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Thrombus age does not differentiate between cardiogenic and atherosclerotic strokes

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ABSTRACT

Objective Interventional stroke therapy made thrombi available for histological analysis. Unfortunately, simple composition aspects such as erythrocyte versus fibrin/platelet rich did not allow a feasible allocation to thrombi's cardiac or carotid origin. Since the mentioned criteria represent characteristics of thrombus age, we used established histological criteria for determining thrombus age in patients who had an atherosclerotic (TOAST (Trial of Org 10172 in Acute stroke Treatment) 1) stroke versus patients who had a cardioembolic (TOAST 2) stroke.

Methods We assessed prospectively data from stroke patients presenting with occlusion of the middle cerebral artery eligible for catheter-based intervention. Besides patient characteristics and stroke workup, extracted thrombi were classified into different age categories according to their cellular to fibrotic transition. Thrombi were collected in an erythrocyte lysing solution to reduce acute clotting effects. Statistics were done with a non-parametric Kolmogorov-Smirnov test.

Results 170 patients were included, of which 50 (38 men; 73±12 years) had a TOAST 1 and 99 (59 women; 75±10 years) had a TOAST 2 categorised stroke. Age, National Institutes of Health Stroke Score (13±7 vs 15±7), Alberta Stroke Program Early CT Score (9±3 vs 9±2), Thrombolysis in Cerebral Infarction Score (2.9±0.2 vs 2.9±0.3), modified Rankin Score on discharge (3.2±2 vs 3.2±2), number of vascular risk factors (0.9±1.4 vs 1.0±1.1) or time span between symptom onset to reperfusion (266±115 vs 260±128 min) remained non-significant. Also, thrombus age did not differ between the groups. The mean age of thrombi was 5–8 days. However, the male–female ratio differed significantly ($p<0.0005$) between groups, with more men in TOAST 1 group and more women in TOAST 2 group.

Conclusion Age aspects of thrombi seem not feasible to allow reliable source allocation. However, the young age of thrombi points to a rapid detachment. The difference in sex relation is in line with previous reports.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ In stroke therapy, the allocation of thrombus source is relevant due to different medication regimes. However, histological composition aspects catheter-retrieved thrombi did not gain clinical feasibility regarding source allocation.

WHAT THIS STUDY ADDS

⇒ With 50% of thrombi classified as younger than a week showed a rapid detachment of thrombi and that thrombi from stroke TOAST 1 or TOAST 2 classified groups did not differ in age aspects.

INTRODUCTION

Endovascular stroke therapy is routine in most stroke centres.[1] Therefore, embolic thrombus material became available for histological analysis. Several studies investigated if distinct features of thrombus histology—such as fibrin/platelet conglomerates and the proportion of red and white blood cells in thrombi—could provide information about the source of the thrombus.[2–8] Thrombi of cardiogenic origin (as defined by TOAST criteria) seemed to be erythrocyte rich, whereas thrombi originating from atherosclerotic carotid stenosis appeared to have a significantly higher fibrin/platelet content.[6 7] A recent publication found a higher percentage of macrophages in cardiogenic emboli, supporting the finding that cardiogenic thrombi are cell rich.[9] An immunohistochemical workup of thrombi regarding granulocytes, macrophages and monocytes did not reveal additional information.[8] Other studies did not find differences in thrombus composition when comparing cardiac or arterial sources of stroke,[2] but these studies were criticised due to an insufficient sample size.[9]

However, the composition of thrombi might not only differ due to their source origin, as suggested by aforementioned papers, but also changes due to thrombus age.

In the present study, we compared thrombus age between TOAST groups of cardiogenic versus atherosclerotic origin. Thrombus age was determined according to the established histological criteria.[10]

METHODS

Study population

We prospectively and consecutively studied 170 patients who had an ischaemic stroke due to occlusion of the middle cerebral artery (MCA) and underwent thrombectomy using a stent retriever. Patient selection and indication for interventional therapy based on established clinical criteria independently from the study protocol. The inclusion criterion for data evaluation for the present study was age between 18 and 85 years.

The cause of stroke was determined according to the TOAST classification. Patients grouped under groups TOAST 1 or 2 were further evaluated. Excluded were patients with multiple potential aetiologies. Patients with criteria 3 (small-vessel occlusion) were not the focus of this study. Patients in groups TOAST 4 (stroke of other determined aetiology) and 5 (stroke of undetermined aetiology) were omitted (n=21).

Routine diagnostic workup for each patient included cerebral CT scan, MRI, duplex ultrasonography of the extracranial and intracranial arteries, 24-hour Holter monitoring and transthoracic and transoesophageal echocardiographies. Laboratory workup evaluated lipid profile, haemoglobin A1c and coagulation tests.

General patient information included age, sex, National Institutes of Health Stroke Scale/Score (NIHSS)[11] at admission, Alberta Stroke Program Early CT Score (ASPECTS)[12] Score, modified Rankin Score (mRS)[13] at admission and Thrombolysis in Cerebral Infarction (TICI)[14] Scale.

