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Original research

Thrombus imaging characteristics within acute ischemic stroke: similarities and interdependence

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ABSTRACT

Background The effects of thrombus imaging characteristics on procedural and clinical outcomes after ischemic stroke are increasingly being studied. These thrombus characteristics – for eg, size, location, and density – are commonly analyzed as separate entities. However, it is known that some of these thrombus characteristics are strongly related. Multicollinearity can lead to unreliable prediction models. We aimed to determine the distribution, correlation and clustering of thrombus imaging characteristics based on a large dataset of anterior-circulation acute ischemic stroke patients.

Methods We measured thrombus imaging characteristics in the MR CLEAN Registry dataset, which included occlusion location, distance from the intracranial carotid artery to the thrombus (DT), thrombus length, density, perviousness, and clot burden score (CBS). We assessed intercorrelations with Spearman's coefficient (ρ) and grouped thrombi based on 1) occlusion location and 2) thrombus length, density and perviousness using unsupervised clustering.

Results We included 934 patients, of which 22% had an internal carotid artery (ICA) occlusion, 61% M1, 16% M2, and 1% another occlusion location. All thrombus characteristics were significantly correlated. Higher CBS was strongly correlated with longer DT ($\rho=0.67$, $p<0.01$), and moderately correlated with shorter thrombus length ($\rho=-0.41$, $p<0.01$). In more proximal occlusion locations, thrombi were significantly longer, denser, and less pervious. Unsupervised clustering analysis resulted in four thrombus groups; however, the cohesion within and distinction between the groups were weak.

Conclusions Thrombus imaging characteristics are significantly intercorrelated – strong correlations should be considered in future predictive modeling studies. Clustering analysis showed there are no distinct thrombus archetypes – novel treatments should consider this thrombus variability.

INTRODUCTION

Multiple thrombus characteristics are increasingly being studied for their effects on procedural and clinical outcomes after ischemic stroke.^{1–3}

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Collinearity of thrombus imaging characteristics in predictive models of acute ischemic stroke is commonly disregarded.

WHAT THIS STUDY ADDS

⇒ We presented distributions, intercorrelations and clustering analysis of thrombus imaging characteristics measured in 937 acute ischemic stroke patients. Thrombus imaging characteristics are significantly intercorrelated, but there are no distinct thrombus archetypes.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Strong correlations between thrombus characteristics should be considered in future predictive models in acute ischemic stroke. The continuous spectrum and specific variability of thrombus characteristics need to be addressed when developing novel treatment approaches.

Currently, the most straightforward way to assess the thrombus is via radiological imaging, mostly computed tomography (CT), since it is part of the standard-of-care diagnostic workup for stroke.⁴ Thrombus imaging characteristics can provide information on the size and (micro)structure of the thrombus, which may be useful to improve and optimize treatment.

After administration of intravenous thrombolysis with alteplase (IVT), the lysis process of the clot can be influenced by the permeability^{5,6} and size of the thrombus.^{7,8} Thrombus density, which has been associated with thrombus histology⁹ and etiology,¹⁰ can also affect the chance of recanalization success after IVT.¹¹ In addition, thrombus density may suggest the use of specific endovascular treatment (EVT) strategies.¹² The optimal size and positioning of the EVT stent-retriever is dependent on thrombus length,¹³ and EVT success can be affected by thrombus volume.¹⁴

As such, there is a whole spectrum of thrombus imaging characteristics influencing treatment, and these characteristics are not independent from

each another. Including multicollinearity in prediction models reduces their statistical significance and should therefore be accounted for. Moreover, in case of strong correlations, there is no added value in measuring all parameters and a reduced number of thrombus measures may be sufficient.

Thrombi are commonly classified by occlusion location, etiology, and/or histopathology.³ Currently, occlusion location is the only thrombus characteristic that is used for treatment selection.⁴ However, the occlusion location does not account for the biomechanical characteristics of the clot, which actually may have a larger effect on treatment response and chance of successful revascularization. Alternatively, thrombi are also classified based on stroke etiology as eg, cardioembolic, atherosclerotic or cryptogenic, according to the TOAST criteria.¹⁵ With different thrombus origins, it could be expected that thrombi show distinct (imaging) features as part of typical thrombus archetypes. Clustering techniques allow for grouping of thrombus characteristics, and therefore, may determine interrelated imaging characteristics that distinguish typical groups of thrombi. Certain sets of thrombus characteristics may be associated with a better response to specific (combinations of) treatments, such as IVT with alteplase or tenecteplase, or specific EVT techniques/devices. For example, thrombolysis was shown to be successful in small, distal, pervious thrombi, whereas its beneficial effect is being debated for large, proximal, impermeable thrombi.

In this study, we present a detailed analysis of interrelations of thrombus imaging characteristics on a large data set of EVT-treated acute ischemic stroke patients. Specifically, we aim to provide information on the distribution and correlation of thrombus imaging characteristics and to study whether thrombi can be grouped based on their imaging characteristics.

