteristics that are distinct from those of noncardioembolic thrombi and (2) that cryptogenic thrombi have the same or at least similar characteristics as those of cardioembolic thrombi, but they are, again, distinct from those of noncardioembolic thrombi. These observations are

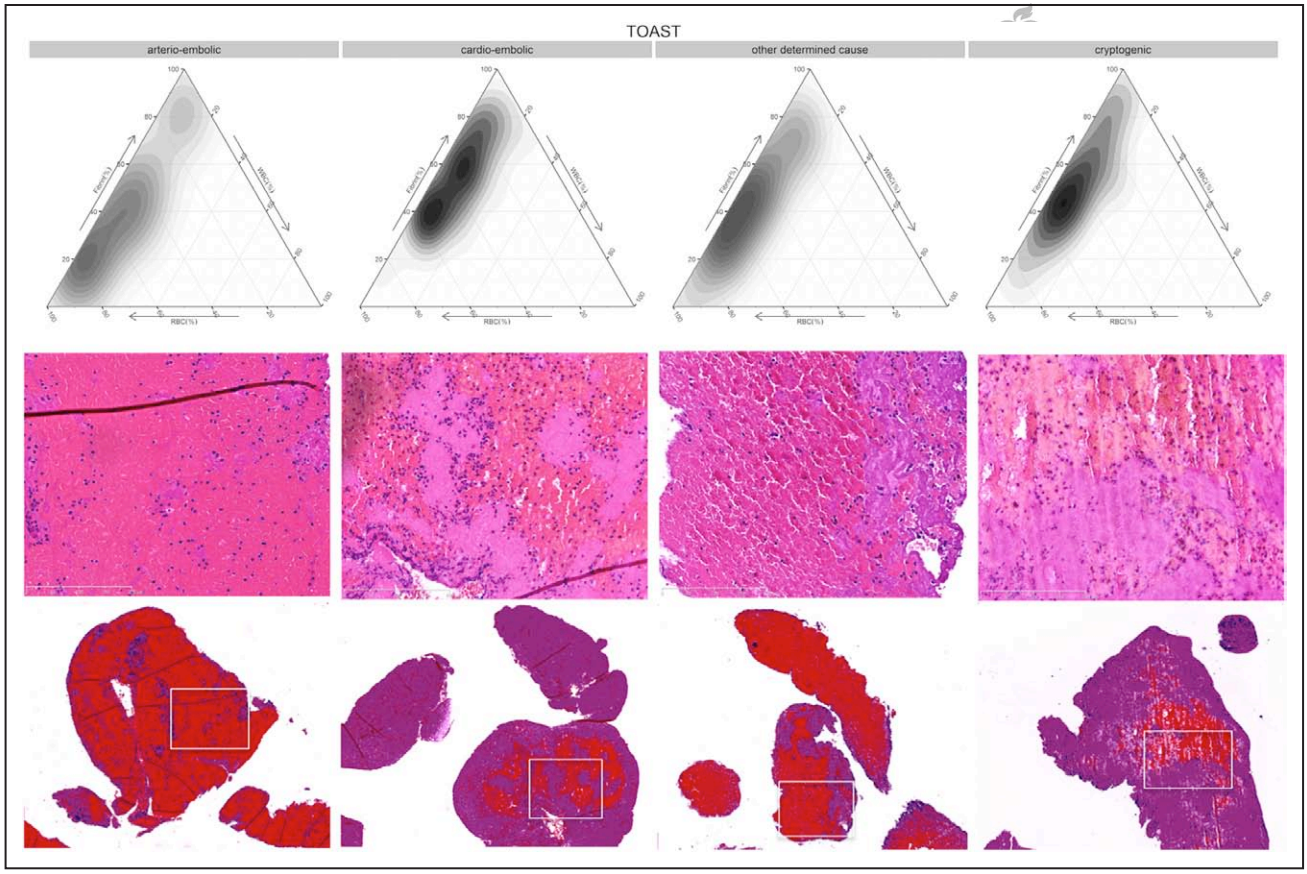

Figure 1. Top, The triangle graphs show similar distributions of the 3 main thrombus components—red blood cells (RBCs), fibrin/platelet, and white blood cells (WBCs)—in Trial of Org 10172 in Acute Stroke Treatment (TOAST) groups 1 and 4 and in TOAST groups 2 and 5, respectively. Dark areas represent higher concentrations of the respective component. **Middle** and **bottom**, The lower row shows 1 thrombus example of each of the respective TOAST groups (magnification, ×50; the components are segmented for better visualization of thrombus organization). Red color represents RBCs; purple regions represent fibrin; and blue dots represent WBCs. **Middle**, The boxes outlined in the lower row are shown in original hematoxylin and eosin staining at ×200 magnification.

