
From the Klinik für Radiologie und Neuroradiologie

Director: Prof. Dr. med. Olav Jansen

at the University Medical Center Schleswig-Holstein, Campus Kiel

at Kiel University

**PREDICTIVE VALUE OF THROMBUS
CHARACTERISTICS ON RECANALIZATION
RATE AND CLINICAL OUTCOME IN ACUTE
POSTERIOR STROKE PATIENTS RECEIVING
ENDOVASCULAR THERAPY**

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LIANG SHU

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1st Reviewer: Prof. Dr. Olav Jansen, Klinik für Radiologie und
Neuroradiologie

2nd Reviewer: Prof. Dr. Ralf Baron, Klinik für Neurologie

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Abbreviations

AICA	Anterior Inferior Cerebellar Artery
BA	Basilar Artery
BAO	Basilar Artery Occlusion
CECT	Contrast-Enhanced Computed Tomography
CSF	Cerebro-Spinal Fluid
CT	Computed Tomography
CTA	Computed Tomography Angiography
CTP	Computed Tomography Perfusion
DSA	Digital Subtraction Angiography
ECASS	European Cooperative Acute Stroke Study
EVT	Endovascular Therapy
HAS	Hyperdense Artery Sign
HBAS	Hyperdense Basilar Artery Sign
HMCAS	Hyperdense Middle Cerebral Sign
HU	Hounsfield Unit
IAT	Intra-Arterial Thrombolysis
IQR	Inter-Quartile Range
IVT	Intra-Vascular Thrombolysis
MCAO	Middle Cerebral Artery Occlusion
MIP	Maximum Intensity Projection
MRA	Magnetic Resonance Angiography
MRI	Magnetic Resonance Imaging
mRS	modified Rankin Scale
NECCT	Non-Enhanced Cranial Computed Tomography

NIHSS	National Institutes of Health Stroke Scale
OTT	Symptom Onset To Treatment
PCA	Posterior Cerebral Artery
ROI	Region of Interest
rtPA	recombinant tissue Plasminogen Activator
sICH	symptomatic Intra-Cranial Hemorrhage
SVS	Susceptibility Vessel Sign
SWI	Susceptibility Weighted Imaging
SUCA	Superior Cerebellar Artery
TICI	Thrombolysis In Cerebral Infarction
TIMI	Thrombolysis In Myocardial Infarction
VA	Vertebral Artery
WBC	White Blood Cell

General Introduction

Acute occlusion of the basilar artery (BAO) is a devastating disease, resulting in really high fatality and morbidity if untreated. Historically, Endovascular therapy (EVT) has been the treatment of choice¹. However, in contrast to the anterior circulation, the optimum treatment strategy for BAO has not been validated in randomized controlled trials since very few patients with BAO have been included in intravenous thrombolysis (IVT) trials. Most of our knowledge on the efficacy and safety on IVT in BAO stems from very few centers routinely performing IVT in these patients. For example, it was recently demonstrated that recanalization rate of occluded basilar artery receiving IVT (62.1%) depends on thrombus length².

More recently, five randomized controlled trials demonstrated the safety and effectiveness of EVT in patients with anterior circulation stroke³⁻⁷. BAO patients again had been almost completely excluded. The ENDOSTROKE registry⁸ claimed similar positive results in BAO patients treated with EVT. The recanalization rate in this study was up to 79% (117 /148) in patients with acute BAO whereas 34% (50/148) had favorable clinical outcome. In contrast, the BASICS registry⁹ could not prove the superiority of EVT. Recent smaller scale studie¹⁰ demonstrated high recanalization rates in patients with large vessel occlusion. However, only sixteen BAO patients were included in the cohort and in addition, six of them (12% of total) underwent thrombectomy alone due to IVT contraindications. Whether EVT is the prime treatment strategy for BAO is still uncertain.

In the past decades, neuroimaging factors have taken on an important role in determining efficacy of different strategies of treatment and predicting clinical and radiological outcome in stroke patients. Since most of the studies have been performed in patients with anterior circulation stroke data on BAO patients is scarce. The aim of this study was to evaluate the prognostic value of thrombus

characteristics in patients with BAO.

Part I Impact of Thrombus Characteristics on Recanalization Rate and Clinical Outcome in Acute Posterior Stroke Patients Receiving Endovascular Therapy.

Introduction

Numerous prognostic factors, such as thrombus length measured on non-enhanced cranial computed tomography (NECCT) images^{11,12}, clot burden score calculated on magnetic resonance imaging (MRI) images¹³, and clot length assessed by using susceptibility weighted imaging (SWI), have been proposed to determine the efficacy of recanalization therapy and clinical outcome in patients with large vessel occlusion. However, the results are somewhat controversial. A key finding reported by Riedel was that there is little chance to reopen the occluded vessel in patients with acute middle cerebral occlusion after IVT if the thrombus length exceeded 8 mm¹². Using computed tomography angiography (CTA), Rohan and his coworkers showed that an optimal cutoff value of 12mm of thrombus length in M1 segment occlusion was an independent predictor of recanalization success¹⁴. However, using SWI, Weisstanner found that the recanalization rate was not dependent on thrombus length in patients with anterior circulation stroke treated with EVT¹⁵. Additionally, in a larger cohort, Spiotta and his colleagues also observed no association between thrombus length and both likelihood of mechanical recanalization and clinical outcome in patients received EVT¹⁶. This “disputable” correlation has been seen in posterior circulation stroke as well. In BAO patients, Strbian reported recanalization success of basilar artery occlusion with IVT depends on thrombus length, although there was no threshold thrombus length². However, no correlation was found between thrombus length and successful recanalization in a small cohort of BAO patients¹⁷. Undoubtedly, a longer thrombus is associated with reduced probabilities of reperfusion following IVT treatment although different or no cutoff values of thrombus length were found. Nevertheless, the correlation between thrombus length and recanalization rate and clinical outcome in the acute stage of BAO remains uncertain.

As one of the thrombus imaging characteristics, thrombus density has emerged as a strong marker for predicting the likelihood of reperfusion in patients treated with IVT. However, findings on the relationship of thrombus density with recanalization rate and clinical outcome were contradictory. Some authors¹⁸⁻²⁰ established that thrombi with lower density (in Hounsfield unit, HU) were more likely resistant to pharmacological lysis. Furthermore, hyperdense thrombi were also shown to associate with higher recanalization rates in patients treated with EVT^{21,22}. Other researchers did not find the same association neither in patients who received IVT¹⁴ or EVT^{16,23}. Additionally, thrombus density did not relate to long-term clinical outcome (90 days or 6-month follow-up) in patients treated with IVT according to others^{14,24,25}. However, the relationship between thrombus density and clinical outcome still needs further determination. Although EVT was widely used for patients with large vessel occlusion in recent years, it still remains uncertain as to how key information about thrombus imaging characteristics might improve treatment decision-making as successful recanalization was independent of a high clot burden²⁶. More substantial insights into thrombus composition or thrombus density should be given to determine therapeutic implications. Therefore, we aimed to evaluate the prognostic value of thrombus characteristics, including thrombus length and thrombus density, regarding recanalization rate and outcome in BAO patients treated with EVT.

Methods and Materials

Patients

The local institutional review board and ethic committee approved the study. We retrospectively studied consecutive patients with BAO treated with EVT between April 2007 and October 2015. Patients who underwent non-enhanced cranial CT (NECCT) scanning with thin-slice reconstructions before treatment were included (n=55). We excluded patients with significant artifacts in the initial NECCT images preventing thrombus evaluation (n=4). Therefore, our cohort comprises 51 BAO patients (Fig. 1).

Clinical data

The relevant demographic data and clinical data of all included patients were extracted from the patients' charts and collected by a board-certified neurologist. Neurological disease severity at admission was scored using the National Institutes of Health Stroke Scale (NIHSS). The site of occlusion proven by Computed Tomography Angiography (CTA) was categorized as proximal [between the confluence of the vertebral arteries (VA) and the origin of the Anterior Inferior Cerebellar Artery (AICA)], mid [between the origin of the AICA and the origin of the Superior Cerebellar Artery (SUCA), but also any occlusion of more than one segment including mid-basilar²], and top of the BA²⁷. Time from onset to treatment (OTT) was defined as the interval between symptom onset to initiation of treatment. If the time of onset was unknown, the time that the patient was last seen asymptomatic was recorded. Stroke etiology was determined using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification²⁸ and a standardized battery of test including follow-up brain imaging (CT and/or Magnetic Resonance Imaging (MRI)), cervical and intracranial vessel imaging (CTA, Magnetic Resonance Angiography (MRA) or ultrasound), 24h ECG Holter monitoring, transthoracic or transesophageal echocardiography and further laboratory tests as

clinically feasible and appropriate. Further relevant demographic data were extracted from the patients' charts.

Recanalization and outcome

A neuroradiology fellow other than the performing neurointerventionalist blinded to clinical outcome interpreted recanalization success on digital subtraction angiography (DSA) images after the intervention. A TICI score of 2b-3 was defined as successful recanalization, and a TICI score of 0-2a was considered as no recanalization or unsuccessful recanalization^{2,9,29}. Clinical outcome was evaluated using the modified Rankin scale (mRS) at 3 months either by face-to-face consultation or telephone interview. An mRS of 0 to 2 was defined as favorable outcome, whereas an mRS of 3-6 was defined as poor clinical outcome.

Image acquisition, analysis and statistics

Thrombus length

NECCT

Thrombi in the basilar artery were detected in all NECCT datasets (Brilliance 64, Philips, Best/The Netherlands, 120 kV, 320 mAs, reconstructed slice thickness 2.5mm). The clot length was calculated by one author as described previously³⁰. In short, a region of interest (ROI) was defined in the course of a hyperdense vessel. All pixels between 55 and 80 HU served as a seed for a region-growing algorithm into adjacent pixels between 40 and 80 HU. Thrombus length was then calculated along the middle axis of this defined volume. The presence of a hyperdense basilar artery sign (HBAS) was rated by two independent raters. If disagreement was present a consensus was reached. Cohen's κ was used to assess the agreement.

CTA

CTA (80 kV, 280 mAs, 80 ml bolus of 350 mg I/ml, followed by a 40-ml saline flush at 5 ml/s) was available in 46 (90.2%) patients. Thrombus length, defined as an opacification gap on CTA, was determined by one investigator and checked for consistency by another, both blinded to thrombus length measurement on NECCT. A Bland-Altman plot was utilized to depict the consensus between thrombus length measurement by CTA and NECCT.

Thrombus density

First, two observers (one is an experienced neuroradiologist and the other a neuroradiology fellow) blinded to clinical data and read the whole series of images on thin-slice NECCT (reconstructed slice thickness 2.5 mm). Second, the HBAS was identified according to the following criteria, which are similar to those used for hyperdense middle cerebral artery sign (HMCAS) recognition³¹: 1) the whole axial view of the BA can be recognized easily; 2) attenuation of the vessel was higher than that of the surrounding tissue; 3) attenuation of the vessel was much higher than that of the vessel without hyperdense sign. Vessel wall calcifications (>100 HU) were excluded from the measurement. Third, the polygonal lasso tool (IMPAX workstation, Agfa Technical Imaging Systems) was utilized to place an Region of Interests (ROIs) encompassing within the whole hyperdense vessel slice by slice and ensure that the outline of the ROI would not surpass the margin of the thrombus (Fig. 2). Fourth, absolute HU values were calculated by adding the obtained HU values and then dividing by the number of observed slices. When available, CTA was used to localize the thrombus, especially a hypodense thrombus (n=9) (synchronous-localization function, Agfa Technical Imaging Systems). In order to correct the influence of hematocrit level on measurements, relative HU values were evaluated by using the HU ratio ($HU_{\text{clot}} / HU_{\text{contralateral}}$). $HU_{\text{contralateral}}$ for calibration was measured in a vessel proximal (intracranial vertebral artery or proximal basilar artery) or distal (distal basilar artery or posterior cerebral artery) to the clot^{20, 22, 32}.

Statistics

Statistical analysis was performed using SPSS software version 22 (IBM, NY). All continuous variables were first tested for normality using the Kolmogorov-Smirnov test. Variables with a normal distribution were noted as mean \pm SD and were tested using an independent t-test. Mann-Whitney U test was used to analyze the variables without a normal distribution. To describe them, median value and the interquartile range (IQR) were calculated. Categorical variables are presented in numbers (percentage, %), and assessed using Fisher's exact test. Spearman correlation test was used to evaluate the relationship between thrombus characteristics with OTT. Logistic regression analysis was performed to determine clinical factors, which might have a prognostic value regarding successful recanalization and favorable outcome. Statistical significance was set at $p < 0.05$.

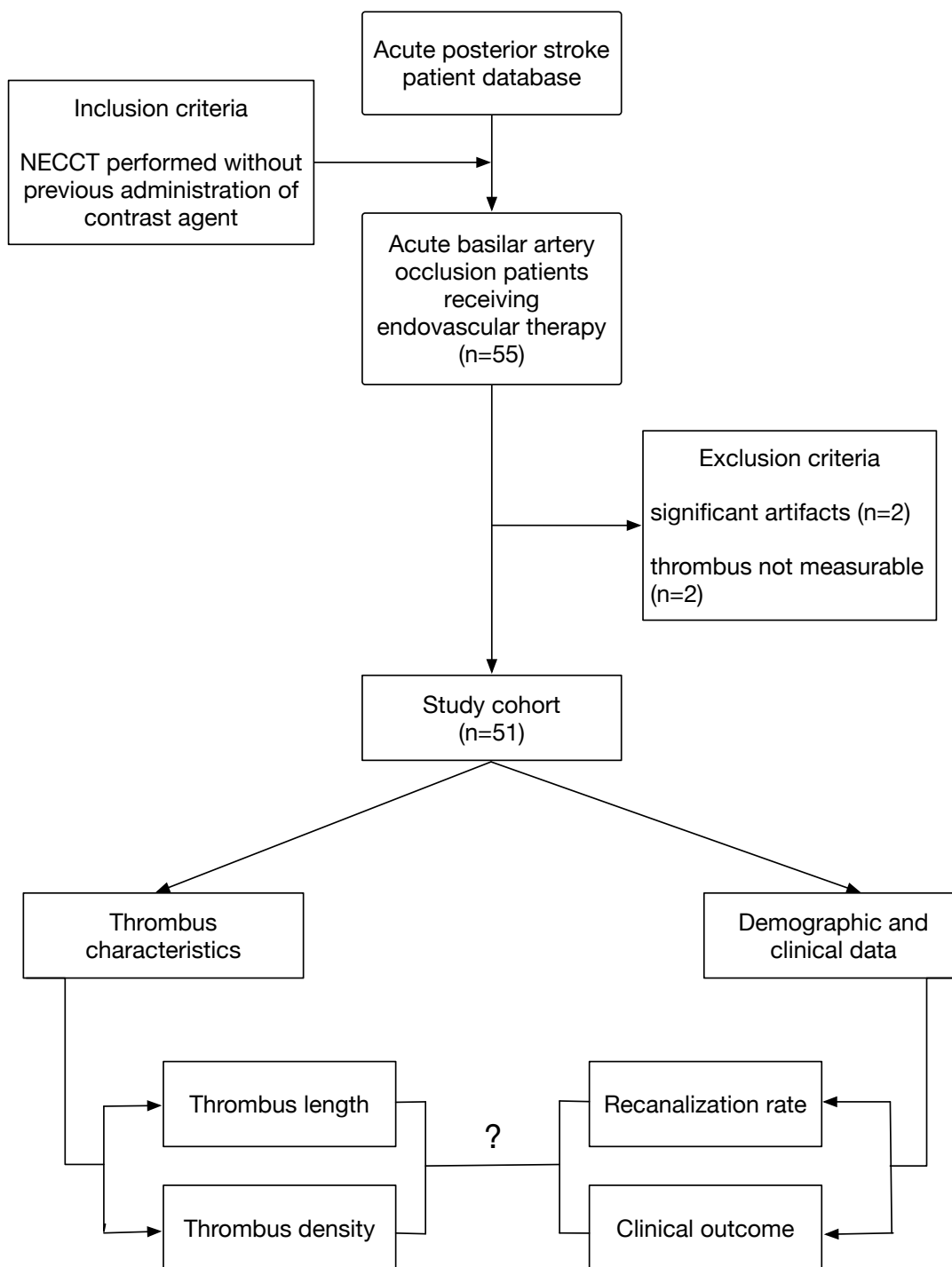


Figure 1. Study design and patient selection.