Interventional thrombectomy

A neuroradiologist performed interventional thrombectomy using the following devices: Neuron MAX System (Penumbra, Alameda, California, USA), ACE 68 (Penumbra, Alameda, California, USA), Prowler Select (Medos International, Le Locle, Switzerland) and Catch-View Mini (Balt, Montmorency, France). The procedure was performed under general anaesthesia using a biplane Neuro X-ray system (Philips Azurion 7 B20/15, Andover, Massachusetts, USA).

Clot analysis

Immediately after extraction from the MCA, the thrombus was stored in a vial containing CytoRich red (Thermo Fisher Scientific, Karlsruhe, Germany) solution. CytoRich lyses red blood cells, that is, agglomerated erythrocytes, due to interventional procedures, which are not originally part of the thrombus itself.[15] For staining, thrombi were cut into 2 µm thick slices. The sections were then stained with H&E and Elastica van Gieson. Then, the slices were scanned at high resolution (×400), and the images were stored digitally. Thrombi were separated into four age classes. The evaluation was performed by an experienced pathologist masked for the clinical information and was based on the following characteristics[10]:

1. Thrombus age group 1: A thrombus was classified as 1–4 days old (d1–4) if features of a layered fibrin pattern, intact platelets, erythrocytes and granulocytes were present. Signs of karyorrhexis of nuclear cells, fragmentation or swelling of histiocytary cells were absent.
2. Thrombus age group 2: A thrombus age was assumed to be in the range of 5–8 days (d5–8) if lytic areas with homogeneous structural elements, with colliquation necrosis and with karyorrhexis of granulocytes (<50% of granulocytes) were present. Single histiocytary cells show swelling, that is, bulking of cytoplasm.
3. Thrombus age group 3: 9–12-day-old thrombi show no clearly separable erythrocytes. Histiocytary cells are swollen, and more than 50% of granulocytes undergo karyorrhexis.
4. Thrombus age group 4: In d13–17-old thrombi, erythrocytes or granulocytes are not separable, granulocyte karyorrhexis, and swelling of histiocytary cells is progressive. Organised areas with features of endothelialisation and ingrowth of capillary vessels, with or without ingrowth of smooth muscle cells and connective tissue deposition, develop. In case of a heterogenous composition of the thrombus, the age was classified by the oldest parts of the thrombus.

Statistical analysis

SAS statistical software package was used for statistics (StatView, Version 5.0.1, SAS Institute). P values≤0.05 were considered statistically significant. The parameters of the two TOAST groups were compared with the non-parametric Kolmogorov-Smirnov test.

RESULTS

Patient demographics

Of the 170 patients included, 50 were grouped under TOAST 1 and 99 were grouped under TOAST 2 groups. Table 1 shows the baseline data of the patients.

Patients in TOAST 1 and 2 groups differed only in gender ratio. No differences appeared regarding age (TOAST 1: 73±12 years vs TOAST 2: 75±10 years; n.s.) or scores for NIHSS (13±7 vs 15±7; n.s.), ASPECTS (9±3 vs 9±2; n.s.), TICI (2.9±0.2 vs 2.9±0.3; n.s.), mRS at discharge (3.2±2 vs 3.2±2; n.s.), number of vascular risk factors (0.9±1.4 vs 1.0±1.1; n.s.) and time between symptom onset and reperfusion (266±155 min vs 260±128 min; n.s.).

Thrombus age data

Thrombus age did not differ between TOAST groups, as given in table 2.

The mean thrombus age class in TOAST 1 group was 1.9±1.1, with 52% of the thrombi being classified as d1–4, 22% as d5–8, 12% as d9–12 and 14% as d13–17 old. In TOAST 2 group, the mean thrombus age class was 2±1, with 40% of the thrombi being classified as d1–4, 28% as d5–8, 21% as d9–12 and 11% as d13–17 old, respectively.

Table 1 Patients characteristics

Parameters	TOAST 1: atherosclerotic mean±SD	TOAST 2: cardioembolic mean±SD	Statistics: p<0.05
N	50	99	
Male/female	38/12	40/59	p<0.0005
Age (years)	73±12	75±10	n.s.
NIHSS	13±7	15±7	n.s.
ASPECTS	9±3	9±2	n.s.
TICI	2.9±0.2	2.9±0.3	n.s.
mRS at demission	3.2±2	3.2±2	n.s.
Sum of risk factor: nicotine, aHT, HLP and DM	0.9±1.4	1.0±1.1	n.s.
Time symptom onset to reperfusion (min)	266±115	260±128	n.s.

aHT, arterial hypertension; ASPECTS, Alberta Stroke Program Early CT Score; DM, diabetes mellitus; HLP, hyperlipidaemia; mRS, modified Rankin Score; NIHSS, National Institutes of Health Stroke Scale/Score; TICI, Thrombolysis in Cerebral Infarction Scale; TOAST, Trial of Org 10172 in Acute Stroke Treatment.