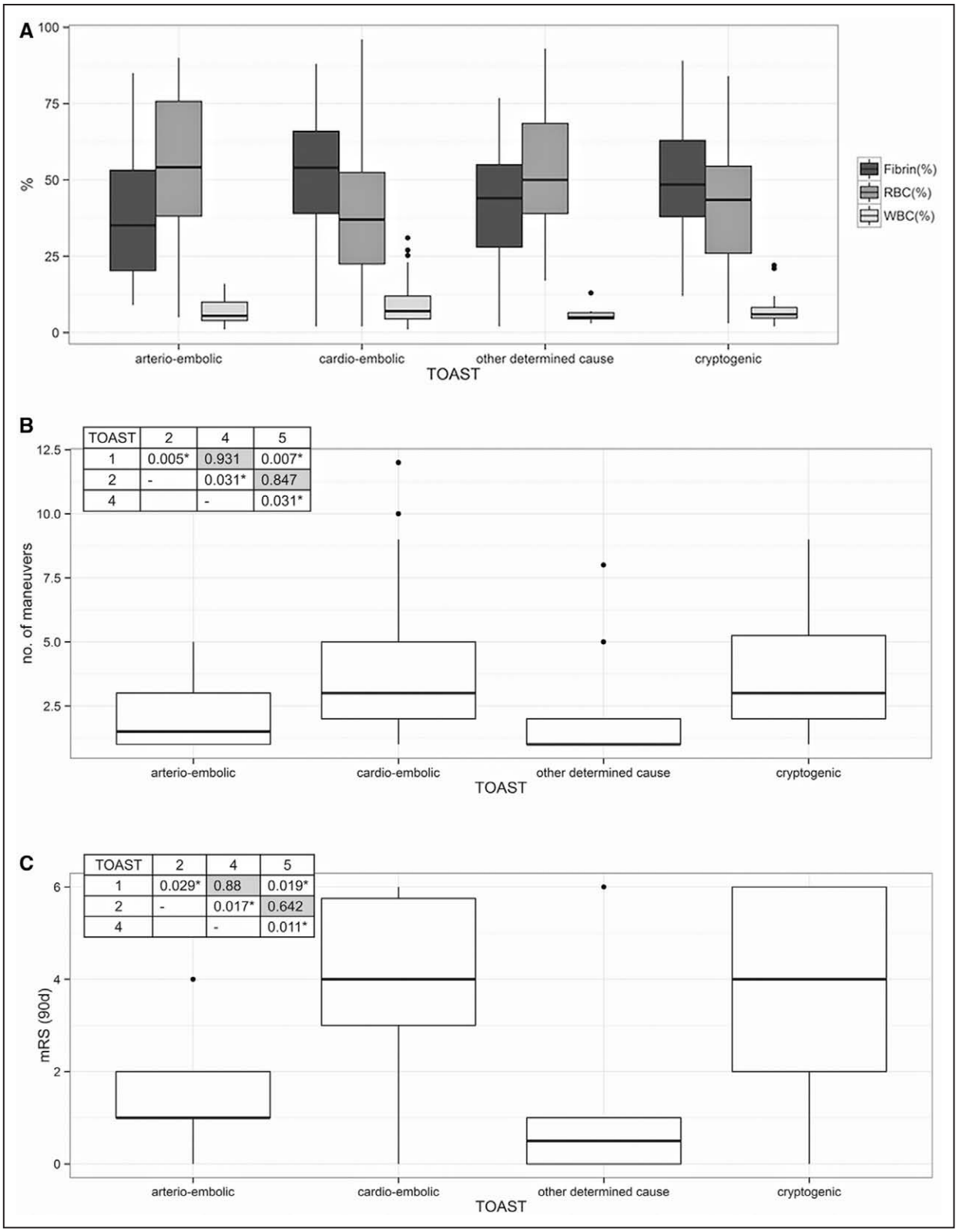

Figure 2. A, Similar to Figure 1, the boxplot shows similar relationships of red blood cells (RBCs; light grey) with fibrin/platelet (dark grey) in Trial of Org 10172 in Acute Stroke Treatment (TOAST) groups 1 and 4 with RBC being the dominant component and in groups 2 and 5 with inverted proportions of the main components. **B**, Boxplot of the number of necessary interventional retraction maneuvers and their relationship with the different TOAST groups, accompanied by a table showing *P* values of the Kruskal–Wallis test for significant differences between each TOAST group. No differences are present between TOAST groups 1 and 4 and groups 2 and 5. All other ratios show significant differences among each other. **C**, Boxplot of modified Rankin Scale (mRS) values ≤90 days after a stroke event and their relationship with the different TOAST groups, accompanied by a table showing *P* values of the Kruskal–Wallis test for significant differences between each TOAST group. (*Continued*)

Figure 2 Coninued. No differences are present between TOAST groups 1 and 4 and groups 2 and 5. All other ratios show significant differences among each other. Significant *P* values <0.05 are indicated with an asterisk in the accompanying table, and similarities between groups with high *P* values are indicated with a grey background. WBC indicates white blood cell.

based on the—to our knowledge—largest published series of histologically analyzed stroke thrombi to date. In contrast to attempts that aim at a clinical differentiation of stroke subtypes[16–18] or trying to improve and escalate poststroke diagnostic efforts,[19,20] we used a distinctly different approach by analyzing the different TOAST groups trying to infer stroke cause from histological and also interventional and clinical outcome parameters. The data strongly support the notion that most cryptogenic strokes are cardioembolic.

The F/P fraction was the dominant clot component in the apparently more organized cardioembolic thrombi, whereas RBCs preponderated in noncardioembolic thrombi. This is in line with our own previous results,[10] with the findings of Niesten et al,[11] and with some restrictions also to the findings of Simons et al[12] but stands in contrast to recently published results by Kim et al,[7] questioning the traditional concept of cardioembolic being mostly red, erythrocyte-rich clots. This discrepancy may be related to differences in methods (2 different