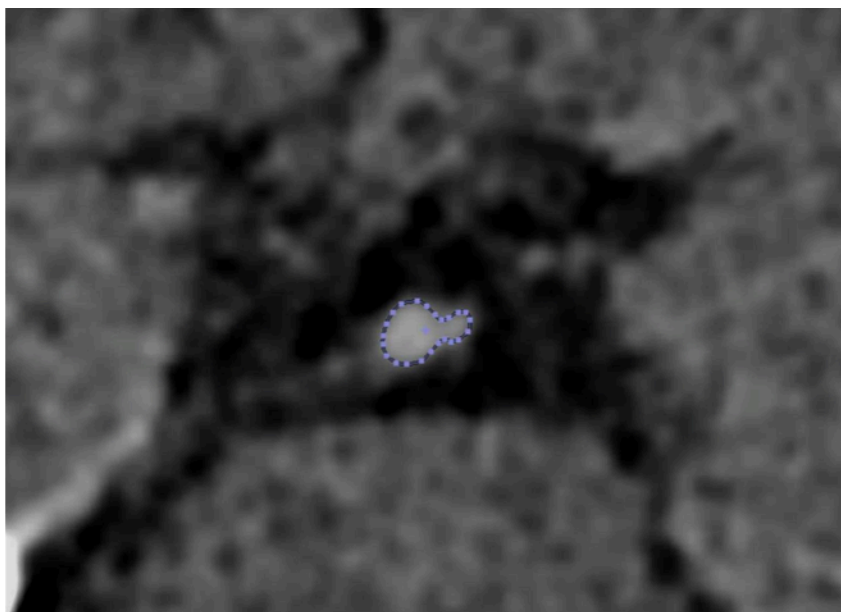


Figure 2. Example of the ROI definition. The outline was drawn along the boundary of the thrombus. This outline should not surpass the margin of the basilar artery, and any calcified plaque ($HU > 100$) was excluded. This was repeated on every slice, the obtained HU values were added and then divided by the number of observed slices.

Results

Fifty-one BAO patients treated with EVT were included in final analysis. EVT was started within 6 hours after symptom onset in most cases (n=37/73%). EVT was performed using three different strategies: intra-arterial rtPA alone (5-80 mg, N=5), mechanical thrombectomy (n=30), and mechanical thrombectomy in combination with intra-arterial rtPA (n=16) (Table 1). IVT (0.9 mg rtPA/kg body weight) was delivered in 15.7% (n=8) of patients prior to EVT when proven BAO occurred within 4.5 h of symptom onset. Baseline characteristics and imaging-related factors of all patients are summarized in Table 2 and Table 3. 70.6% of the patients were male (n=36), median age was 72 years (66-78 years, IQR), and median NIHSS at admission was 18 (11-23points, IQR). There was no relationship between baseline NIHSS and recanalization rate. However, low baseline NIHSS was a strong predictive factor for favorable outcome ($P=0.04$). Median thrombus length measured on NECCT was 7.5 mm (5-14.4 mm, IQR). Absolute thrombus density was 51.6 ± 6.2 HU (mean value), whereas mean relative thrombus density was 1.34 ± 0.15 . There was no correlation between thrombus length and density ($P=0.16$). CTA was utilized to determine the site of BA occlusion before treatment. Proximal BA occlusion was observed in 19.6% (n=10) while mid-BA occlusion was 41.2% (n=21) and top of BA occlusion was 39.2% (n=20). No evidence was found in support of a different site of occlusion being relevant to recanalization rate or clinical outcome (Table 2 and 3). In 42 patients (82.4%) HBAS was present (Cohen's $\kappa = 0.74$, Table 4). Median time from onset to treatment (OTT) was 222 min (178-462 min, IQR). Shorter OTT showed a trend, but not a statistically significant difference towards higher recanalization rate ($P=0.13$), whereas no relationship was found between OTT and clinical outcome. Neither the presence of a HBAS nor OTT was correlated with recanalization and outcome. In the Spearman correlation test, neither thrombus length nor thrombus density was correlated with OTT (Fig.3 and 4). BAO patients only treated with IAT tended to hardly achieve successful recanalization ($P=0.04$). However, the population was quite small (n=5 and successful recanalization was only in one patient). Bland-Altman plot demonstrated a good agreement

between thrombus length measured on NECCT and on CTA (Fig. 5). Interestingly, the thrombi of the three patients whose data points were outside the limits of agreement, proved to be of heterogeneous composition (Fig. 6). Stroke etiology was cardiogenic (58.5%, $N=30$), atherosclerotic large artery (25.5%, $N=13$), other confirmed (9.8%, $N=5$), and unknown (5.9%, $N=3$) according to TOAST criteria²⁸.

Recanalization

Successful recanalization was achieved in 34 patients (66.7 %). In forty-six patients, first-generation mechanical devices (such as Penumbra) were applied in 30.4% ($n=14$), whereas second-generation devices (e.g. stent retrievers such as Trevo or Solitaire) were used in 69.6% ($n=32$) (one device alone or in combination with other devices). In seven patients (21.8%) no or minimal recanalization was achieved. In one patient (3.1%) the exact number of passes was not documented. For the remaining twenty-four patients, we did not find a correlation between thrombus density and number of stent-retriever passes ($P=0.197$). However, there was a trend towards longer thrombus needed more number of stent-retriever passes ($r=0.37$, $P=0.08$). The recanalization rate in our study is slightly lower than those established in other studies^{8,17}. However, after dichotomizing our series according to mechanical devices used for patients, we found that recanalization was achieved in 78.1% of patients treated with stent retrievers ($n=32$) and in 57.1% of patients in whom stent retrievers were not applied ($n=14$). Favorable clinical outcome was reached by 13 patients (25.5%) and moderate clinical outcome (90 days mRS 0-3) was in 18 patients (35.3%). Mortality was 37.3% ($n=19$) (Table 5). In univariate analysis, thrombus length measured on NECCT or CTA showed no significant difference between patients with ($n=34/66.7%$) or without ($n=17/33.3%$) successful recanalization (7.9 mm vs. 7.5mm, $P=0.94$) (Table 2 and Fig. 7). In univariate analysis, absolute thrombus density showed a trend toward higher recanalization rate ($P=0.08$) (Table 2). However, relative thrombus density showed no statistically significant correlation with successful recanalization ($P=0.11$) (Fig. 9). Other clinical

variables did not have a correlation with successful recanalization as well. In binary logistic regression analysis, neither recanalization rate nor clinical outcome showed a significant association with thrombus length (odds ratio [OR] 1.03, 95% confidence interval [CI] 0.97-1.10, $P=0.37$; OR 0.94, 95% CI 0.86-1.03, $P=0.20$, respectively, Table 6).

Clinical Outcome

Favorable outcome was observed in 13 patients (25.5%). There was no difference of thrombus lengths between patients with and without favorable outcome (7.3 mm vs. 8.3 mm, $P=0.23$, Table 3 and Fig. 8). In univariate analysis, absolute thrombus density was statistically different between patients with ($n=13/25.5\%$) and without ($n=38/74.5\%$) favorable outcome (55.7 HU vs. 50.1 HU, $P=0.004$) (Table 3 and Fig. 10). This statistically significant difference was also present when relative thrombus density was compared (1.49 vs. 1.28, $p<0.001$). In a secondary analysis, no differences were found in absolute thrombus density when patients with local atherosclerotic changes were compared to those without (51.0 ± 7.0 v. 51.8 ± 5.9 , $P=0.70$) and there was no difference regarding recanalization rates ($P=0.25$) or clinical outcomes ($P=0.73$). This statistically significant difference was also present when relative thrombus densities were compared (1.49 vs. 1.28, $p<0.001$). All patients without a HDBAS showed poor outcome ($n=13/100\%$) even though no statistical difference was found ($P=0.09$). In the logistic regression analysis, thrombus length did not show a correlation with favorable outcome (OR 0.94, 95% CI 0.86-1.03, $P=0.20$). However, high density of thrombi was a significant predictor of favorable outcome (OR 1.31, 95% CI 1.08-1.59, $P=0.006$) (Table 3 and Fig. 10). Symptomatic intracranial hemorrhage per ECASS II³³ occurred in five patients (9.8%) (three patients with SAH and two patients with PH I) and all of them (100%) showed poor outcome. There was a strong association between successful recanalization and favorable outcome ($P=0.04$) (Table 3).

Table 1. Treatment strategy and devices.

Treatment strategy	<i>n</i> (%)
IAT only	5 (9.8%)
Mechanical thrombectomy only	23 (45.1%)
IVT + Mechanical thrombectomy	7 (13.7%)
IAT + Mechanical thrombectomy	15 (29.4%)
IVT + IAT + Mechanical thrombectomy	1 (2.0%)
Overall	51
Devices	
Mechanical thrombectomy	30 (58.9%)
Trevo	15 (29.4%)
Penumbra	4 (7.8%)
Solitaire	3 (5.9%)
Trevo + Solitaire	3 (5.9%)
Trevo + Penumbra	2 (3.9%)
Other	3 (5.9%)
Mechanical thrombectomy + IAT	16 (31.4%)
Penumbra + IAT	9 (17.6%)
Trevo + IAT	4 (7.8%)
Solitaire + IAT	2 (3.9%)
Other + IAT	1 (2.0%)
Stent	
“Coronary Integrity”	5 (9.8%)
Mechanical devices	46 (90.2%)
First-generation	14 (30.4%)
Second-generation	32 (69.6%)
Overall	51

IVT: Intravenous thrombolysis with recombinant tissue plasminogen activator.

IAT: Intra-arterial thrombolysis with recombinant tissue plasminogen activator.

Table 2. Baseline characteristics of BAO patients with regard to recanalization.

Characteristics	Overall (n=51)	No or unsuccessful recanalization TICI 0-2a (n=17)	Successful recanalization TICI 2b-3 (n=34)	P Value
Age, yr, median (IQR)	72 (66-78)	73 (61-83)	72 (67-76)	0.42
Male, <i>n</i> (%)	36 (70.6)	13 (76.5)	23 (67.6)	0.75
Baseline NIHSS, median (IQR)	18 (11-23)	18 (16-24)	17 (10-20)	0.30
Site of occlusion				
Proximal BA, <i>n</i> (%)	10 (19.6)	3 (17.6)	7 (20.6)	1.00
Mid BA, <i>n</i> (%)	21 (41.2)	7 (41.2)	14 (41.2)	1.00
Top of BA, <i>n</i> (%)	20 (39.2)	7 (41.2)	13 (38.2)	1.00
Thrombus length, mm, median (IQR)	7.5 (5-14.4)	7.5 (6.5-11.5)	7.9 (5-16.2)	0.94
Density, absolute HU	51.6 ± 6.2	49.2 ± 6.8	52.7 ± 5.6	0.08
Density, relative HU	1.34 ± 0.15	1.29 ± 0.13	1.36 ± 0.16	0.11
HBAS, <i>n</i> (%)	42 (82.4)	13 (76.5)	29 (85.3)	0.46
Onset-to-treatment, min, median (IQR)	222 (178-462)	205 (184-803)	221 (165-365)	0.68
Strategy of EVT				
I.A. rtPA only, <i>n</i> (%)	5 (9.8)	4 (23.5)	1 (2.9)	0.04
Mechanical only, <i>n</i> (%)	30 (58.9)	8 (47.1)	22 (64.7)	0.25
Mechanical + I.A. rtPA, <i>n</i> (%)	16 (31.4)	5 (29.4)	11 (32.4)	1.00
Etiology				
Cardioembolic, <i>n</i> (%)	30 (58.8)	11 (64.7)	19 (55.9)	0.76
Large artery atherosclerotic, <i>n</i> (%)	13 (25.5)	3 (17.6)	10 (29.4)	0.50
Other confirmed etiology, <i>n</i> (%)	5 (9.8)	2 (11.8)	3 (8.8)	1.00
Unknown, <i>n</i> (%)	3 (5.9)	1 (5.9)	2 (5.9)	1.00

IQR: interquartile range; NIHSS: National Institutes of Health Stroke Scale scores; BA: basilar artery; HBAS: hyperdense basilar artery sign; I.A.: intra-arterial; rtPA: recombinant tissue plasminogen activator.

Table 3. Baseline characteristics of BAO patients with regard to outcome.

Characteristics	Overall (n=51)	Poor outcome 3-mo mRS 3-6 (n=38)	Favorable outcome 3-mo mRS 0-2 (n=13)	P Value
Age, yr, median (IQR)	72 (66-78)	71 (66-80)	73 (67-74)	0.72
Male, n (%)	36 (70.6)	28 (73.7)	8 (61.5)	0.49
Baseline NIHSS, score, median (IQR)	18 (11-23)	18 (16-23)	15 (9-19)	0.04
Site of occlusion				
Proximal BA, n (%)	10 (19.6)	9 (23.7)	1 (7.7)	0.42
Mid BA, n (%)	21 (41.2)	16 (42.1)	5 (38.5)	1.00
Top of BA, n (%)	20 (39.2)	13 (34.2)	7 (53.8)	0.32
Thrombus length, mm, median (IQR)	7.5 (5-14.4)	8.3 (6-15.6)	7.3 (4.9-10.3)	0.23
Density, absolute HU	51.6 ± 6.2	50.1 ± 6.1	55.7 ± 4.3	0.004
Density, relative HU	1.34 ± 0.15	1.28 ± 0.12	1.49 ± 0.12	<0.001
HBAS, n (%)	42 (82.4)	29 (76.3)	13 (100)	0.09
Onset-to-treatment, min, median (IQR)	222 (178-462)	221 (183-479)	244 (175-515)	0.90
Strategy of EVT				
I.A. rtPA only, n (%)	5 (9.6)	5 (12.8)	0 (0)	0.31
Mechanical only, n (%)	30 (57.7)	22 (56.4)	8 (61.5)	1.00
Mechanical + I.A. rtPA, n (%)	16 (32.7)	12 (30.8)	5 (38.5)	0.73
Etiology				
Cardioembolic, n (%)	30 (58.8)	23 (60.5)	7 (53.8)	0.75
Large artery atherosclerotic, n (%)	13 (25.5)	9 (23.7)	4 (30.8)	0.72
Other confirmed etiology, n (%)	5 (9.8)	4 (10.5)	1 (7.7)	1.00
Unknown, n (%)	3 (5.9)	2 (5.3)	1 (7.7)	1.00
sICH per ECASS II, n (%)	5 (9.8)	5 (13.2)	0	0.31
Recanalization, TIC1 2b-3, n (%)	34 (66.7)	22 (57.9)	12 (92.3)	0.04

IQR: interquartile range; NIHSS: National Institutes of Health Stroke Scale score; mRS: modified Rankin Scale; BA: basilar artery; HBAS: hyperdense basilar artery sign; I.A.: intra-arterial; rTPA: recombinant tissue plasminogen activator; sICH: symptomatic intracranial hemorrhage; ECASS: European Cooperative Acute Stroke Study.

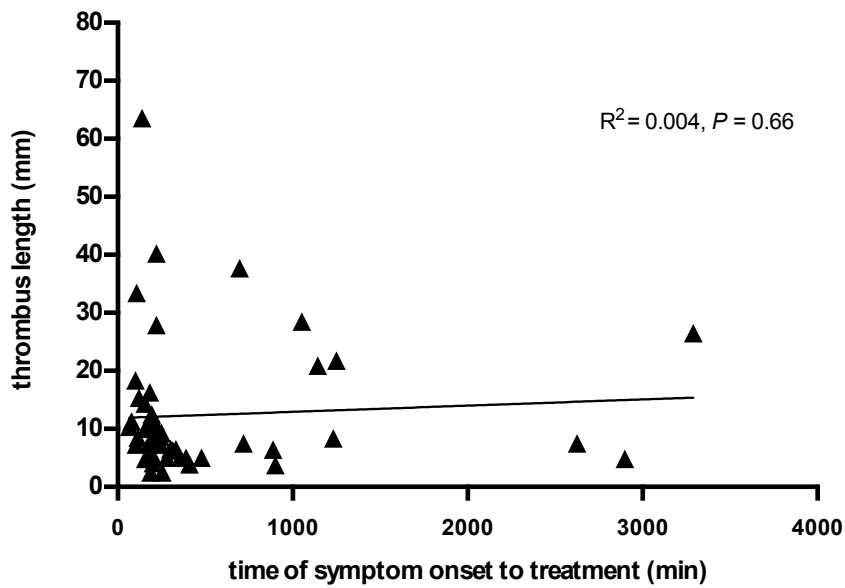


Figure 3. No correlation between thrombus length and time from onset to treatment.

