

ONLINE SUPPLEMENT

Thrombus histology suggests cardio-embolic etiology in cryptogenic stroke

Tobias Boeckh-Behrens, MD¹, Justus F. Kleine MD¹, Claus Zimmer, MD¹, Frauke Neff, MD², Fabian Scheipl, PhD³, Jaroslav Pelisek, PhD⁴, Lucas Schirmer, MD⁵, Kim Nguyen⁵, Deniz Karatas⁵, Holger Poppert, MD⁵

¹Department of Neuroradiology, University Hospital Rechts der Isar, Technical University Munich, Germany

²Department of Pathology, German Research Centre for Environmental Health, Munich, Germany

³ Department of Statistics, Ludwig-Maximilians-University Munich, Germany

⁴Clinic of Vascular and Endovascular Surgery, University Hospital Rechts der Isar, Technical University Munich, Germany

⁵Department of Neurology, University Hospital Rechts der Isar, Technical University Munich, Germany

Corresponding author: Tobias Boeckh-Behrens, MD

Department of Neuroradiology

Klinikum Rechts der Isar

Technical University Munich

Ismaninger Str. 22

D-81675 Munich/Germany

Tel: +49-89-4140-5274

Fax: +49-89-4140-4887

Email: boeckh-behrens@tum.de

Cover Title: Thrombus histology and cryptogenic stroke

List of Tables and Figures:

Table 1: Clinical characteristics

Table 2: Basic interventional and histological parameters

Table 3: Differences between non-cardio-embolic and cardio-embolic stroke patients

Table 4: Differences between cryptogenic and non-cardio-embolic stroke patients

Figure 1

Figure 2 A-C

Key Words: Stroke, Etiology, Thrombus, Histopathology, Mechanical recanalization

Subject Codes: 53, Embolic Stroke; 63, Pathology of Stroke; 172, Arterial Thrombosis

Supplementary Data with 1 Supplementary Figure

Number of Words: 5744

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 TICl 2b or 3; TICl 2b being defined as complete revascularization of more than two-thirds of the target area, and TICl 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- μ m 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 ($\times 400$) 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	
<i>Time to treat (min):</i> <i>median, range</i>	220, 15–625
<i>Recanalization time (min):</i> <i>median, range</i>	50, 10–308
<i>Number of maneuvers:</i> <i>median, range</i>	3, 1–12
<i>TICI score (percentage), n = 137</i>	
TICI 0	2.2%
TICI 2a	8%
TICI 2b	35%
TICI 3	54.7%
Histological parameters	
<i>Thrombus components , median</i> <i>percentage, range</i>	
F/P	47%, 2-89
RBC	43%, 2-96
WBC	7%, 1-31