stainings, thereby including platelet content; possibly different quantification method and component assignment) and to the much smaller sample size in this latter study with only 8 clots defined as arterioembolic. Furthermore, our results seem to be in contrast to the imaging study by Cho et al[21] that describes a relationship between the blooming artifact of the vessel-occluding thrombus in magnetic resonance imaging with cardioembolic stroke cause. On the other hand, they concur perfectly with a CT study,[22] showing a clear relationship between the hyperdense artery sign (which undoubtedly reflects a higher erythrocyte content) and noncardioembolic stroke cause.

Nevertheless, as our study addresses basic differences and similarities in clot composition, possible discrepancies with some of these studies do not affect the derived conclusions.

In addition, cardioembolic thrombi were associated with different interventional and outcome characteristics when compared with noncardioembolic thrombi (Figure 2B and 2C). The higher number of retraction maneuvers required for the extraction of cardioembolic thrombi may be because of a higher organizational degree of these thrombi, which is congruent with our histopathologic findings. Worse outcome values, reflected by higher mRS and NIHSS scores in these groups, are also in line with published findings: several studies report worse (also long-term) outcome and higher NIHSS values of patients with cardioembolic strokes, especially if caused by atrial fibrillation.[16–18,23,24]

Thrombi from cryptogenic stroke patients showed the same basic pattern as cardioembolic thrombi, with higher proportions of F/P and smaller fractions of RBCs, in clear distinction from noncardioembolic thrombi (arterioembolic and TOAST 4 other determined cause; Figures 1 and 2). Analogous similarities between cryptogenic and cardioembolic and differences to noncardioembolic strokes were also observed regarding interventional and outcome parameters (Figure 2B and 2C). Differences between cryptogenic and noncardioembolic strokes were somewhat less pronounced than those between cardioembolic and noncardioembolic strokes, but still they are significant for F/P and most of the prespecified clinical and interventional parameters. Importantly, the histological differences were even more distinct when patients with incomplete diagnostic workup were excluded, providing strong support for a genuine similarity between cryptogenic and cardioembolic thrombi.