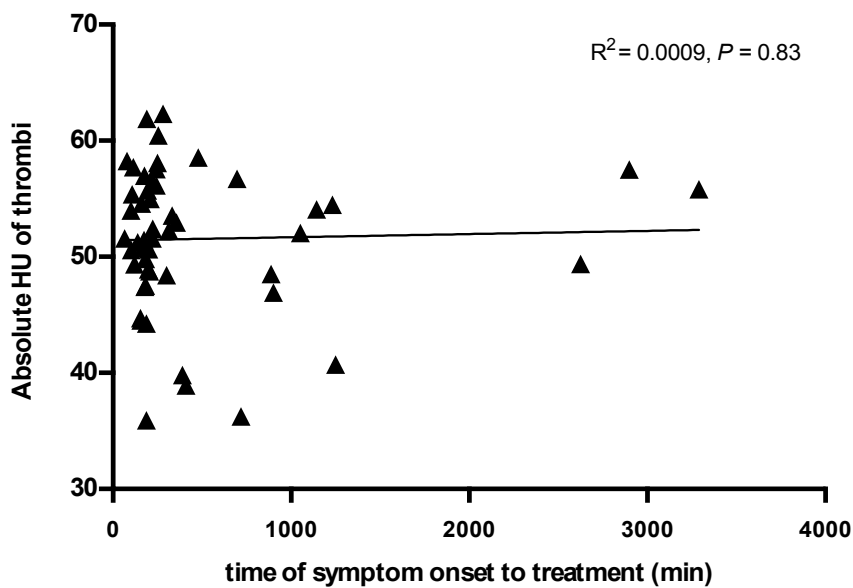


Figure 4. No correlation between thrombus density and time from onset to treatment.

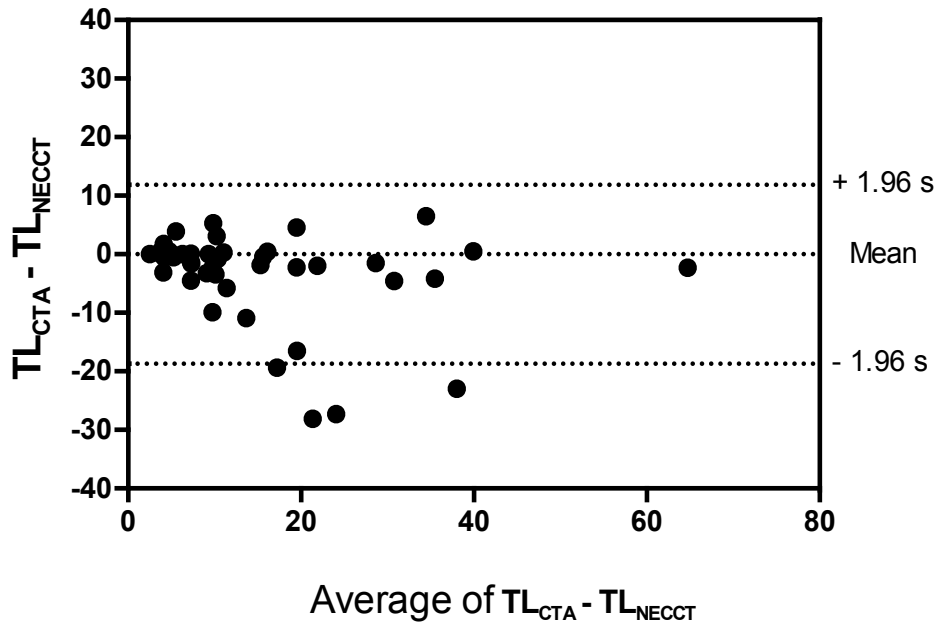


Figure 5. Bland-Altman plot demonstrates a good agreement between the reference thrombus lengths measured on CT angiography (TL_{CTA}) and thrombus lengths measured on non-enhanced cranial CT (TL_{NECCT}). TL: thrombus length (in mm).

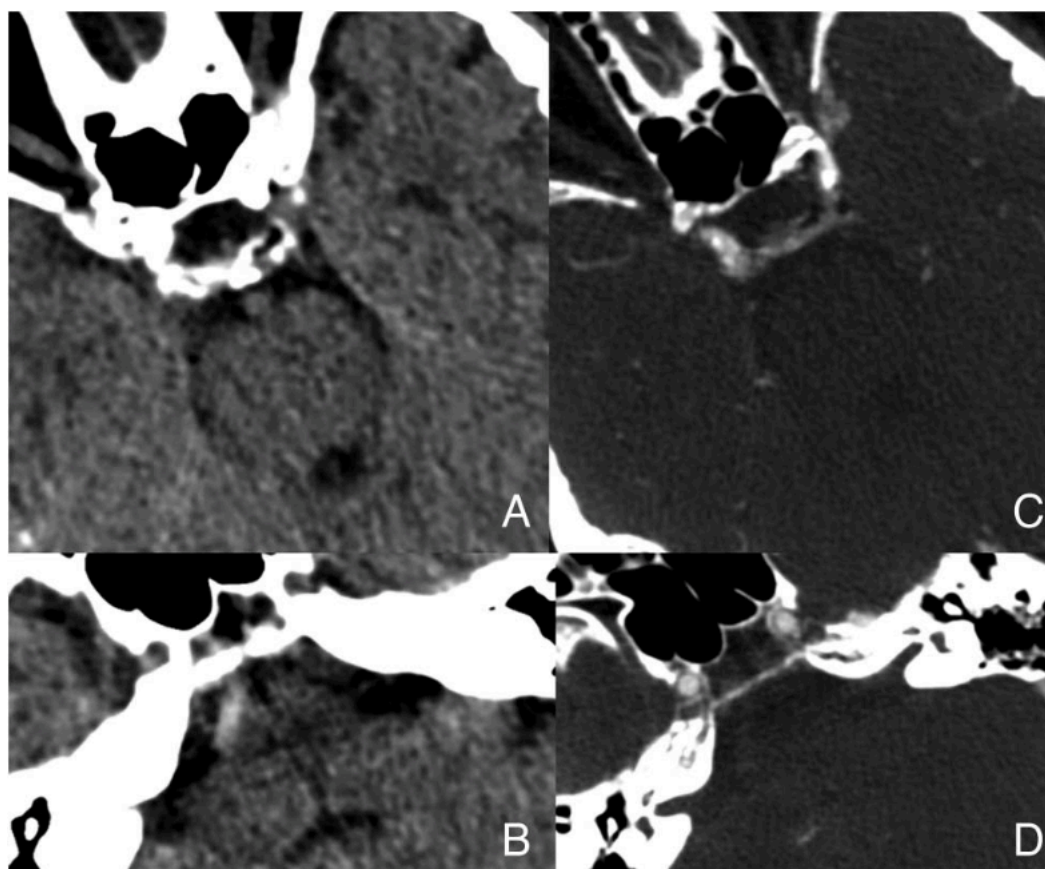


Figure 6. Heterogeneous thrombus. NIHSS 30 at admission. A, B: A hypodense thrombus is present in the midbasilar level, a hyperdense thrombus in the proximal basilar artery. C, D: CTA demonstrates multi-segmental occlusion.

Table 4. Inter-rater coefficient of HBAS identification.

		Observer 2		Cohen's κ
		HBAS present	HBAS absent	
Observer 1	HBAS present	37	5	0.74
	HBAS absent	0	9	

Table 5. Clinical outcome data.

	No recanalization TICI 0-2a, $n=17$	Successful recanalization TICI 2b-3, $n=34$	P value
sICH per ECASS II, n (%)	5 (29.4)	0	0.003
90 days mRS 0-2, n (%)	1 (5.9)	12 (35.3)	0.04
90 days mRS 0-3, n (%)	1 (5.9)	17 (50)	0.002
90 days mortality, n (%)	10 (58.8)	9 (26.5)	0.034

sICH: symptomatic intracranial hemorrhage. ECASS: European Cooperative Acute Stroke Study. mRS: modified Rankin Scale.

Table 6. Logistic regression for successful recanalization and favorable outcome.

Predictive Factors	Odds Ratio (95% Confidence Interval)	<i>P</i> Value
Successful Recanalization		
Thrombus Length	1.03 [0.97-1.10]	0.37
Thrombus Density	1.10 [0.99-1.22]	0.06
Favorable Outcome		
Thrombus Length	0.94 [0.86-1.03]	0.20
Thrombus Density	1.31 [1.08-1.59]	0.006

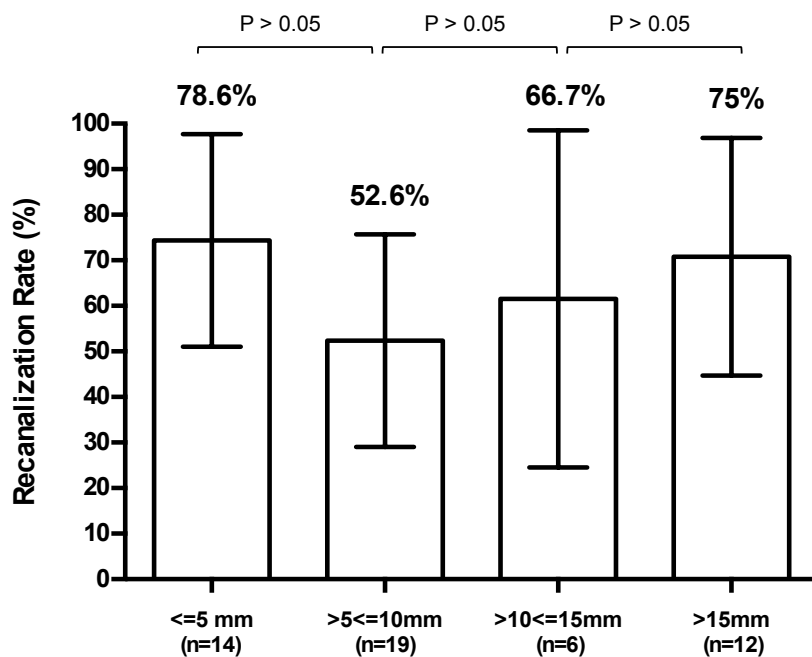


Figure 7. Bar graph illustrates recanalization rate and 95% confidence interval of BAO patients regarding different thrombus lengths.

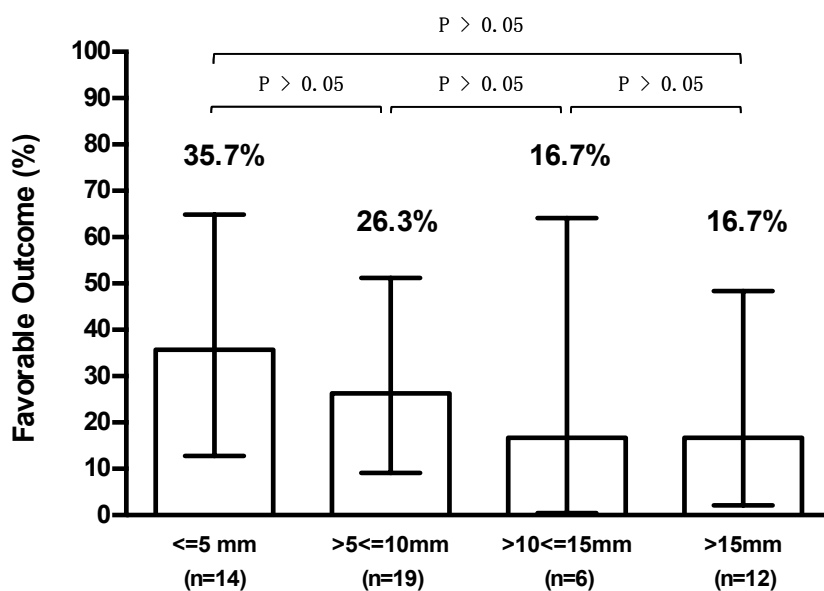


Figure 8. Bar graph demonstrates favorable outcome rate and 95% confidence interval of BAO patients regarding different thrombus lengths.

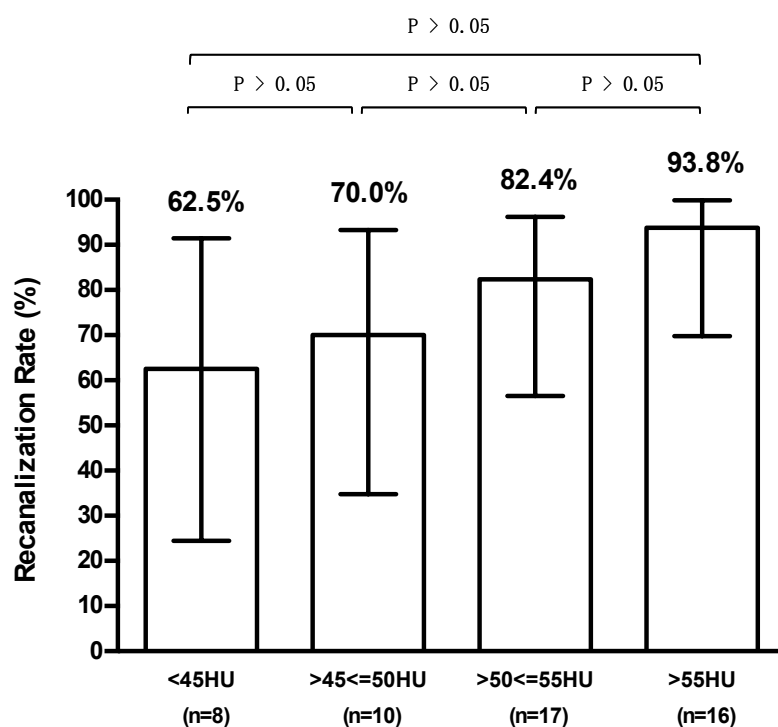


Figure 9. Bar graph demonstrates a trend towards higher density of the thrombus correlated with higher recanalization rate. (HU: Hounsfield units.).

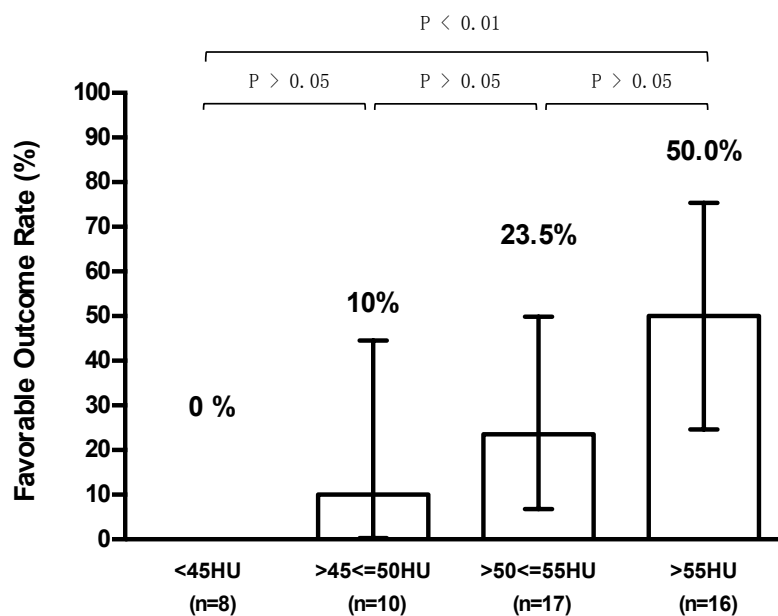


Figure 10. Bar graph demonstrates increasing rate of favorable outcome with higher density of the thrombus. (HU: Hounsfield units).

Discussion

In this study, we analyzed the dependency of thrombus properties for recanalization rate and clinical outcome in 51 patients with acute occlusion of the basilar artery treated with endovascular therapy. The main results are twofold. First, successful recanalization was independent of thrombus length. The effectiveness of EVT is well documented and was reported to range from 68-82%^{10,34-37} even with a high clot burden³⁸. This is different in patients treated with IVT. Our previous study¹² suggested that patients with MCA occlusion rarely benefited from IVT if the length of thrombi exceeded 8 mm. Strbian and coworkers demonstrated that the recanalization rate of BAO patients after IVT was dependent on thrombus length although no threshold could be established beyond which IVT was deemed futile. Similar to our results, Gilberti and colleagues reported that recanalization is independent of thrombus length in BAO treated with EVT¹⁷. However, they determined thrombus length on DSA, whereas we measured thrombus length on initial NECCT.