DISCUSSION

Comparing patient groups according to the TOAST criteria for cardiogenic or atherosclerotic stroke, we found no statistically significant age-related aspects of thrombi. Our data, therefore, do not support reports that erythrocyte-rich thrombi (ie, suggesting 'young' thrombi) are likely to be of cardiogenic origin and fibrin-dominant thrombi (ie, 'old' thrombi) are of atherosclerotic origin. Although not all studies came to the same clear findings, we could possibly explain the discrepancies through the erythrocyte lysing effect of CytoRich: Acute generation of erythrocyte conglomerates is an often periprocedural effect of the catheter technique leading to a bias in most previous reports which did not control for this effect. To the best of our knowledge, our study is the first to use CytoRich, whereas it is commonly used in gynaecological cervical PAP (Papanicolaou) smears or in haemorrhagic fine needle aspiration for fixation of cells alongside lysis of background red blood cells without compromising the interpretation of the histologic specimen.[15] Furthermore, differences in the use of recombinant tissue-type plasminogen activator (rtPA) may also affect thrombus composition: Bridging rtPA treatment before endovascular thrombectomy was associated with a significantly greater erythrocyte composition and lower fibrin composition[16–18] and thinner fibrin layers.[19] Moreover, rtPA pretreatment activates platelets in up to 34% of patients[20] and releases plasminogen activator inhibitor-1 and alpha2-antiplasmin, which, in turn, may lead to paradoxical thrombosis.[21] Recent review reports on the matter of composition aspects of thrombi seem to disbelieve in a clinically feasible source allocation.[22 23] However, the histological evaluation addresses morphological aspects of thrombi, which are not influenced by the above-mentioned periprocedural erythrocyte conglomerates. Therefore, we did not find an rtPA effect between groups: Intervention with prior rtPA bridging was performed in 34% of patients in TOAST 1 group and 37% of patients in TOAST 2 group. Bridging with rtPA did not affect thrombus age class results (TOAST 1: no rtPA bridging: 1.9±1.3vs rtPA bridging: 1.8±1.2; p=n.s.; TOAST 2: no rtPA bridging: 2.1±1 vs rtPA bridging: 2±1; p=n.s.).

Interestingly, the mean age of thrombi was about 1 week, independent of the TOAST group. This suggests rapid detachment of thrombus material from the primary location and is in line with findings that thrombotic material on arterial plaques or in the left atrial appendage excessively increases the risk of stroke.[24–26] Older thrombi than those of group 4 (d13–17) were not seen. Older thrombi stabilise by invasion of smooth muscle cells and fibrinocytes along with a network matrix of collagen, elastin and other molecules. In addition, endothelial cells begin to coat the thrombus material.[27] Plaque rupture might therefore be also a possible reason for acute MCA

Table 2 Clot data: age and age distribution

	TOAST 1: atherosclerotic mean±SD	TOAST 2: cardioembolic mean±SD	Statistics: p<0.05
Thrombus age group	1.9±1.1	2±1	n.s.
	n=50	n=99	
Thrombus age group 1 (%)	52	40	
Thrombus age group 2 (%)	22	28	
Thrombus age group 3 (%)	12	21	
Thrombus age group 4 (%)	14	11	

TOAST, Trial of Org 10172 in Acute Stroke Treatment.

occlusion, but was not seen in the present study, possibly due to the infrequent occurrence of a rupture.

The significant difference in gender ratio between atherosclerotic and cardioembolic patient groups is consistent with the literature[28]: Men account for approximately 60% of atherosclerosis-related stroke cases and only 40% of cardioembolic stroke.

Limitations of the study might be a selection bias due to the exclusion of stroke patients without occlusion of main cerebral vessels. Excluded were also patients with basilar artery occlusion because pathological conditions (ie, local thrombotic occlusion of basilar artery) as well as intervention procedures differ from that of patients with MCA occlusion. Another limitation is the skewed distribution of the different stroke types, which only allowed a comparison between TOAST 1 and 2 groups.

A further bias to older reports may result from a more generous use of catheter intervention: Today, the time window of catheter intervention has extended, and intervention is more frequently used in addition to intravenous lysis at an early time window.

CONCLUSION

Histologically determined age aspects of thrombi did not differ between atherosclerotic and cardiac origins. However, the young age of thrombi supports the recommendation of a fast clinical workup of patients to prevent further strokes.

Contributors BR is the guarantor. BR and JJS: conception and design and drafted the article. SS and JJS: acquisition of data. SB: interventional radiologist. OD: thrombus workup and thrombus age determination. BR: analysis and statistics. All authors: interpretation of data and critically reviewed the article.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the Declaration of Helsinki 1964 and its later amendments or comparable ethical standards. The Saxonian Ethics Committee approved the study (EK-BR-30/1B1). The written informed consent was obtained from all patients or their legal representatives.

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Data availability statement Data are available upon reasonable request. Due to local privacy policy conditions data are not publicly available. In case of interest a request should be sent to the corresponding author.

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