These findings suggest that the majority, although not all, of cryptogenic strokes have a cardioembolic origin. This is in line with recent reports, indicating that the detection rate of atrial fibrillation in cryptogenic stroke patients increases substantially when these patients are examined by multimodal long-term recording.[25]

Advanced age is associated with poorer clinical outcome, possibly with less favorable technical thrombectomy results and also with a higher prevalence of atrial fibrillation and therefore higher incidence of cardioembolic events.[26,27] Conversely, patients with cardioembolic strokes have a higher mean age than patients with strokes of other causes.[23,24] The observed associations of technical and clinical outcome parameters and stroke causes might, therefore, reflect a confounding influence of age. However, in line with other reports,[28] the mean age of cardioembolic stroke patients in our sample was significantly higher than that of all other TOAST groups, including cryptogenic strokes. Likewise, TOAST 4 patients were significantly younger than patients in all other TOAST groups, including TOAST 1 (arterioembolic). Thus, the similarities in technical and clinical outcome parameters of cryptogenic and cardioembolic strokes on the one hand and of TOAST 1 and TOAST 4 patients on the other hand cannot be explained by overlapping age distributions.

As the application of intravenous lysis (recombinant tissue-type plasminogen activator) may directly influences thrombus composition by interfering with the coagulation system, differences in histological clot composition in patients with and without intravenous thrombolysis are conceivable. However, as we did not observe any histological differences between these patient groups, such a possible bias can be ruled out.

Taken together, our congruent histopathologic, clinical, and interventional data support the hypothesis that cardioembolic and so-called cryptogenic strokes have overlapping causes but are distinct from the TOAST groups 1 and 4 (Figure II in the online-only Data Supplement).

Without doubt, secondary prevention measures are more effective if they are specifically adapted to identified stroke causes.[29,30] Two thirds of our cryptogenic cases fulfill the criteria of embolic strokes of undetermined source (ESUS).[15] In these patients, the decision of anticoagulant versus antiplatelet therapy as secondary prevention still remains a matter of debate. Two large studies comparing direct anticoagulants with standard therapy of antiplatelet therapy in ESUS have recently been initiated (https://clinicaltrials.gov/ct2/show/NCT02313909 and https://clinicaltrials.gov/ct2/show/NCT02239120).

Presently, the worldwide standard of secondary prevention in cryptogenic stroke relies on platelet inhibition,[31] a strategy that might be revisited, at least for the group of patients with large-vessel occlusions.

The previously discussed findings are mainly based on group comparisons, accounting for the considerable high interindividual variabilities of the evaluated parameters. However, it would obviously be desirable, if thrombus histology would allow to make inferences to stroke causes also in individual patients.

The receiver operating characteristic analysis with a selected cutoff value of 60% F/P content resulted in a positive predictive value of 80.7% for distinguishing cardioembolic strokes. Accordingly, the diagnostic benefit of such an approach may be substantial even for individual patients. Thrombus histology alone may not allow to make treatment decisions in individual patients. Nevertheless, it may be worthwhile to keep and conservate thrombus specimens retrieved during endovascular stroke therapy (rather than to simply discard them) for subsequent analysis in cases in which the routine workup does not clarify stroke cause.

Limitations

Using only hematoxylin and eosin stain for the quantitative analysis allowed direct comparison of the different components without the risk of methodological problems but came at the cost of insufficient discrimination between platelets and fibrin. This, however, might limit the interpretability of the data but does not affect the main conclusions, as the observed group similarities/differences still clearly reflect organizational differences in thrombus structure. Nevertheless, assessment of platelets may inhere additional value in the differentiation of stroke cause warranting further studies, especially with respect to previous studies showing that platelet content also has an effect on the probability of embolization of cardiac thrombi.[32]

The low number of cryptogenic cases meeting the ESUS criteria is another limitation. Nevertheless, as the observation of similarities between cryptogenic and cardioembolic thrombi became even clearer after exclusion of patients with incomplete workup, our conclusions seem not be affected by this limitation.