Contrary to the result that long thrombi showed a linear association with poor outcome¹⁷, we did not observe the same correlation in this study. In the ENDOSTROKE registry, the authors found that baseline NIHSS and collateral status were significant predictors of clinical outcome⁸. However, the relationship between imaging markers and clinical outcome was not evaluated. Thus, a large trial is still needed to validate the influence of thrombus length on clinical outcome.

During the past few years, thrombus length was considered as a useful predictor of recanalization rate and outcome but the results remain controversial (Table 7).

Indeed, several points require special interpretation. First, some of these studies measured thrombus length on CTA images. However, the contrast gap as assessment of clot burden on CTA images depends both on clot length and the status of the pial collaterals³⁹. Regularly, the distal end of a clot will be delineated less clearly than the proximal face. This implies that clot length might be overestimated on CTA images if the vasculature back to the distal clot face was very blurry due to a poor leptomeningeal collateral. Secondly, measuring thrombus length on Susceptibility Weighted Imaging (SWI) images relies on determining the Susceptibility Vessel Sign (SVS). However, the accuracy of quantification is limited by blooming artifacts and a large variation in sensitivity of SVS. These aspects might explain why these studies came to different conclusions by using same imaging.

Table 7. Thrombus length and its correlation with recanalization rate and clinical outcome established in recent major studies.

	Modality	Slice thickness	Treatment	Site of occlusion	Mean or median length of thrombi	Correlation with recanalization	Threshold of thrombus length as a predictor of successful recanalization	Correlation with outcome
Riedel et al. ¹² (n=138)	NECCT	2.5 mm	IVT	Anterior circulation	NR	Longer thrombus correlated with lower recanalization rate	8 mm	N/A
Kamalian et al. ¹¹ (n=53)	NECCT	0.625 mm	IVT	Anterior circulation	18.5 mm	Longer thrombus correlated with lower recanalization rate	8 mm	N/A
Rohan et al. ³⁶ (n=80)	CTA	2 mm, MIP	IVT	Anterior circulation	M1: 12.7 mm M1/M2: 15.4 mm	Longer thrombus correlated with lower recanalization rate	12 mm	Longer thrombus correlated with worse clinical outcome
Yan et al. ⁴⁰ (n=74)	dGE-T1, MRI	5mm - 20 mm	IVT	Anterior circulation	8.18 mm	Longer thrombus correlated with lower recanalization rate	14 mm	N/A
Strbian et al. ² (n=140)	CTA	NR	IVT	Posterior circulation	Median: 10 mm Mean: 12 mm	Longer thrombus correlated with lower recanalization rate	N/A	N/A
Spiotta et al. ¹⁶ (n=141)	CTA	2 mm, MIP	EVT	NR	13.3 ± 9.5 mm	No correlation	N/A	No correlation
Weisstanner et al. ¹⁵ (n=88)	GE-MRA	0.9 mm	EVT	Anterior circulation	10.9 mm	No correlation	N/A	N/A
Jindal et al. ⁴¹ (n=28)	SWI	1 mm	EVT	Anterior circulation	19 ± 7 mm	No correlation	N/A	N/A
Gilberti et al. ¹⁷ (n=32)	DSA	NA	EVT	Posterior circulation	NR	No correlation	N/A	Longer thrombus correlated with worse clinical outcome

N/A: not applicable.

NR: not reported.

Recently, Yan et al. demonstrated the response to alteplase in MCA occlusion patients by measuring thrombus length on delayed gadolinium-enhanced (dGE) T1 MRI images⁴⁰. This special method measures thrombus length more accurately than SWI does. The authors introduced a novel approach to demarcate the distal face of the thrombus clearly on magnetic resonance angiography (MRA) images using dGE T1 sequences although collateral flow was poor in these patients. However, this particular method is not part of a routine scan in clinical practice. Moreover, the application of this special dGE T1 sequence would be limited by difficulties in accessing MRI in a timely fashion in many clinical centers. Nowadays, a slice thickness less than 1 mm can be achieved with modern multi-detector CT scanners so that clot length can be reliably determined and visualized without interference from partial volume effects⁴². Only a few seconds of additional reconstruction time was needed and time was saved for urgent treatment rather than long acquisition duration of a MR protocol. In our series, we evaluated thrombus length on thin-slice NECCT images based on excellent agreement with identification of HBAS (Table 4). The Bland-Altman plot demonstrated a great consensus between thrombus length measured on NECCT and on CTA. Interestingly, the thrombi of the three patients whose data points were outside the limits of agreement proved to be of heterogeneous composition (Fig. 6). Usually we registered thrombus segmentation on NECCT images according to HBAS, which would commonly show a “mismatch” of clot length estimation between thrombus with low density or a heterogeneous component and that with high density.

The second finding of our study is that high-attenuating thrombi correlated with better clinical outcome. Several previous studies had already demonstrated that a thrombus with higher density was associated with better recanalization rates either by IVT or EVT (Table 8). However, this finding is in dispute. Yilmaz²³ reported on 70 patients treated with EVT and thrombus density did not predict recanalization success. It is widely accepted that erythrocyte-rich clots show a higher density than platelet-rich thrombi on NECCT⁴³⁻⁴⁵. It was accordingly claimed that erythrocyte-rich clots with low

fibrin content are more amenable to pharmacological⁴⁶ and mechanical therapy⁴⁴. However, we did not find a correlation with recanalization rate but rather with clinical outcome. We hypothesized that low-attenuating thrombi might be caused by atherosclerotic changes (Fig. 11) and partial volume effects may have blurred high-attenuating thrombi but our secondary analysis did not substantiate this hypothesis. Patients with atherosclerotic changes in the basilar artery did not have lower-attenuating thrombi nor did they experience lower recanalization rates and clinical outcome.

There are several reasons why the influence of thrombus density on clinical outcome and recanalization rate should be regarded with caution. First, in the posterior circulation -- especially in the basilar artery -- beam-hardening artifacts caused by the temporal bones and vessel calcifications are fairly common and may substantially alter density measurements. Secondly, there is no standard procedure to measure thrombus density and results are thus more or less subjective. Thirdly, since a high hematocrit level influences the accuracy of thrombus density measurement, this effect needs to be calibrated. However, it is difficult to choose the same vessel as a reference, such as the contralateral MCA: for instance, if the mid-BA was occluded, the VA or the top of the BA or even the PCA would be considered as the “vessel on contralateral side” for calibration. This kind of uncertainty might render a thrombus density estimation less accurate. In addition, the validation of this method should be evaluated. In summary, more sophisticated and standardized methods to measure thrombus density, including filtering, might be needed⁴⁷

In contrast to anterior circulation stroke, recanalization did not automatically translate into good clinical outcome. We thus suggest that thrombus length may not be the pivotal factor when it comes to clinical outcome. Patients should be treated irrespectively of thrombus length with the treatment modality according to national or local guidelines.

Table 8. Thrombus density and its correlation with recanalization rate and clinical outcome established in recent major studies.

	Treatment	Site of occlusion	Different methods used for thrombus density measurement	Absolute HU	Relative HU	Correlation with recanalization	Correlation with outcome
Kim EY et al. ¹⁸ (n=51)	IVT	Anterior circulation	One ROIs around the margin of the thrombi	51.4 HU	1.33	Lower HU of thrombi was more likely resistant to alteplase (relate to lower recanalization rate)	N/A
Puig et al. ²⁰ (n=87)	IVT	Anterior circulation	One ROIs around the margin of the thrombi	50 (recanalization group) and 42 (persistent occlusion group)	1.57 (recanalization group) and 1.11 (persistent occlusion group)	Higher HU of thrombi correlated with higher recanalization rate	N/A
Rohan et al. ¹⁴ (n=80)	IVT	Anterior circulation	One small round ROIs throughout the occluded artery	64 HU	1.26	No correlation	No correlation
Nielsen et al. ¹⁹ (n=88)	IVT	Anterior circulation	Three small round ROIs within the thrombi (mean value)	63.1 HU (recanalization group) and 52.2 HU (no recanalization group)	1.54 (recanalization group) and 1.29 (no recanalization group)	Lower absolute HU and relative HU related to lower recanalization rate	N/A
Mair et al. ²⁴ (n=109)	IVT	Anterior /Posterior circulation	One ROIs in the thrombi	51.0 HU	1.38	N/A	No correlation
Topcuoglu et al. ²⁵ (n=105)	IVT or IA. rtPA	Anterior circulation /vertebrobasilar stroke	One voxel-sized ROIs over occluded artery	47.4 HU (favorable outcome group) and 49.4 HU (poor outcome group)	1.13 HU (favorable outcome group) and 1.17 HU (poor outcome group)	N/A	No correlation

Moftakhar et al. ³² (n=90)	IVT alone, EVT alone and EVT followed with IVT	Anterior circulation /Basilar artery	One ROIs around the margin of the thrombi	N/A	1.58 in patients with IV rtPA; 1.66 in patients with IA rtPA; 1.7 in patients with EVT in successful recanalization group,	Lower HU of thrombi tended to be resistant to alteplase (relate to lower recanalization rate after both IVT and EVT)	N/A
Froehler et al. ²¹ (n=67)	EVT (MERC device)	Anterior circulation /Basilar artery	Three circular or oval ROIs within the thrombi (mean value)	N/A	1.46 in patients with hyperdense vessel sign and 1.03 in patients with hypodense vessel sign	Hyperdense thrombi correlated with successful recanalization	N/A
Yilmaz et al. ²³ (n=70)	EVT	Anterior circulation	ROIs were placed perpendicular to the vessel on sagittal reconstructions of NECCT	50.1 HU (recanalization group) and 48.7 HU (no recanalization group)	N/A	No correlation	N/A
Spiotta et al. ¹⁶ (n=141)	EVT	NR	One round ROIs which cover the most-dense part of the whole thrombi in three slices (mean value)	59.9 HU	N/A	No correlation	No correlation
Mokin et al. ²² (n=41)	EVT	Anterior circulation	One ROIs around the margin of the thrombi	49.9 HU (recanalization group) and 43.8 HU (no recanalization group)	1.2 (recanalization group) and 1.0 HU (no recanalization group)	Higher absolute and relative HU of thrombi correlated with higher recanalization	N/A

N/A: not applicable.

NR: not reported

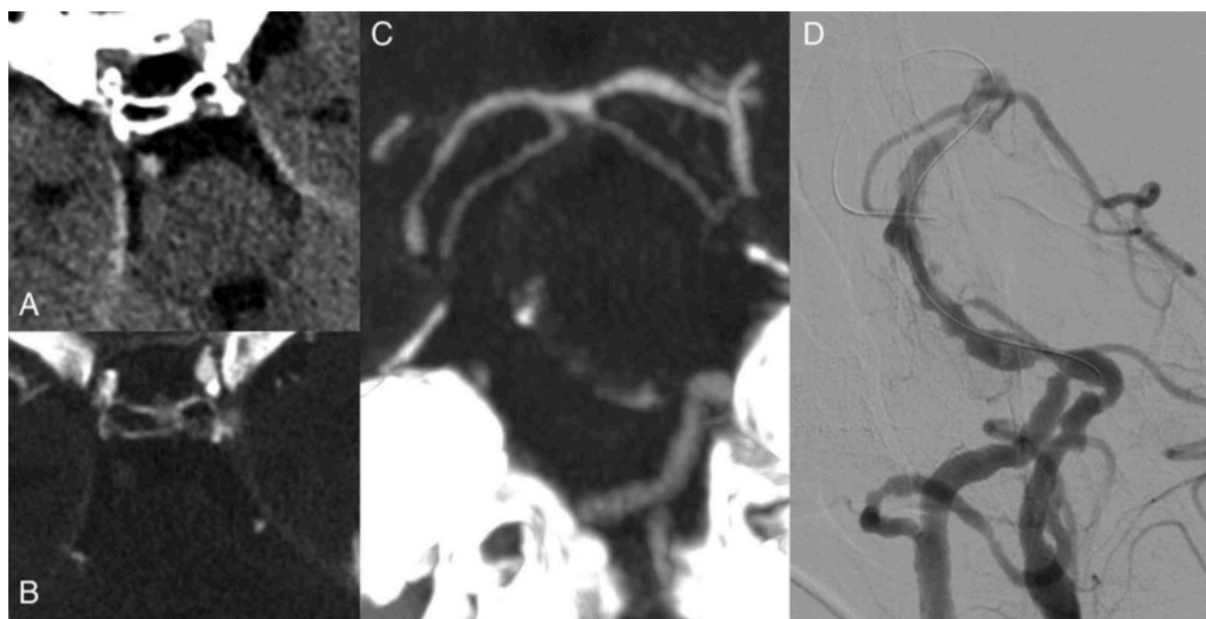


Figure 11. Severe atherosclerotic changes in a 69-year-old male with NIHSS 30 at admission. **A.** Hyperdense thrombus with hypodense rim in the mid-basilar artery on NECCT. **B.** CTA demonstrates complete occlusion of the basilar artery. **C.** DSA image demonstrates severe atherosclerotic changes in the basilar artery.

Four patients in our cohort were excluded from this study because of substantial artifacts that prevented us from measuring thrombus length and density. After including their OTT into a secondary analysis, patients whose OTT was less than 3 hours were more likely to achieve successful recanalization than those more than 3 hours (Fig. 12). Our result is in line with that of Sheth⁴⁸ who reached the same conclusion after further analysis of the SWIFT and STAR trials. For acute ischemia stroke patients, although effective second-generation mechanical devices were widely used, several minutes' delay in EVT would result in a worse clinical outcome.

This study has some limitations. The retrospective nature of the study and limited sample size (Type II

error) does not allow for generalization of our results. Further, the study period from 2007-2015 limits the comparability of devices used as the effectiveness dramatically changed. In our cohort, we achieved a higher rate of recanalization with second generation devices (n=32) of 78.1 % as compared to 57.1 % with first generation devices (n=14) (Table 9). In addition, we did not evaluate every possible variable such as collateral status, medical history, etc. that may be predictive of recanalization and outcome. Furthermore, considering the limited number of patients, multi logistic model were not used for further analysis of independent predictive value of thrombus density toward favorable outcome. Four patients in our cohort were excluded from this study because of substantial artifacts preventing thrombus length and density measurement. With regard to their successful recanalization and poor clinical outcome, the relationship between thrombus properties and recanalization and outcome might be underestimated.

Conclusions

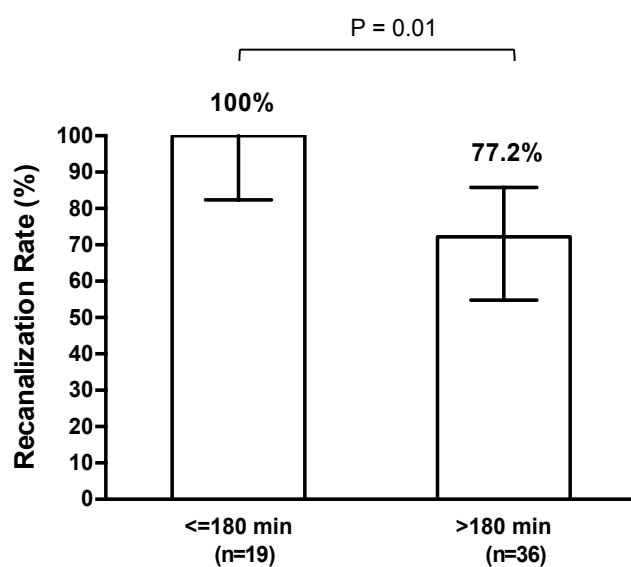
Successful recanalization with EVT can be achieved despite a high clot burden in patients with acute occlusion of the BA. High density of thrombi seems to be a predictor of better clinical outcome.

Standardized methods to measure thrombus density are needed.

Table 9. Difference between first-generation and second-generation mechanical devices regarding recanalization rate.

Mechanical devices	Overall (n=46)*	No or partial recanalization TICI 0-2a (n=13)	Successful recanalization TICI 2b-3 (n=33)	P Value
First-generation (non-stent retrievers), n (%)	14 (30.4)	6 (46.2)	8 (24.2)	0.17
Second-generation (stent retrievers), n (%)	32 (69.6)	7 (53.8)	25 (75.8)	
Thrombus length in patients with non-stent retrievers, mm, median (IQR)		7.5 (6.1-8.1)	5 (4.3-6.9)	0.11
Thrombus length in patients with stent retrievers, mm, median (IQR)		8.6 (6.4-20.9)	11.2 (6.5-24.2)	0.75

*5 patients received intra-arterial thrombolysis only.