Supplemental Figures

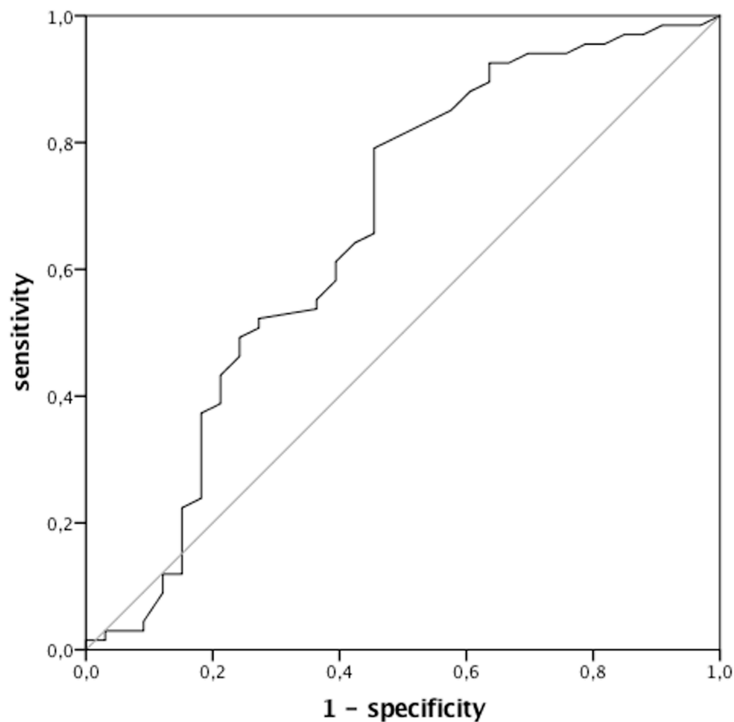


Fig. I: ROC analysis for F/P content and cardioembolic stroke origin, AUC-value 0.661, $p = 0.009$.

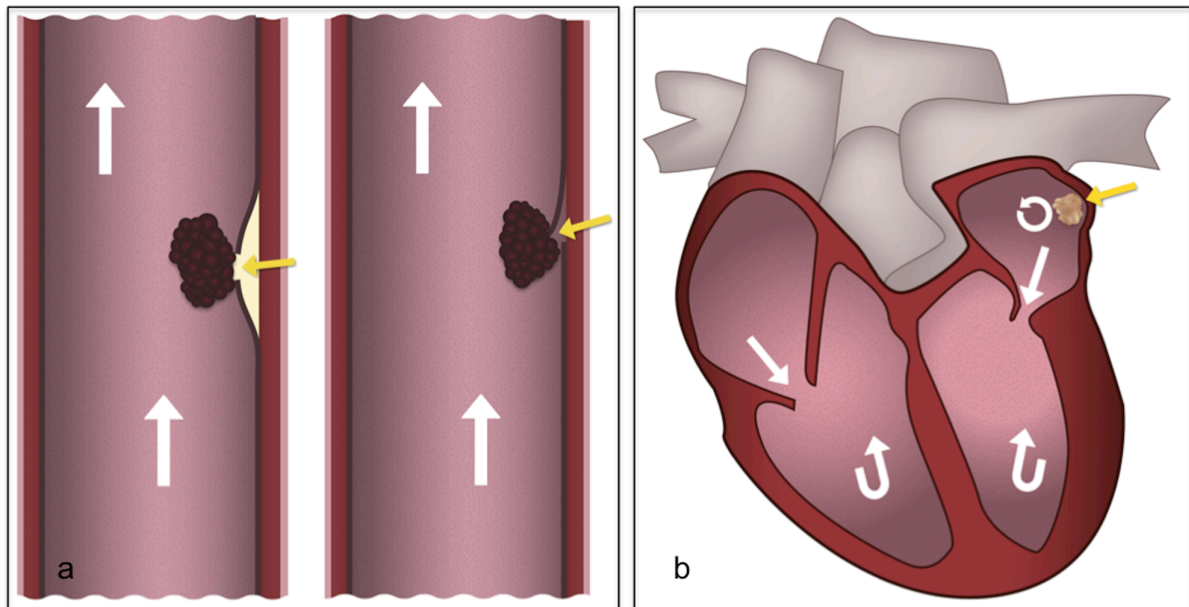


Fig. IIa: Arterioembolic clot formation due to a local stimulus of a ruptured plaque (left image, TOAST 1) and clot formation in dissection due to an intima lesion (right image, TOAST 4) show fundamental analogies, possibly explaining similar thrombus characteristics.

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.

Supplemental references

1. Boeckh-Behrens T, Schubert M, Forschler A, Prothmann S, Kreiser K, Zimmer C, et al. The impact of histological clot composition in embolic stroke. [published online ahead of print September 27, 2014]. *Clinical neuroradiology*. 2014. February 15, 2016
2. Higashida RT, Furlan AJ, Roberts H, Tomsick T, Connors B, Barr J, et al. Trial design and reporting standards for intra-arterial cerebral thrombolysis for acute ischemic stroke. *Stroke; a journal of cerebral circulation*. 2003;34:e109-137