As the retrieved thrombus material not always reflect the whole occlusive thrombus, a certain bias toward more stable clot components is possible. Moreover, given the broad variability of clot composition also in the evaluated sections of individual specimens, quantitative component assignments may not always be perfectly representative for the entire clot volume. However, we always tried to obtain sections in the optimal longitudinal plane as representative specimens, so that systematic biases are unlikely.

Conclusions

Retrieved thrombus specimens of cardioembolic and noncardioembolic stroke patients differ significantly in their histopathologic characteristics. Cryptogenic and cardioembolic strokes show strong overlap in both histopathologic thrombus characteristics and interventional and clinical outcome parameters, and both are clearly distinct from other stroke subtypes. These findings suggest that cardioembolic mechanisms account for the majority of so-called cryptogenic strokes.

Disclosures

None.

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Thrombus Histology Suggests Cardioembolic Cause in Cryptogenic Stroke

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ONLINE SUPPLEMENT

Thrombus histology suggests cardio-embolic etiology in cryptogenic stroke

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Supplementary Data with 1 Supplementary Figure

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Supplemental Methods

Patients were eligible for endovascular stroke treatment according to our institutional standard operating procedure: Main-stem occlusion of internal carotid artery (ICA), median cerebral artery (MCA), anterior cerebral artery (ACA), basilar artery (BA) or posterior cerebral artery (PCA), National Institutes of Health Stroke Scale (NIHSS) > 4, symptom onset < 5 h in the anterior circulation, < 8 h in the posterior circulation, no early signs of stroke demarcation in more than one-third of the dependent territory. All procedures of mechanical recanalization were performed according to our institutional guidelines, and have already been described in detail [1]. All procedures were done with stent retrievers as recanalization tools. Successful treatment was defined as modified TICI 2b or 3; TICI 2b being defined as complete revascularization of more than two-thirds of the target area, and TICI 3 defined as complete revascularization with no persistent occlusions [2].

The processing of the retrieved specimens has already been described in detail [1]. In brief, formalin-fixed and paraffin-embedded thrombus material was cut into 2-µm sections using a Microm HM335 E microtome (Microm International GmbH, Walldorf, Germany) followed by hematoxylin-eosin (HE) staining. Because the thrombus material was inhomogeneous in some specimens with several thrombus fragments, the most suitable cutting plane—preferably in the longitudinal axis of the thrombus material—was chosen to give the most representative slice regarding overall clot composition. After high-resolution scanning (×400) with a Hamamatsu Nano-Zoomer 2.0 RS scanner (Hamamatsu Photonics K.K., Hamamatsu City, Japan), entire images of the stained specimens were stored digitally.

In the semi-automated, quantitative component analyses, all thrombus fragments were included after exclusion of folded and unevaluable areas (median percentage of analysed thrombus fraction 86%, range 54% - 100%).

Supplemental Tables

Table I: Basic interventional and histological parameters

Interventional parameters	
Time to treat (min): *median, range*	220, 15–625
Recanalization time (min): *median, range*	50, 10–308
Number of maneuvers: *median, range*	3, 1–12
TICI score (percentage), n = 137 TICI 0 TICI 2a TICI 2b TICI 3	 2.2% 8% 35% 54.7%
Histological parameters	
Thrombus components , median *percentage, range* F/P RBC WBC	 47%, 2-89 43%, 2-96 7%, 1-31