**Figure 12.** Recanalization rate in patients whose OTT less than 3 hours was significantly higher than in those in whom it was more than 3 hours.

PART II Thrombus density measurements on non-enhanced cranial computed tomography: comparison of three methods

Introduction

In basilar artery occlusion (BAO) patients, early diagnosis and rapid, successful reperfusion are the primary goals of therapy. Since successful recanalization was achieved in 50–70% BAO patients treated with IVT^{9, 49, 50}. Recently, mechanical treatment or bridging therapy (IVT is administered before IAT or thrombectomy or both) was explored further. However, neuroimaging markers valuable in acute ischemic stroke for selecting efficient and appropriate treatment necessitate further investigation. Although numerous prognostic factors have emerged, few of those provided information for improving the decision-making with regard to treatment²⁶. Previous studies focused on the estimation of clot length or volume wherein, a long thrombus was considered to correlate with a low rate of recanalization^{2, 12, 14, 17, 51}. Interestingly, a certain proportion of short thrombi needed more thrombectomy maneuvers during the intervention than those with identical length. This implies that additional thrombus characteristics, such as thrombus histopathological composition, were essential.

HBAS is a visual marker of BAO on NECCT images⁵². Although the specificity is high, the sensitivity of HBAS is low. The hyperdense vessel sign indicated a high likelihood of arterial obstruction when observed on NECCT images; whereas its absence indicated only 50% arterial patency²⁴. However, thin-slice NECCT scan supported by modern multi-detector CT may improve the detection of the hyperdense vessel sign⁴². Ernst et al. found that approximately 90% sensitivity of HBAS can be achieved by the combination of a cut-off visual value of 46.5 HU (absolute HU) or 1.32 ratio (absolute HU/hematocrit level) on thin-slice volumetric NECCT⁵³. Owing to the reliable function of qualitative identification, HBAS might be a valuable tool in quantitatively measuring the density of the thrombus. Notably, some studies found that a higher density of thrombi was associated with a higher probability

of recanalization^{18-20, 22, 32}, which could not be confirmed by other studies^{14, 16, 23}.

In all the studies described above, these controversial results might be attributed to different methods used for the measurement of the thrombus density. The most widely used methods based on ROI are as follows: 1) Placing one circular ROI in the dense part (usually exhibiting the most intense signal) of the thrombi^{16, 18}. 2) Placing multiple (usually three) circular ROIs into the hyperdense vessel^{19, 21}. 3) Placing multiple irregular ROIs encompassing the whole hyperdense vessel slice-by-slice^{20, 54}.

Although all three methods above were commonly used in most studies, there is no doubt that the single mean/median value of the thrombus density calculated from the measurements of one or multiple ROIs could not represent the real density of the whole thrombus, especially when the composition of the thrombus was heterogeneous. Therefore, in this study, the density of the thrombus in BAO patients was compared among these three different methods. We hypothesized that these methods yield significantly different results and might preclude the comparison among studies conducted with different methods, and the whole thrombus density measurements could provide additional information about the composition of the thrombus.

Methods and Materials

Patients

The study was approved by the local ethics committee. Consecutive patients with basilar artery occlusion receiving EVT between April 2007 and October 2015 were studied retrospectively. All BAO patients who underwent thin slice non-enhanced cranial CT (NECCT) (Brilliance 64, Philips, Best/The Netherlands, 120 kV, 320 mAs, reconstructed slice thickness 2.5mm) prior to mechanical thrombectomy were included (n=36). Patients whose initial NECCT presented significant artifacts preventing thrombus density evaluation were excluded (n=2). In addition, patients with unavailable CTA scan (n=5) or DSA images showing severe atherosclerotic changes (n=5) were also excluded. Subsequently, two commonly used methods for density measurement and density distribution of the whole thrombus were performed in 24 patients sorted alphabetically to avoid selection bias (Fig. 1).

Clinical data

Demographics data and other baseline characteristics (initial NIHSS, recanalization rate, and outcome) were evaluated according to the criteria described in Part 1.

Image acquisition

Three different methods for thrombus density assessment

Method 1: The whole hyperdense vessel encompassed within a ROI.

In order to determine the density distribution of the whole thrombus, we evaluated thrombus density by using a ROI encompassed the whole hyperdense vessel slice by slice. In the case of thrombi with high density (HBAS present), first, the thrombus was detected on thin-slice NECCT images according to HBAS (Fig. 2A). Second, these NECCT raw images were imported into Image J (National Institutes of Health, <https://imagej.nih.gov/ij/>). Third, polygonal lasso tool of Image J was used to place the consecutive multiple dots encompassing the margin of the whole hyperdense thrombus on

every slice in which thrombus could be identified. (Fig. 2B). Fourth, the bin values of all pixels within the ROIs were automatically recorded by Image J and converted into independent datasets. For thrombi with low density (HBAS absent), the following steps were performed. First, CTA images (reconstruction slice thickness 2 mm) were utilized to co-localize with the thrombus in all corresponding NECCT slices (IMPAX workstation, Agfa Technical Imaging Systems) (Fig. 3). Second, the polygonal selection tool (IMPAX workstation) was used to define all ROIs on these NECCT images (Fig. 2A). Third, steps two to four are repeated to ensure that the borderline of all ROIs defined by Agfa Imaging systems well-overlapped with those defined by Image J (Fig. 2C). In the end, the whole thrombus density measurements were calculated by using the independent datasets and represented as density distribution graph (Fig. 4). For thrombi that could be detected in several consecutive slices, thrombus density was determined by adding the density values of the ROIs divided by the number of the slices. All independent datasets representing the density distribution of the whole thrombus in every slice were combined and merged into integrated distribution.

Method 2: Three circular ROIs placed into the most hyperdense appearing part of the vessel. The second method was performed by placing multiple (usually three) circular ROIs into the hyperdense vessel. First, step one and two performed in Method 1 were repeated. Second, three circular ROIs were placed using the oval tool of Image J (Fig. 5). In the event that the thrombi could be detected in only one slice, three ROIs would be defined in the same slice. The overlap of any two ROIs should be prevented and the borderline should not surpass the margin of the thrombi. For thrombi that could be detected in any two consecutive slices, two ROIs would be defined in one slice and the third ROI would be defined in the other. The long thrombi that could be detected in three or more consecutive slices, all the ROIs would be defined slice-by-slice. Third, the density of the thrombus was described as the mean/median value of three or more ROIs in all corresponding slices.

Method 3: One circular ROIs placed into the most hyperdense appearing part of the vessel.

The third method for thrombus density measurement was performed similarly by placing a large circular region of interest into the most hyperdense-appearing part of the vessel (commonly with the most intense signal) (Fig. 5). If the thrombus could be detected on several consecutive slices, the maximum HU value among those derived from all slices would be considered as thrombus density.

Absolute and relative thrombus density

In order to achieve consistency in the quantitative measurements, one investigator defined ROIs with three different methods described above and evaluated both absolute thrombus density and relative thrombus density in all the 24 BAO patients. The absolute density was determined by the method discussed above. A second reader, blinded to the results of the first investigator, redrew 50 randomly selected ROIs. The mean density difference was 2.8 ± 1.2 HU. Thus, only the ROIs, defined by the first investigator, were used for subsequent analysis. The three different methods discussed above were repeated in the normal-appearing contralateral or distal/proximal artery ($HU_{\text{calibration}}$) in all 24 BAO patients. The relative density was calculated with $HU_{\text{thrombus}} / HU_{\text{calibration}}$ described in Part I.

Statistics

Normally distribution was assessed by the Kolmogorov–Smirnov test. Both density distribution of the whole thrombus method and density derived from the two other methods were expressed by the mean value (standard deviation, SD) and median value (interquartile range, IQR). The difference in the densities among different methods was expressed as the median value. To evaluate the difference in the density distributions of the whole thrombus method as compared to the measurements from the other methods, Mann–Whitney U test was used. Prism GraphPad software 6.0 (GraphPad Software Inc., La Jolla, CA, USA) and R (R software, Development Core Team, Vienna, Austria; <http://www.R-project.org>, 2011) was conducted. The values of the relative thrombus density within

patients were compared using the paired *t*-test and represented as mean \pm standard deviation or median and range where appropriate. Statistical significance was set as *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

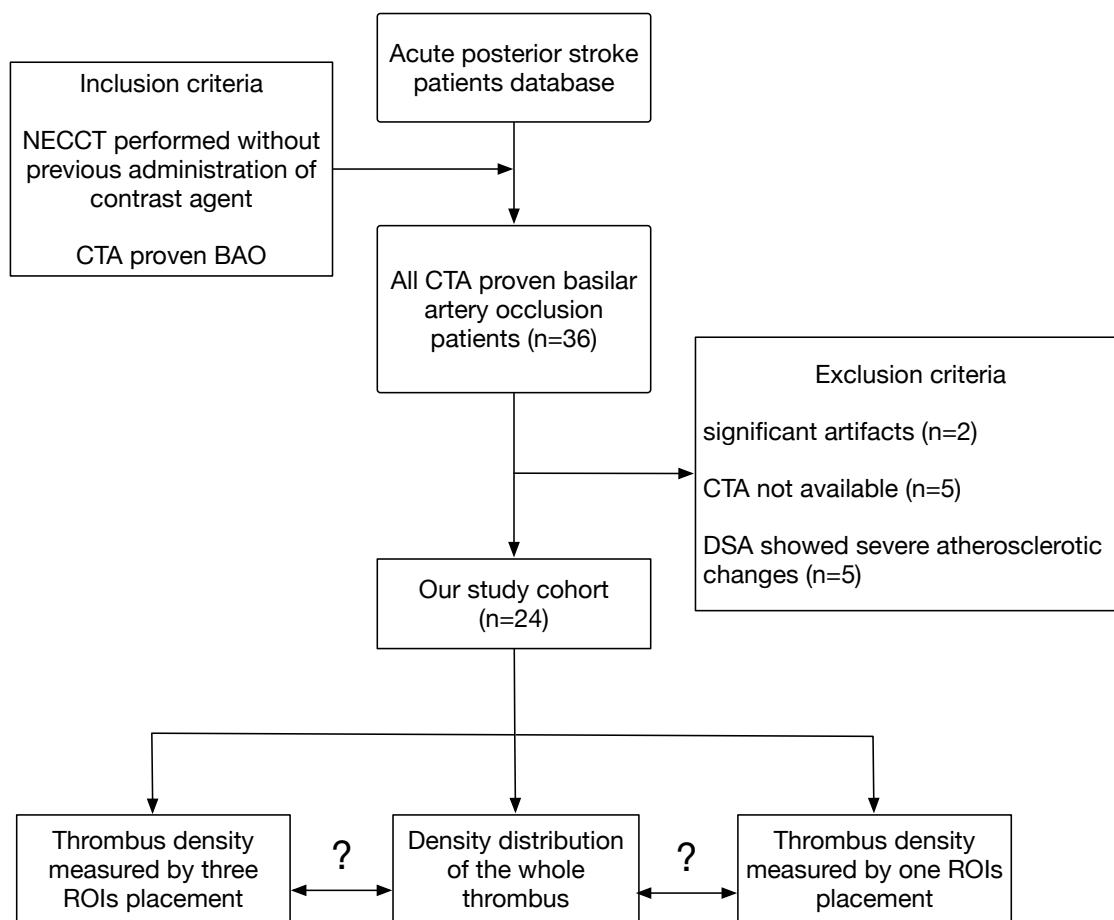


Figure 1. Study design and patient selection.

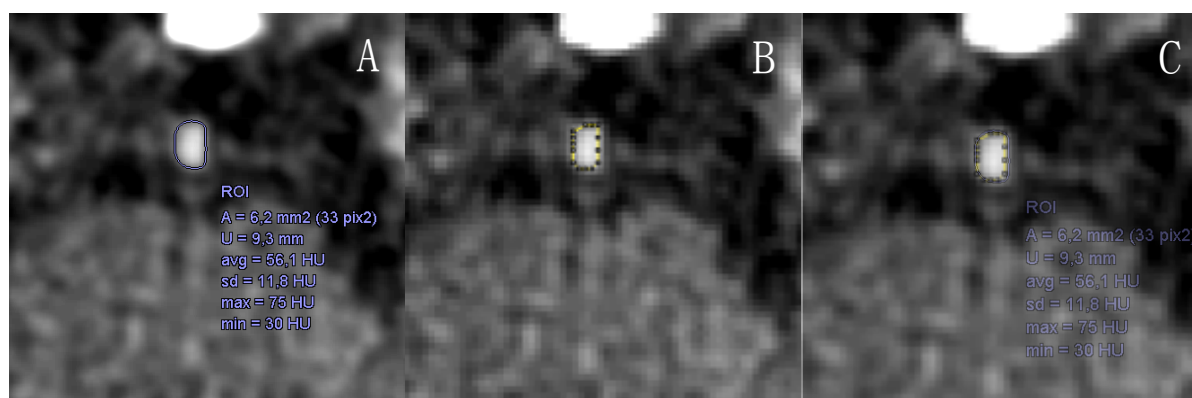


Figure 2. The whole thrombus density measurement in mid-BA occluded patient with high-density thrombus (HBAS present). A: The borders of the whole thrombus drawn by polygonal selection tool in IMPAX workstation. B: The borders of the whole thrombus drawn by polygonal lasso tool in Image J. C: The borders in image B and image A showed overlap after the two images were merged.

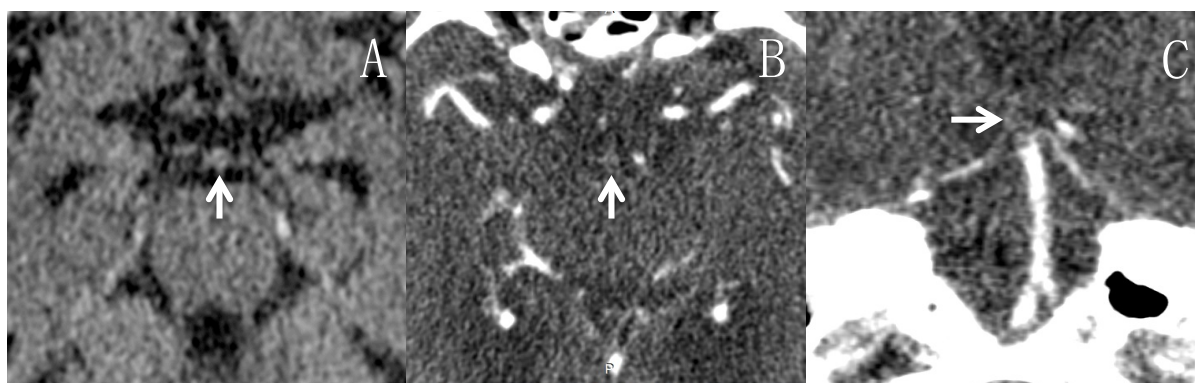


Figure 3. Illustration of co-localization function of Agfa Technical Imaging System. A: White arrow indicates a transection of thrombus with low density in NECCT images. B: The cross line was used to identify an occlusion (an opacification gap) on CTA image (the corresponding slice to image A). C: An opacification gap at the top of BA in coronal view after multi-planar reconstruction (slice thickness 0.45mm). The cross-line emerged automatically when co-localization function was used.