METHODS

Patient selection

We included patients from the MR CLEAN Registry, a multicenter prospective observational registry of all patients undergoing EVT for acute ischemic stroke in the Netherlands.¹⁶ This registry was approved by the central medical ethics committee of the Erasmus Medical Center Rotterdam, which served as the review board of all participating centers and granted permission to carry out the study as a registry (MEC-2014–235). All patients or legal representatives were provided with oral and written information on the registry and had the opportunity to withdraw consent to use their data.

We included acute ischemic stroke patients aged 18 or older, with an anterior circulation occlusion who underwent EVT (defined as groin puncture with the intent of thrombectomy) less than 390 minutes after stroke onset between March 2014 and November 2017. Patients without contraindications received 0.9 mg/kg of intravenous alteplase before EVT. The EVT approach and choice of material was left to the individual interventionalist. All patients underwent a standardized stroke imaging protocol at baseline, consisting of baseline non-contrast computed tomography (NCCT) followed by single arterial-phase computed tomography angiography (CTA). Other imaging modalities, such as CT perfusion, were acquired at the discretion of the treating physician. Patients with thick-slice (>2.5 mm) CT imaging, and with NCCT and CTA images not acquired within 30 minutes from each other were excluded. Source data for this study are not available as we did not obtain patient approval for sharing individual, coded patient data. All analytic methods,

codes, and results are available from the corresponding author on reasonable request.

Data collection

The thin-slice NCCT and CTA images were acquired in GE Medical Systems, Philips, Siemens, or Toshiba scanners with a median tube peak voltage of 100 (100–120) kV for CTA, and 120 (100–120) kV for NCCT. The median exposure was 156 (73–221) mA·s for CTA and 301 (240–394) mA·s for NCCT. All details on the image acquisition parameters and protocols can be found in online supplemental table S1.

The MR CLEAN Registry imaging core laboratory consisting of 31 interventional neuroradiologists (with at least 5 years of experience assessing CTA scans in routine clinical practice) assessed the occlusion location and the clot burden score (CBS) based on the contrast-filling defects found on baseline CTA. The CBS is a 10-point scale assessing the extension of the occlusion: the 10 points are divided between vessel segments in the anterior circulation, and for each occluded segment the corresponding points are deducted from 10.¹⁶ Therefore, more occlusive thrombi have lower CBS. Core-lab members were blinded to all clinical information (demographic, treatment and outcome data) except symptom side.¹⁶ Each rater assessed a subset of the total number of cases following the provided training and guidelines.

We additionally measured the following radiological thrombus characteristics: distance from the internal carotid artery (ICA) terminus to the thrombus (DT), thrombus length, thrombus density, and thrombus perviousness. Thrombus measurements of patients included between March 2014 and June 2016 were already available.¹ For patients included between June 2016 and November 2017, additional measurements were performed similar to Bruna *et al.*¹ These measurements were performed by trained observers (AAEB, JB, JWH, KRK, MK, MLT, NA, NAT, NB, and PRK) who were blinded to all clinical information except symptom side. Each observer was assigned a unique subset of the total number of cases. To ensure consistency and homogeneity across the measurements, the observers received training and guidelines to place the markers, including definitions of each vessel segment and a scheme similar to the one shown in figure 1A,B. Difficult cases (such as bifurcated or very long thrombi and cases without distal collateral filling) were marked and discussed among the group of observers. If the marker-placement remained unclear, the case was reviewed by a senior radiologist.

For these measurements, the thin-slice NCCT and CTA images were coregistered using Elastix' rigid-registration.¹⁷ Scans with uncorrectable registration errors, imaging artifacts (eg, beam hardening, metal artifacts), excessive noise, poor CTA contrast opacification, or an incomplete field of view were excluded. Partial occlusions (thrombi occluding 50% of the vessel diameter), bilateral thrombi, too short thrombi (<2 mm), thrombi very close to bone (affected by partial volume/blooming artifact of bone), and/or calcified emboli were also excluded.¹

For each occlusion, we placed up to nine markers along the occluded artery: one marker defining the ICA-terminus, up to three markers along the vessel segment between the ICA-terminus and the proximal thrombus border, two markers defining the thrombus' proximal and distal borders, and three markers in the proximal, middle, and distal parts of the thrombus (figure 1). These markers were simultaneously placed on the coregistered NCCT and CTA scans using ITK-snap software.¹⁸ We used three views (axial, sagittal, and coronal) for the marker placement. The occlusion extension was determined based on the contrast-filling