Supplemental Figures

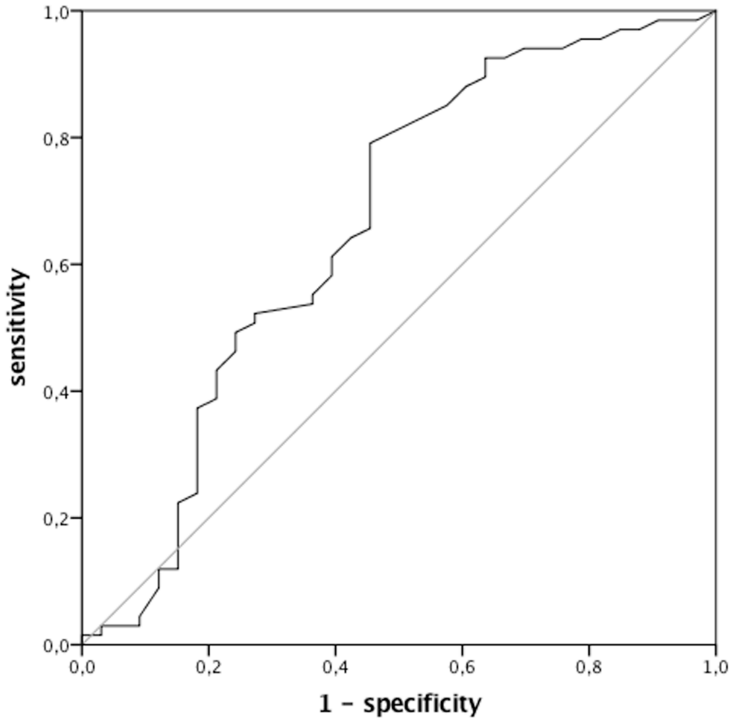

Fig. I: ROC analysis for F/P content and cardioembolic stroke origin, AUC-value 0.661, p = 0.009.

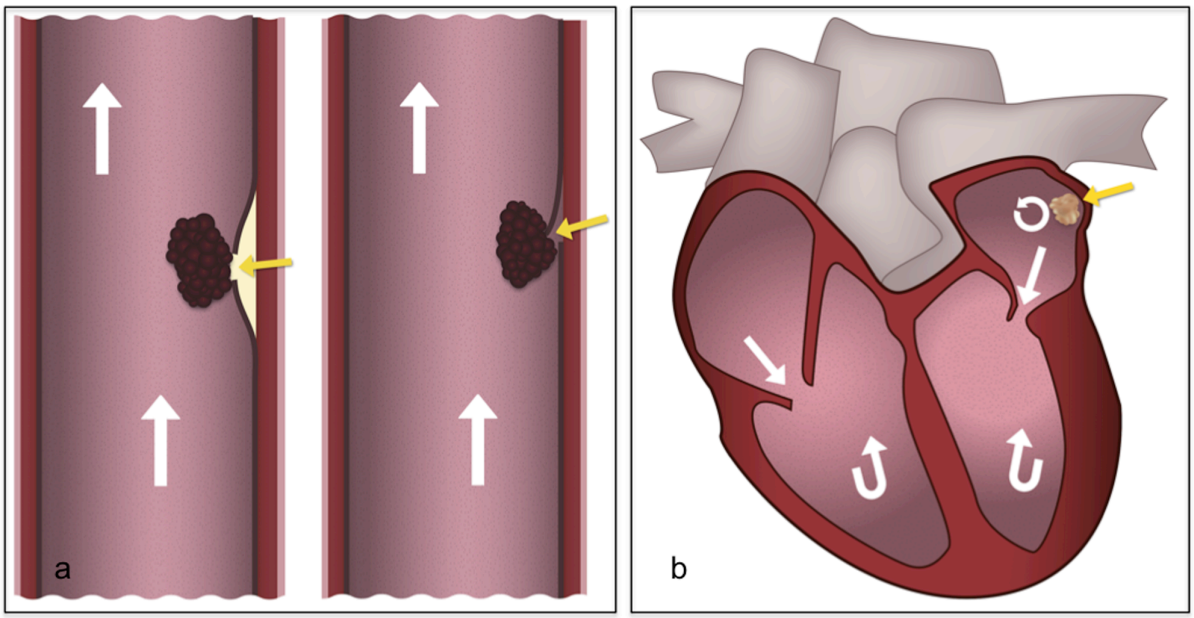

Fig. IIa: Arterioembolic clot formation due to a local stimulus of a ruptured plaque (left image, TOAST 1) and clot formation in dissection due to an intima lesion (right image, TOAST 4) show fundamental analogies, possibly explaining similar thrombus characteristics.

Fig. IIb: The fundamentally different clotting mechanism in cardioembolic strokes (compared with local clot formation by plaque rupture or intima violation) is predominantly based on local "low-flow" or "circular-flow" areas with possible continuous thrombus growth.

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