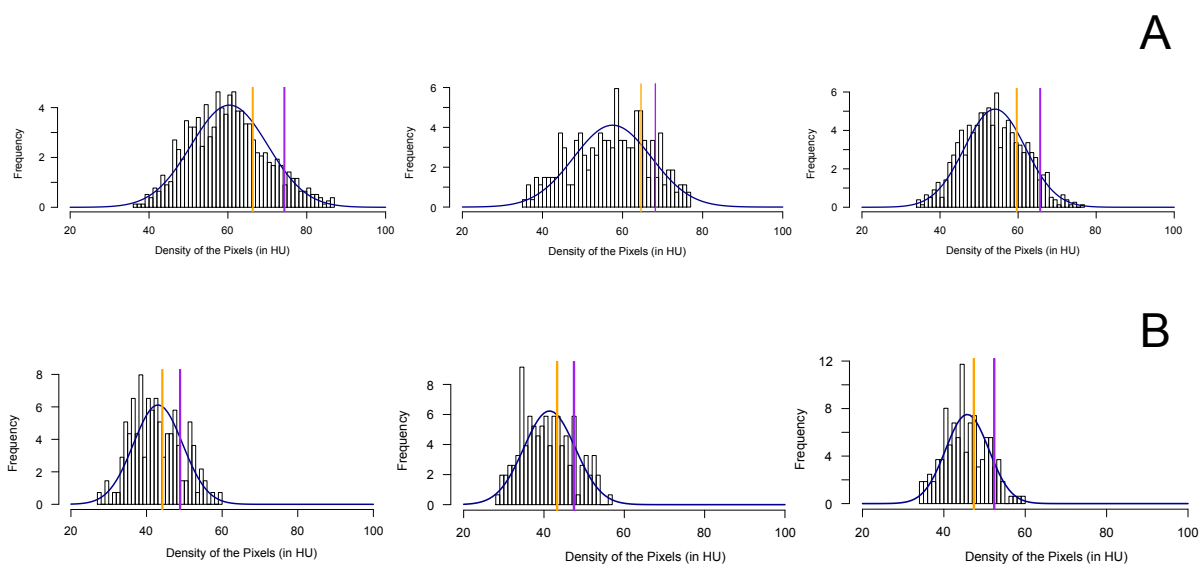


Figure 4. Histograms of the density distribution of whole thrombi in three patients with high-density thrombi (A) and others with low-density thrombi (B). The intense blue curve indicates the fitted normal distribution curve. The orange line indicates the mean value of thrombus density deriving from three ROIs placed into the hyperdense vessel. The purple line indicates the mean value of thrombus density deriving from one ROI placement into the maximum dense part of the thrombi. The purple line is systematically shifted towards the right.

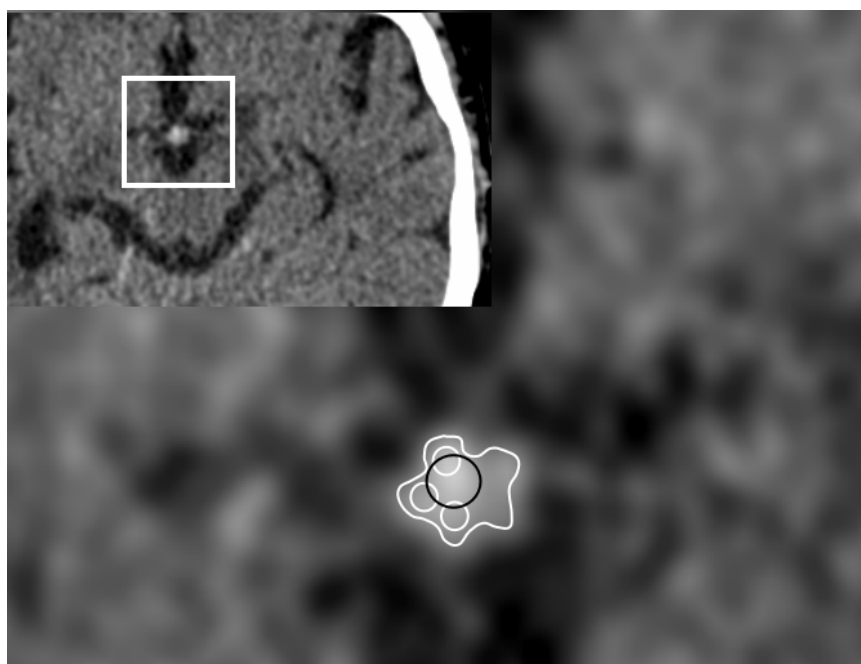


Figure 5. Illustration of three different methods of thrombus density measurement in one patient with top of basilar artery occlusion. The whole hyperdense vessel was encompassed within a ROI out of shape (Method 1, ☁). Three (Method 2, ⦿) or One (Method 3, ○) circular ROIs were placed into the most hyperdense appearing part of the vessel. This was repeated in the normal-appearing contralateral or distal/proximal artery ($HU_{\text{calibration}}$). Hyperdensities with >100 HU were excluded. Absolute density (HU_{thrombus} and $HU_{\text{calibration}}$) was determined by adding the density values of the ROIs divided by their numbers. The relative density was calculated by $HU_{\text{thrombus}} / HU_{\text{calibration}}$.

Results

Baseline characteristics of all 24 BAO patients were summarized in Table 1. The mean age of the patients was 69 years, and 2/3rd (n=16) were male. Proximal BA occlusion was observed in 29.2% (7/24), mid-BA occlusion was 50% (12/24), and top of the BA occlusion was 20.8% (5/24). HBAS was identified in 16 patients (66.7%, n=24). The median initial NIHSS was 18 (9–19 score, IQR), and the median time from onset to treatment (OTT) was 201 min (155–376 min, IQR). Successful recanalization was achieved in 15 patients (62.5%, n=24) and 6 patients (25%) resulted in the favorable outcome. The etiology of the stroke was cardiogenic (n=13), large atherosclerotic artery (n=7), and unknown (n=4), respectively, as assessed by the TOAST criteria.

Normal distribution of thrombus density was observed in 9 patients (37.5%, n=24). An example of density distribution of the whole thrombus measurements is illustrated in Fig.4. The average value of the whole thrombus density (Method 1, in absolute Hounsfield values) was 53.8 ± 10.7 HU (mean) and 53.9 (46–61, IQR) (median). Both the average density measured by Method 2 (three ROIs placement) (58.7 ± 10.5 HU (mean) and 59 (52–66, IQR) (median)) or that measured by Method 3 (one ROI placement) (63.1 ± 12.2 HU (mean) and 65 (54–72, IQR) (median)) are significantly higher than those derived from density distribution of the whole thrombus (Method 1) ($P < 0.0001$, $P < 0.0001$, respectively) (Table 2 and Fig. 6).

The comparison of the relative density for all 24 BAO patients were represented in the order methods 1 and 2, 3 (Fig. 4). The relative thrombus and normal vessel densities are summarized in Table 2. The relative densities were 1.64 ± 0.16 , 1.58 ± 0.16 , and 1.53 ± 0.15 respectively. After excluding the influence of the level of hematocrit on the measurement, the statistically significant differences were found among methods 1 and 2 and between methods 1 and 3 with respect to relative density measurements (Fig. 7).

Table 1. Baseline characteristics of 24 BAO patients.

	BAO patients (n=24)
Mean age	69 ± 14 years
Sex	8 females, 16 males
Site of occlusion	Proximal of BA: <i>n</i> =7 Mid of BA: <i>n</i> =12 Top of BA: <i>n</i> =5
HBAS, <i>n</i> (%)	16 (66.7%)
Median onset to treatment	201 (155–376, IQR) mins
Median initial NIHSS	18 (9-19, IQR)
Stroke etiology	Cardioembolic: <i>n</i> =13 Large artery: <i>n</i> =7 Unknown: <i>n</i> =4
Recanalization (TICI)	0-1: <i>n</i> =5 2a-3: <i>n</i> =19 2b-3: <i>n</i> =15
Outcome (mRS at 90 days)	0-2: <i>n</i> =6 0-3: <i>n</i> =10 4-6: <i>n</i> =14

IQR: Interquartile range.

Table 2. Mean and median values of the three methods (both absolute and relative thrombus density).

		Density distribution (method 1)	Three ROIs placement (method 2)	One ROI placement (method 3)	<i>P</i> value (method 2 vs. method 1)	<i>P</i> value (method 3 vs. method 1)
Absolute thrombus density (n=24)	Mean \pm SD, (HU)	53.8 \pm 10.7	58.7 \pm 10.5	63.1 \pm 12.2	<i>P</i> < 0.0001	<i>P</i> < 0.0001
	Median (HU)	53.9	59	65		
	IQR	46-61	52-66	54-72		
Normal vessel (n=24)	Mean \pm SD, (HU)	38.8 \pm 3.1	41.5 \pm 3.1	43.1 \pm 3.8		
Relative thrombus density (n=24)	Mean \pm SD, (HU)	1.53 \pm 0.15	1.60 \pm 0.16	1.65 \pm 0.15	<i>P</i> = 0.0003	<i>P</i> = 0.0004
	Median (HU)	1.51	1.57	1.59		
	IQR	1.41-1.60	1.47-1.71	1.53-1.77		

IQR: Interquartile range.

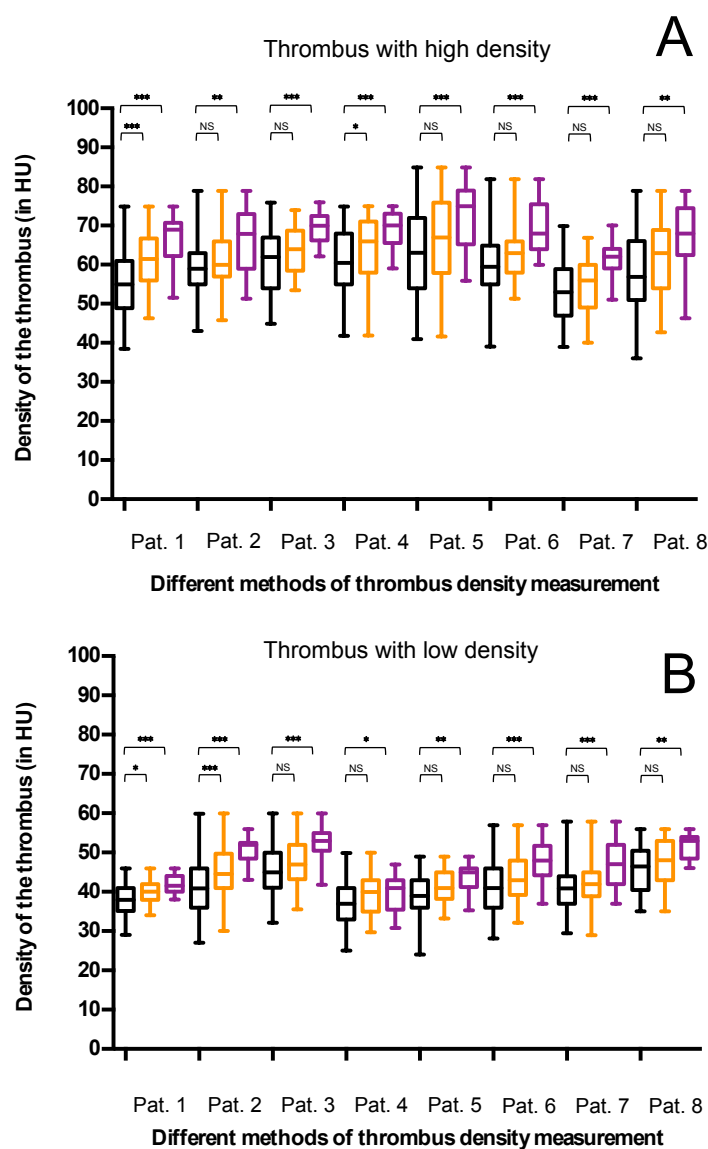


Figure 6. Boxplots demonstrated the differences of density of whole thrombus measurements (black boxplots) in comparison with density measured with three ROIs (orange boxplots) and one ROI (purple boxplot) placements both in high-density thrombi (A) and low-density thrombi (B). The horizontal line in the boxplot indicates median value, and the upper and lower lines of the box indicate Q1 and Q3 (IQR, respectively). NS: not significant. */**/***: significant at $P < 0.05$ / $P < 0.01$ / $P < 0.001$, respectively.

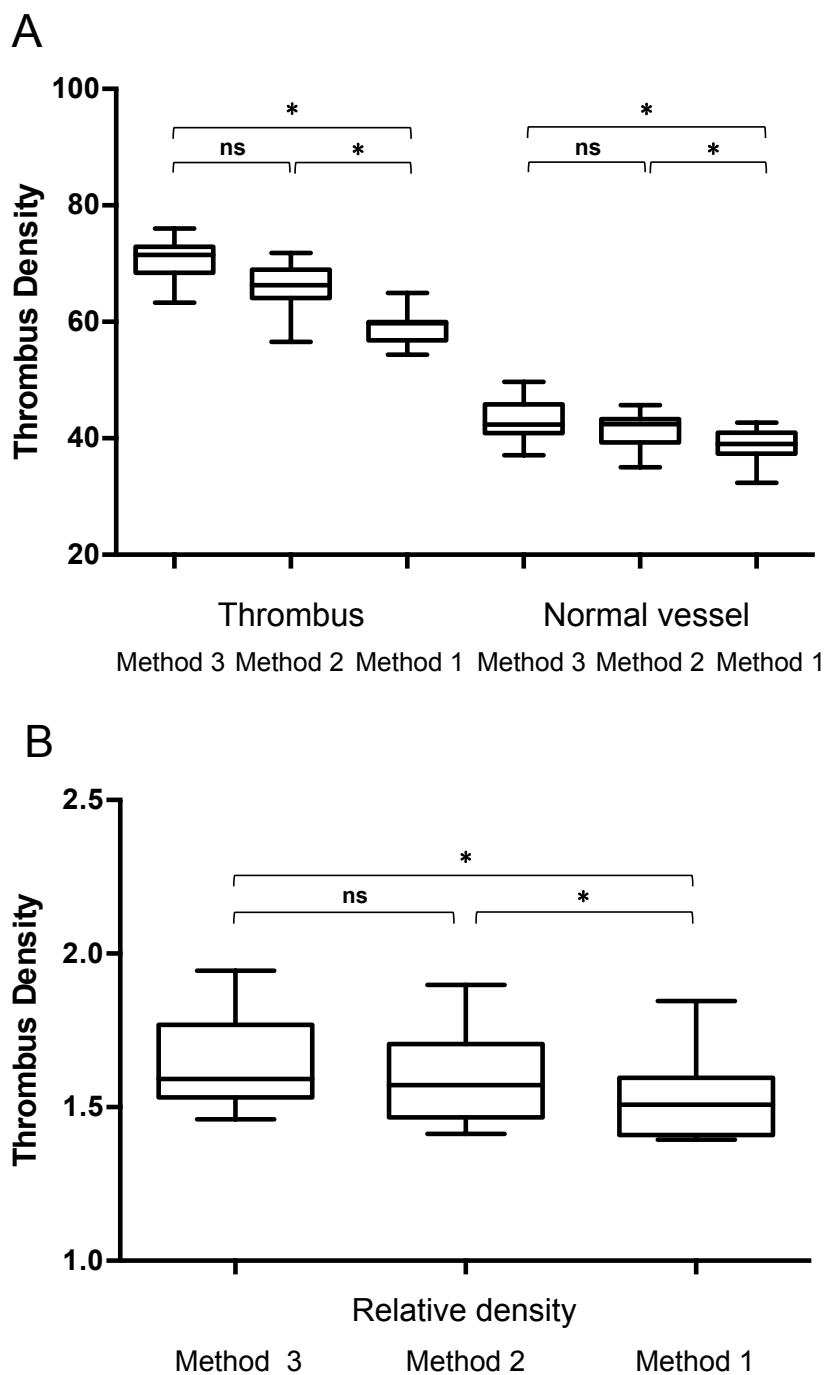


Fig. 7. Boxplots for absolute thrombus density, normal vessel density (A) and relative thrombus density (B) displaying mean, quartiles, and extreme values. *: significant at $P < 0.001$, ns: not significant.

Discussion

In this study, we compared three different methods for thrombus density measurement on thin-slice NECCT images. We found that ROI-based methods of thrombus density measurement yield largely different results in both absolute and relative density. Furthermore, in this small cohort, the density distribution of the whole thrombi showed a range of variation in the thrombus density rather than a single mean/median value, as calculated from three or one ROIs placements. Hence, the whole thrombus density measurements supplied more features of the thrombi than other two methods.

Presently, limited studies are available on the thrombus density in BAO patients. To our knowledge, this is the first study comparing three different methods with respect to the measurement of thrombus density (both absolute and relative density) slice-by-slice on NECCT in BAO patients. Santos⁵⁴ reported that the average median of the thrombus density distribution was lower than those from two commonly used methods in patients with MCA occlusion, which is similar to our results found in BAO patients. However, our study revealed that the difference between three different methods could also be found in relative density. Moreover, the density distribution measurements in every slice provided supplementary information on thrombus density distribution than an average value in only one slice (Fig. 8). Since the RBC component was considered to correlate with thrombus attenuation⁴⁵, the whole thrombus density measurement in every slice provided further insights on the thrombus composition. Previous studies measured the thrombus density slice-by-slice in patients with MCA occlusion²⁰. However, a single mean/median value (in absolute density), rather than a range of variation, was used to represent the thrombus density.

In this study, we found a significant density difference between the whole thrombus density measurements and median values of density measured by the other two methods (Table 2). This implies that the thrombus density calculated from the placements of multiple or one ROIs into the

maximum density part of the thrombi was overestimated as compared to the density distribution values; the results of the previous methods were rarely included in the analysis in the low-density parts of the thrombi. Consequently, this overestimation in the thrombus density might partially explain the correlation between thrombus density and successful recanalization and was demonstrated in some studies²⁰⁻²², but not in others^{16, 23}.

The histological composition of thrombi has been demonstrated to be crucial for successful recanalization. A high content of red blood cells has been associated with successful mechanical thrombectomy and pharmacological thrombolysis^{43, 44}. Thus, thrombus imaging has thus been the target of pretreatment imaging approaches. Compared to other two methods, the variation of thrombus distribution illustrated in the present study provided additional information about the heterogeneity of thrombus composition. Recently, Boeckh-Behrens⁵⁵ reported that high percentages of WBCs, rather than RBCs, were related to organized thrombi of cardioembolic origin. The thrombi with HBAS (with high density) were RBC-prominent, and those without HBAS (with low density) were platelet-prominent. Thus, the whole density distribution measurement, which places the ROIs in both the high dense part and more importantly, the low dense part of the thrombi, might indicate the order of organization of the thrombi. Consequently, more mechanical features of the thrombus⁵⁶ and the response of different compositions to various corresponding thrombectomy devices^{57, 58} could be determined⁴⁷.

In addition to different methods for the measurement of thrombus density, several other factors such as slice thickness were demonstrated to be crucial. In the literatures, a slice thickness between 2.5 and 5 mm²⁰⁻²³ was used. The quantitative estimation of the whole thrombus density distribution on thin-slice NECCT images provided valuable details about thrombus composition. Furthermore, the differing and increasing efficacy of treatment modalities, such as intravenous thrombolysis and

mechanical thrombectomy, with new devices may also affect the observed density of successfully treated thrombi. Nevertheless, assessing the influence of these and additional factors⁵⁹ was beyond the scope of this study, thereby necessitating further investigations.

The present study has some limitations. First, due to the retrospective nature and small cohort, our results require substantiation in large population-based studies. Second, for some thrombi with blurred boundary or atherosclerotic changes in an obstructed BA, a part of blood or thickened endothelium of vessels around the thrombi was inevitably drawn into the density estimation by manual identification of the borderline of thrombi, thereby resulting in bias with respect to the assessment of thrombus density. Third, a standard method for thrombus density evaluation on NECCT images is lacking due to technical limitations. Some recent studies^{44, 45, 55} established the correlation between different dominated components in thrombi (for example, erythrocyte, WBC, platelet, and fibrin) and thrombus attenuation through histopathological analysis of the retrieved thrombus. However, due to the retrospective nature, the histological analysis for the retrieved clot could not be conducted. Moreover, although the density distribution of the whole thrombus instead of measurements using multiple or one ROIs placement provided sufficient information for estimation, the specific confidence interval of the distribution for the specific range of real thrombus density is yet to be determined. Further studies would develop a gold standard that might provide an accurate knowledge of the whole thrombus density, and then, the density range of radiographic estimation and different proportions of components in thrombi from histological analysis could be compared. Subsequently, the responsiveness to appropriate mechanical devices by the thrombus with different compositions would be investigated. As a result, efficient devices might lead to a reduced time taken for the intervention as well as increased incidence of favorable clinical outcome.

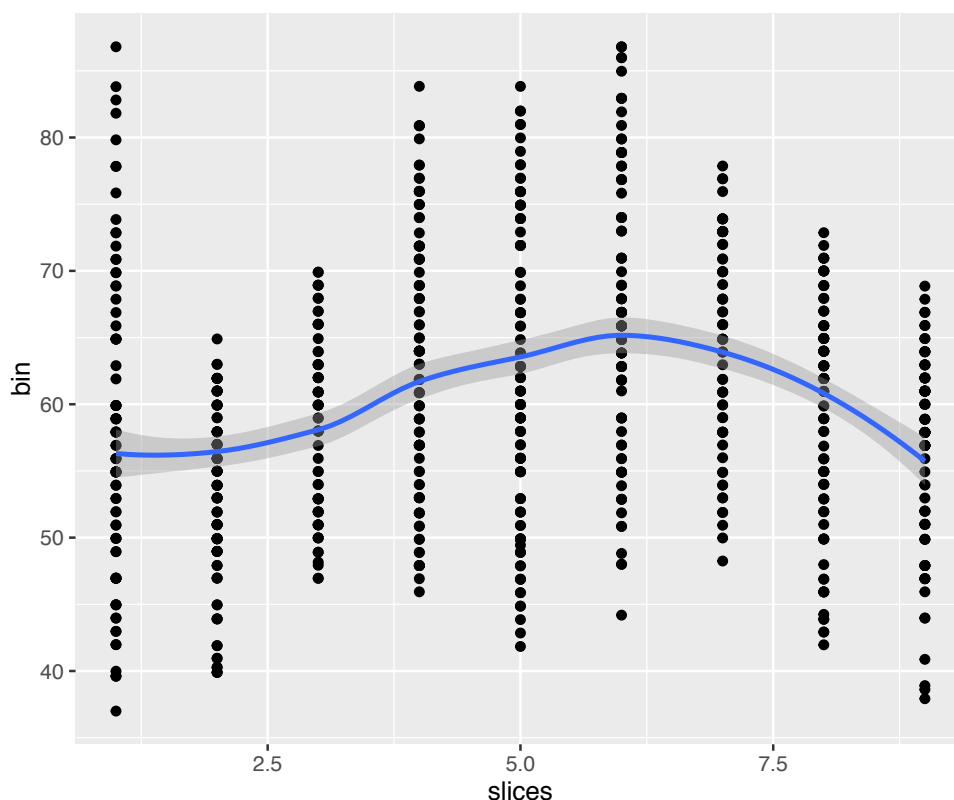


Figure 8. The point graph demonstrated density distribution of the whole thrombus derived from all consecutive slices ($n=9$) in which thrombus can be identified in a BAO patient. Each black dot represents one bin value within the thrombus by using Image J software (discussed in “Methods” section, Part I). All black dots in one column demonstrate the density distribution in one slice. The blue line that runs across the graph signifies the smoothed line of all mean value within the nine columns. For this BAO patient, the density distribution measurements provided different mean values of thrombus density from nine consecutive slices rather than one value from either slice.

Conclusion

Different methods of the measurement of thrombus density on NECCT yield significantly varied results. Thus, the comparison among recently published studies is controversial. The whole thrombus density measured slice-by-slice should be taken into account the heterogeneity of the thrombus composition. While automated algorithms⁵⁴ might be valuable, our results demonstrate that technical standards are needed.

General Discussion

In this thesis, the prognostic value of thrombus characteristics including thrombus length and thrombus density on recanalization rate and clinical outcome in BAO patients were demonstrated (Part I). Furthermore, the comparison of three different methods of thrombus density measurement on thin-slice NECCT images was investigated (Part II).

PART I. Prognostic value of thrombus characteristics on recanalization rate and clinical outcome in BAO patients treated with EVT.

In this part, we evaluated the correlation between thrombus length and thrombus density with recanalization rate and clinical outcome. The results of Part 1 were two-fold. Our first finding is that successful recanalization could be achieved despite high clot burden in BAO patients treated with EVT. Similar to other studies^{16, 17}, we did not find any relationship between thrombus length and the likelihood of recanalization. Clot length measurement might be useful for neurointerventionalists to plan the intervention and choose an appropriate mechanical device for thrombectomy, but the role of it in decision making is limited²⁶. In contrast to other series¹⁶, the association between clot length and clinical outcome was not found in our study. This may be due to the rate of favorable clinical outcome in our series, which was a little lower (25.5%) than that published in previous studies^{9, 60-63} (around 30~56%) and different methods used for thrombus length measurement. Rapid reperfusion could be accomplished by short OTT, effective mechanical devices, and experienced neurointerventionalist irrespective of clot burden. However, besides quick recanalization, patient characteristics, such as age, neurological deficit and collateral status should also be considered as predictors of clinical outcome. Therefore, the prognostic value of thrombus length on clinical outcome as an independent factor is limited and needs to be tested in larger trials.

The second finding of our series showed that high density of thrombi is a strong predictor of favorable

clinical outcome, whereas the correlation between thrombus density and recanalization rate was not found in our cohort, which is in line with other series²³. In contrast with previous study¹⁶, a significant association between high thrombus density and good outcome was found in our series. We hypothesized that low-density thrombi might be caused by atherosclerotic changes and partial volume effects may have blurred high attenuating thrombi but our secondary analysis didn't substantiate this hypothesis. Nevertheless, due to our small cohort and lack of verification of thrombus composition, further studies could validate our finding in comparison of radiographic assessment and histopathological analysis of retrieved thrombi.

PART II. Thrombus density measurements on non-enhanced cranial computed tomography: Comparison of three different methods.

In this part, we compared three different methods for thrombus density measurement. We found the whole thrombus density measurements was statistically lower than single value measured by multiple or one ROIs placements, which indicated thrombus density might be overestimated by two methods that widely used in many studies. This overestimation of measured density might explain why a correlation between thrombus density and success recanalization found in some studies was not confirmed in others. On the other hand, different-ranged variation of density deriving from all corresponding slices of thrombi, as presented in Part II, revealed heterogeneity of thrombus composition. As a consequence, more mechanical features of the thrombus and the response of different thrombus composition to different corresponding thrombectomy devices should be evaluated.

General Conclusion of this thesis

It remains uncertain how thrombus characteristics assessment might alter clinical decision-making and optimize treatment strategies. This thesis provided evidence that successful recanalization could be achieved despite high clot burden in BAO patients treated with EVT, which indicate that there should

be no impact of thrombus length on treatment decision in the acute BAO patient. On the other hand, more insights should be given into thrombus density or thrombus composition, which could have clinical and technical implications. Automated whole thrombus algorithms could be helpful⁵⁶. However, a gold standard method for thrombus density measurement is still needed.

Bibliography

Uncategorized References

1. Zeumer H, Hacke W, Kolmann HL, Poeck K. Lokale fibrinolysetherapie bei basilaris-thrombose. *DMW - Deutsche Medizinische Wochenschrift*. 1982;107:728-731
2. Strbian D, Sairanen T, Silvennoinen H, Salonen O, Lindsberg PJ. Intravenous thrombolysis of basilar artery occlusion: Thrombus length versus recanalization success. *Stroke*. 2014;45:1733-1738
3. Berkhemer OA, Fransen PS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. *N Engl J Med*. 2015;372:11-20
4. Campbell BC, Mitchell PJ, Kleinig TJ, Dewey HM, Churilov L, Yassi N, et al. Endovascular therapy for ischemic stroke with perfusion-imaging selection. *N Engl J Med*. 2015;372:1009-1018
5. Goyal M, Demchuk AM, Menon BK, Eesa M, Rempel JL, Thornton J, et al. Randomized assessment of rapid endovascular treatment of ischemic stroke. *N Engl J Med*. 2015;372:1019-1030
6. Jovin TG, Chamorro A, Cobo E, de Miquel MA, Molina CA, Rovira A, et al. Thrombectomy within 8 hours after symptom onset in ischemic stroke. *N Engl J Med*. 2015;372:2296-2306
7. Saver JL, Goyal M, Bonafe A, Diener HC, Levy EI, Pereira VM, et al. Stent-retriever thrombectomy after intravenous t-pa vs. T-pa alone in stroke. *N Engl J Med*. 2015;372:2285-2295
8. Singer OC, Berkefeld J, Nolte CH, Bohner G, Haring HP, Trenkler J, et al. Mechanical recanalization in basilar artery occlusion: The endostroke study. *Ann Neurol*. 2015;77:415-424
9. Schonewille WJ, Wijman CA, Michel P, Rueckert CM, Weimar C, Mattle HP, et al. Treatment and outcomes of acute basilar artery occlusion in the basilar artery international cooperation study (basics): A prospective registry study. *Lancet Neurol*. 2009;8:724-730
10. Costalat V, Machi P, Lobotesis K, Maldonado I, Vendrell JF, Riquelme C, et al. Rescue, combined, and stand-alone thrombectomy in the management of large vessel occlusion stroke using the solitaire device: A prospective 50-patient single-center study: Timing, safety, and efficacy. *Stroke*. 2011;42:1929-1935
11. Kamalian S, Morais LT, Pomerantz SR, Aceves M, Sit SP, Bose A, et al. Clot length distribution and predictors in anterior circulation stroke: Implications for

- intra-arterial therapy. *Stroke*. 2013;44:3553-3556
12. Riedel CH, Zimmermann P, Jensen-Kondering U, Stingle R, Deuschl G, Jansen O. The importance of size: Successful recanalization by intravenous thrombolysis in acute anterior stroke depends on thrombus length. *Stroke*. 2011;42:1775-1777
 13. Tan IY, Demchuk AM, Hopyan J, Zhang L, Gladstone D, Wong K, et al. Ct angiography clot burden score and collateral score: Correlation with clinical and radiologic outcomes in acute middle cerebral artery infarct. *AJNR Am J Neuroradiol*. 2009;30:525-531
 14. Rohan V, Baxa J, Tupy R, Cerna L, Sevcik P, Friesl M, et al. Length of occlusion predicts recanalization and outcome after intravenous thrombolysis in middle cerebral artery stroke. *Stroke*. 2014;45:2010-2017
 15. Weisstanner C, Gratz PP, Schroth G, Verma RK, Kochl A, Jung S, et al. Thrombus imaging in acute stroke: Correlation of thrombus length on susceptibility-weighted imaging with endovascular reperfusion success. *Eur Radiol*. 2014;24:1735-1741
 16. Spiotta AM, Vargas J, Hawk H, Turner R, Chaudry MI, Battenhouse H, et al. Hounsfield unit value and clot length in the acutely occluded vessel and time required to achieve thrombectomy, complications and outcome. *J Neurointerv Surg*. 2014;6:423-427
 17. Gilberti N, Gamba M, Premi E, Costa A, Vergani V, Delrio I, et al. Endovascular mechanical thrombectomy in basilar artery occlusion: Variables affecting recanalization and outcome. *J Neurol*. 2016;263:707-713
 18. Kim EY, Heo JH, Lee SK, Kim DJ, Suh SH, Kim J, et al. Prediction of thrombolytic efficacy in acute ischemic stroke using thin-section noncontrast ct. *Neurology*. 2006;67:1846-1848
 19. Niesten JM, van der Schaaf IC, van der Graaf Y, Kappelle LJ, Biessels GJ, Horsch AD, et al. Predictive value of thrombus attenuation on thin-slice non-contrast ct for persistent occlusion after intravenous thrombolysis. *Cerebrovasc Dis*. 2014;37:116-122
 20. Puig J, Pedraza S, Demchuk A, Daunis IEJ, Termes H, Blasco G, et al. Quantification of thrombus hounsfield units on noncontrast ct predicts stroke subtype and early recanalization after intravenous recombinant tissue plasminogen activator. *AJNR Am J Neuroradiol*. 2012;33:90-96
 21. Froehler MT, Tateshima S, Duckwiler G, Jahan R, Gonzalez N, Vinuela F, et al. The hyperdense vessel sign on ct predicts successful recanalization with the merci device in acute ischemic stroke. *J Neurointerv Surg*. 2013;5:289-293
 22. Mokin M, Morr S, Natarajan SK, Lin N, Snyder KV, Hopkins LN, et al. Thrombus density predicts successful recanalization with solitaire stent retriever

- thrombectomy in acute ischemic stroke. *J Neurointerv Surg*. 2015;7:104-107
23. Yilmaz U, Roth C, Reith W, Papanagiotou P. Thrombus attenuation does not predict angiographic results of mechanical thrombectomy with stent retrievers. *AJNR Am J Neuroradiol*. 2013;34:2184-2186
 24. Mair G, von Kummer R, Lindley RI, Sandercock PA, Wardlaw JM, Group ISTC. Effect of x-ray attenuation of arterial obstructions on intravenous thrombolysis and outcome after ischemic stroke. *PLoS One*. 2015;10:e0145683
 25. Topcuoglu MA, Arsava EM, Kursun O, Akpinar E, Erbil B. The utility of middle cerebral artery clot density and burden assessment by noncontrast computed tomography in acute ischemic stroke patients treated with thrombolysis. *J Stroke Cerebrovasc Dis*. 2014;23:e85-91
 26. Campbell BC. Clot length assessment in stroke therapy decisions. *Stroke*. 2016;47:643-644
 27. Archer CR, Horenstein S. Basilar artery occlusion: Clinical and radiological correlation. *Stroke*. 1977;8:383-390
 28. Adams HP, Jr., Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. Toast. Trial of org 10172 in acute stroke treatment. *Stroke*. 1993;24:35-41
 29. Nagel S, Schellinger PD, Hartmann M, Juettler E, Huttner HB, Ringelb P, et al. Therapy of acute basilar artery occlusion: Intraarterial thrombolysis alone vs bridging therapy. *Stroke*. 2009;40:140-146
 30. Riedel CH, Jensen U, Rohr A, Tietke M, Alfke K, Ulmer S, et al. Assessment of thrombus in acute middle cerebral artery occlusion using thin-slice nonenhanced computed tomography reconstructions. *Stroke*. 2010;41:1659-1664
 31. Leys D, Pruvo JP, Godefroy O, Rondepierre P, Leclerc X. Prevalence and significance of hyperdense middle cerebral artery in acute stroke. *Stroke*. 1992;23:317-324
 32. Moftakhar P, English JD, Cooke DL, Kim WT, Stout C, Smith WS, et al. Density of thrombus on admission ct predicts revascularization efficacy in large vessel occlusion acute ischemic stroke. *Stroke*. 2013;44:243-245
 33. Hacke W, Kaste M, Fieschi C, von Kummer R, Davalos A, Meier D, et al. Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischaemic stroke (ecass ii). Second european-australasian acute stroke study investigators. *Lancet*. 1998;352:1245-1251
 34. Castano C, Dorado L, Guerrero C, Millan M, Gomis M, Perez de la Ossa N, et al. Mechanical thrombectomy with the solitaire ab device in large artery occlusions

- of the anterior circulation: A pilot study. *Stroke*. 2010;41:1836-1840
35. Penumbra Pivotal Stroke Trial I. The penumbra pivotal stroke trial: Safety and effectiveness of a new generation of mechanical devices for clot removal in intracranial large vessel occlusive disease. *Stroke*. 2009;40:2761-2768
 36. Roth C, Papanagiotou P, Behnke S, Walter S, Haass A, Becker C, et al. Stent-assisted mechanical recanalization for treatment of acute intracerebral artery occlusions. *Stroke*. 2010;41:2559-2567
 37. Smith WS. Safety of mechanical thrombectomy and intravenous tissue plasminogen activator in acute ischemic stroke. Results of the multi mechanical embolus removal in cerebral ischemia (merci) trial, part i. *AJNR Am J Neuroradiol*. 2006;27:1177-1182
 38. Meyne JK, Zimmermann PR, Rohr A, Riedel C, Hansen HC, Pulkowski U, et al. Thrombectomy vs. Systemic thrombolysis in acute embolic stroke with high clot burden: A retrospective analysis. *Rofo*. 2015;187:555-560
 39. Miller TS, Brook AL, Riedel CH, Hirsch JA, Yoo AJ. Expanding the role of ncct in acute stroke imaging: Thrombus length measurement and its potential impact on current practice. *J Neurointerv Surg*. 2014;6:5-6
 40. Yan S, Chen Q, Xu M, Sun J, Liebeskind DS, Lou M. Thrombus length estimation on delayed gadolinium-enhanced t1. *Stroke*. 2016;47:756-761
 41. Jindal G, Miller T, Shivashankar R, Mitchell J, Stern BJ, Yarbrough K, et al. Relationship of thrombus length to number of stent retrievals, revascularization, and outcomes in acute ischemic stroke. *J Vasc Interv Radiol*. 2014;25:1549-1557
 42. Riedel CH, Zoubie J, Ulmer S, Giethmuehlen J, Jansen O. Thin-slice reconstructions of nonenhanced ct images allow for detection of thrombus in acute stroke. *Stroke*. 2012;43:2319-2323
 43. Kirchhof K, Welzel T, Mecke C, Zoubaa S, Sartor K. Differentiation of white, mixed, and red thrombi: Value of ct in estimation of the prognosis of thrombolysis phantom study. *Radiology*. 2003;228:126-130
 44. Liebeskind DS, Sanossian N, Yong WH, Starkman S, Tsang MP, Moya AL, et al. Ct and mri early vessel signs reflect clot composition in acute stroke. *Stroke*. 2011;42:1237-1243
 45. Niesten JM, van der Schaaf IC, van Dam L, Vink A, Vos JA, Schonewille WJ, et al. Histopathologic composition of cerebral thrombi of acute stroke patients is correlated with stroke subtype and thrombus attenuation. *PLoS One*. 2014;9:e88882
 46. Tomkins AJ, Schleicher N, Murtha L, Kaps M, Levi CR, Nedelmann M, et al. Platelet rich clots are resistant to lysis by thrombolytic therapy in a rat model of embolic stroke. *Exp Transl Stroke Med*. 2015;7:2

47. Angermaier A, Langner S. Thrombus density measurement is promising but technical standards are needed. *J Neurointerv Surg.* 2017;9:e9-e10
48. Sheth SA, Jahan R, Gralla J, Pereira VM, Nogueira RG, Levy EI, et al. Time to endovascular reperfusion and degree of disability in acute stroke. *Ann Neurol.* 2015;78:584-593
49. Lindsberg PJ, Mattle HP. Therapy of basilar artery occlusion: A systematic analysis comparing intra-arterial and intravenous thrombolysis. *Stroke.* 2006;37:922-928
50. Sairanen T, Strbian D, Soenne L, Silvennoinen H, Salonen O, Artto V, et al. Intravenous thrombolysis of basilar artery occlusion: Predictors of recanalization and outcome. *Stroke.* 2011;42:2175-2179
51. Behrens L, Mohlenbruch M, Stampfl S, Ringleb PA, Hametner C, Kellert L, et al. Effect of thrombus size on recanalization by bridging intravenous thrombolysis. *Eur J Neurol.* 2014;21:1406-1410
52. Connell L, Koerte IK, Laubender RP, Morhard D, Linn J, Becker HC, et al. Hyperdense basilar artery sign—a reliable sign of basilar artery occlusion. *Neuroradiology.* 2012;54:321-327
53. Ernst M, Romero JM, Buhk JH, Cheng B, Herrmann J, Fiehler J, et al. Sensitivity of hyperdense basilar artery sign on non-enhanced computed tomography. *PLoS One.* 2015;10:e0141096
54. Santos EM, Niessen WJ, Yoo AJ, Berkhemer OA, Beenen LF, Majoie CB, et al. Automated entire thrombus density measurements for robust and comprehensive thrombus characterization in patients with acute ischemic stroke. *PLoS One.* 2016;11:e0145641
55. Boeckh-Behrens T, Schubert M, Forschler A, Prothmann S, Kreiser K, Zimmer C, et al. The impact of histological clot composition in embolic stroke. *Clin Neuroradiol.* 2016;26:189-197
56. Gersh KC, Nagaswami C, Weisel JW. Fibrin network structure and clot mechanical properties are altered by incorporation of erythrocytes. *Thromb Haemost.* 2009;102:1169-1175
57. Chueh JY, Puri AS, Wakhloo AK, Gounis MJ. Risk of distal embolization with stent retriever thrombectomy and adapt. *J Neurointerv Surg.* 2016;8:197-202
58. Chueh JY, Wakhloo AK, Gounis MJ. Effectiveness of mechanical endovascular thrombectomy in a model system of cerebrovascular occlusion. *AJNR Am J Neuroradiol.* 2012;33:1998-2003
59. Grams AE, Rehwald R, Schmittnagel C, Schmidt T, Tanislav C, Berghoff M, et al. Factors influencing intracranial vessel densities on unenhanced computed tomography: Differences between hemispheres. *Clin Imaging.* 2016;40:1081-1085

60. Arnold M, Nedeltchev K, Schroth G, Baumgartner RW, Remonda L, Loher TJ, et al. Clinical and radiological predictors of recanalisation and outcome of 40 patients with acute basilar artery occlusion treated with intra-arterial thrombolysis. *J Neurol Neurosurg Psychiatry*. 2004;75:857-862
61. Jung S, Mono ML, Fischer U, Galimanis A, Findling O, De Marchis GM, et al. Three-month and long-term outcomes and their predictors in acute basilar artery occlusion treated with intra-arterial thrombolysis. *Stroke*. 2011;42:1946-1951
62. Mordasini P, Brekenfeld C, Byrne JV, Fischer U, Arnold M, Heldner MR, et al. Technical feasibility and application of mechanical thrombectomy with the solitaire fr revascularization device in acute basilar artery occlusion. *AJNR Am J Neuroradiol*. 2013;34:159-163
63. Pfefferkorn T, Holtmannspotter M, Schmidt C, Bender A, Pfister HW, Straube A, et al. Drip, ship, and retrieve: Cooperative recanalization therapy in acute basilar artery occlusion. *Stroke*. 2010;41:722-726

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Academic Curriculum Vitae

Liang Shu
Master of Medicine

FIRST NAME: Liang

SURNAME: Shu

GENDER: Male

DATE AND PLACE OF BIRTH: October 11, 1980, Shanghai

NATIONALITY: People's Republic of China

MARITAL STATUS: Married

HOSPITAL: Working in the Department of Neurology, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Stay in the Department of radiology and neuroradiology, Universitätsklinikum Schleswig-Holstein Campus Kiel, Kiel, Germany as a fellow from 09.2014 to 09.2016

EMAIL : shuliang_9@hotmail.com

MOBILE PHONE: +86 13671799246

MEDICAL SCHOOL:

1999-2004 Bachelor of Medicine, Specialty of Clinical Medicine, Tong Ji University, School of Medicine

2008-2011 Master of Medicine, Specialty of Neurology, Shanghai Jiao Tong University, School of Medicine

FURTHER DEVELOPMENT/EMPLOYMENTS:

2004.8-2011.11 Staff member and Neurologist
Department of Neurology, Shanghai Ninth People's Hospital

2011.12-now Attending Doctor
Department of Neurology, Shanghai Ninth People's Hospital

2010.1-now Specialism clinic of Benign Paroxysmal Positional
Vertigo, Department of Neurology, Shanghai Ninth People's Hospital

2010.7-2011.6 Studied in Institutes of Brain Science and State Key Laboratory of
Medical Neurobiology, Fudan University

SCIENTIFIC RESEARCH FOUNDATION:

2018 The mechanism of cofilin rod induced synaptic dysfunction in oxygen glucose deprivation (OGD)/reperfusion injured neurons.
(Shanghai Jiao Tong University Medicine and Engineering Collaboration Foundation, No. ZH2018QNB01, 2019-2021)

2012 Participate in the study of “The regulation mechanisms on expression and divergent roles of bradykinin B2 receptor by HIF-1 α in a murine model of cerebral ischemic reperfusion”
(National Natural Science Foundation of China, No.81200941, 2012-2015)

2011 Study of the relationship between Cofilin rod formation and the loss of Neuron synapse.
(Hospital Scientific Research Foundation, No. JY2011A18, 2011-2014)

PUBLICATIONS IN RECENT FIVE YEARS

1. Shu L, Dong Y-R, Yan W-H, Zhai Y, Wang Y, Li W. [long term depression of the recurrent inhibition of monosynaptic spinal reflexes after sciatic nerve crush in adult rats]. *Sheng li xue bao : [Acta physiologica Sinica]*. 2011;63:291-299
2. Gong H, Shu L, Xu H, Chen B, Mao R, Zhang F, et al. Bilateral internal carotid arteries ligation temporary impairs brain vasculature in young rats. *Autonomic Neuroscience*. 2013;173:39-44

3. Shu L, Su J, Jing L, Huang Y, Di Y, Peng L, et al. Reduced rensaw recurrent inhibition after neonatal sciatic nerve crush in rats. *Neural Plasticity*. 2014;2014:786985
4. Shu L, Wang Y. Highlights in basic autonomic neuroscience: Insular cortex injury leads to cardiovascular dysfunction. *Autonomic Neuroscience*. 2014;185:1-4
5. Zhao R, Liu FD, Wang S, Peng J-LL, Tao XX, Zheng B, et al. Blood pressure reduction in the acute phase of an ischemic stroke does not improve short- or long-term dependency or mortality: A meta-analysis of current literature. *Medicine*. 2015;94(23):e896
6. Chen W, Shu L, Wang Q, Pan H, Wu J, Fang J, et al. Validation of 5-item and 2-item questionnaires in chinese version of dizziness handicap inventory for screening objective benign paroxysmal positional vertigo. *Neurological Sciences*. 2016;37:1241-1246
7. Liu F-D, Shen X-L, Zhao R, Li G-F, Wu Y-L, Tao X-X, et al. Predictive role of chads2 and cha2ds2-vasc scores on stroke and thromboembolism in patients without atrial fibrillation: A meta-analysis. *Annals of medicine*. 2016:1-9
8. Liu F-D, Shen X-L, Zhao R, Tao X-X, Wang S, Zhou J-J, et al. Pulse pressure as an independent predictor of stroke: A systematic review and a meta-analysis. *Clinical Research in Cardiology*. 2016;105:677-686
9. Shu L, Riedel C, Meyne J, Jansen O, Jensen-Kondering U. Successful recanalization in acute basilar artery occlusion treated with endovascular therapy is independent of thrombus length. *J Neurointerv Surg*. 2017 Nov;9(11):1047-1052
10. Su J, Shu L, Zhang Z, Cai L, Zhang X, Zhai Y, et al. A small deletion in SERPINC1 causes type I antithrombin deficiency by promoting endoplasmic reticulum stress. *Oncotarget*. 2016 Nov 22;7(47):76882-76890
11. Shu L, Meyne J, Jansen O, Jensen-Kondering Ulf. Manual thrombus density measurement depends on the method of thrombus delineation. *J Stroke*. 2018 Sep;20(3):411-412
12. Shu L, Chen B, Chen B, Xu H, Wang G, Huang Y, Zhao Y, Gong H, Jiang M, Chen L, Liu X, Wang Y. Brain ischemic insult induces cofilin rod formation leading to synaptic dysfunction in neurons. *J Cereb Blood Flow Metab*. 2018 Jun 22:271678X18785567 (Epub ahead of print)

List of Publications

Published Papers in Peer-reviewed Journals

Liang Shu, Christian Riedel, Johannes Meyne, Olav Jansen, Ulf Jensen-Kondering.
Successful recanalization in acute basilar artery occlusion treated with endovascular therapy is independent of thrombus length.

Journal of NeuroInterventional Surgery. 2017 Nov;9(11):1047-1052

Liang Shu, Johannes Meyne, Olav Jansen, Ulf Jensen-Kondering.
Manual thrombus density measurement depends on the method of thrombus delineation.

Journal of Stroke. 2018 Sep;20(3):411-412

Poster Presentations

Liang Shu, Christian Riedel, Johannes Meyne, Olav Jansen, Ulf Jensen-Kondering.
Korreliert die mechanische Rekanalisierungsrate und das klinische Outcome mit den Thrombuseigenschaften oder der Zeit zur Rekanalisierung bei Patienten mit akutem Verschluss der A. basilaris?

Arbeitstagung NeurointensivMedizin 2016, Berlin, Germany

Liang Shu, Christian Riedel, Johannes Meyne, Olav Jansen, Ulf Jensen-Kondering.
Successful Recanalization is Independent of Clot Characteristics In Acute Basilar Artery Occlusion Treated With Endovascular Therapy.

European Stroke Organization Congress 2016, Barcelona, Spain

Ulf Jensen-Kondering, Ruwen Böhm, **Liang Shu**, Ulrich Katscher, Olav Jansen.

Electric Properties Tomography in Ischemic Stroke: Results of an ex-vivo Pilot Study.

European Stroke Organization Congress 2016, Barcelona, Spain

Liang Shu, Christian Riedel, Johannes Meyne, Olav Jansen, Ulf Jensen-Kondering.

Successful Recanalization is Independent of Clot Characteristics In Acute Basilar Artery

Occlusion Treated With Endovascular Therapy.

51st Annual Meeting of the German Society of Neuroradiology, Köln, Germany

Ulf Jensen-Kondering, Ruwen Böhm, **Liang Shu**, Olav Jansen, and Ulrich Katscher

Electric properties tomography in a rodent model of ischemic stroke: Results of a combined ex-vivo and in-vivo pilot study.

25th ISMRM Annual Meeting & Exhibition, Honolulu, Hawaii