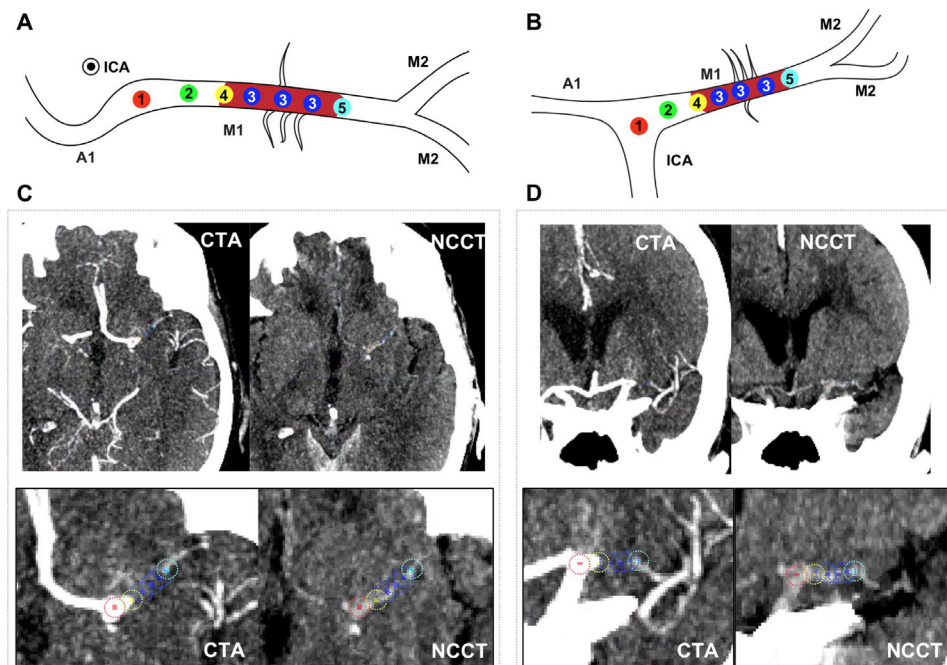


Figure 1 Thrombus markers. (A) Schematic overview of thrombus marker placement, axial view. (B) Schematic overview of thrombus marker placement, coronal view. (C) Axial view of CTA and NCCT scans showing a left-MCA occlusion and placed markers. To visualize the markers, a zoom-in of the occlusion is displayed and the markers are circled in their corresponding color in the image in the lower row. These circles do not represent the 1 mm radius spherical regions of interest used to compute density and perviousness. (D) Coronal view of the CTA and NCCT scans displayed in C showing a left-MCA occlusion and placed markers. A closer view of the occlusion is displayed in the image in the lower row, with the markers circled in their corresponding color. A1, anterior cerebral artery A1 segment; CTA, computed tomography angiography; ICA, internal carotid artery; M1, middle cerebral artery M1 segment; M2, middle cerebral artery M2 segment; MCA, middle cerebral artery; NCCT, non-contrast computed tomography.

defects found on CTA, and aided by the NCCT hyperdense artery sign if present. Because of NCCT was performed before CTA, hyperdensities are expected to come from the thrombus rather than residual contrast.

DT was computed as the total path length between the ICA-terminus and the proximal thrombus border marker. DT is thereby a measure of thrombus distality with respect to the ICA-terminus. If the proximal part of the thrombus was located proximal to the ICA-terminus, DT was set to zero. Thrombus length was computed as the total path length between the proximal and distal thrombus border markers. The three in-thrombus markers were used to define 1 mm radius spherical regions of interest, which were used to compute the average thrombus density and perviousness values, as described previously.¹

Statistical analyses

Our analysis includes three main sections: summary statistics which describes the methods to present characteristic distributions, correlation analyses to assess the collinearity of these characteristics, and clustering of thrombus imaging characteristics to determine whether typical thrombus archetypes with distinct imaging features can be found. We used Python's statistics library for the statistical analysis, Seaborn library for data visualizations and scikit-learn library for clustering analysis. We set the significance level for all analyses at $p < 0.05$.

Summary statistics

For numerical variables, we presented summary statistics as median and interquartile range (IQR). For categorical variables, we presented summary statistics as frequencies and percentages. Baseline characteristics were compared between the currently included patient sample and the overall MR CLEAN Registry

population. For between-group comparisons, the Kruskal-Wallis and/or Mann-Whitney-U tests were used to compare medians, and χ^2 tests to compare frequencies. Post-hoc analyses were adjusted with Bonferroni corrections for multiple testing.

Correlation analyses

We included the following variables in our correlation analysis: DT, thrombus length, thrombus density, thrombus perviousness, and CBS. We generated bivariate scatterplots and computed the correlation using the pairwise Spearman's correlation coefficient, ρ , for all occlusion locations. We evaluated the correlation strength as follows: $0.10 \leq |\rho| < 0.40$, weak correlation; $0.40 \leq |\rho| < 0.60$, moderate correlation; $|\rho| \geq 0.60$, strong correlation. In addition, we built pairwise linear regressions to further assess the association and variability between the variables. Although a smooth polynomial relationship like LOESS would be more appropriate to further assess the association and trend of the correlated variables, linear regressions provide simple models to explore the spectrum of thrombus characteristics. These analyses were performed for all thrombi combined and also per occlusion location (ICA, M1, and M2).

Clustering analysis

We used a classical, widely-used, unsupervised learning algorithm to perform the clustering: Hartigan-Wong k -means unsupervised clustering.¹⁹ For n number of features and k cluster centroids, this method aims to minimize the Euclidean distance between each point in the n -dimensional space and its closest cluster centroid k , that is, to minimize the sum of the squared error (SSE). The following thrombus characteristics were used as features: thrombus length, density, and perviousness. All variables were standardized (transformed to a mean of 0 and a standard

deviation of 1), such that their weights evenly contribute. We selected the optimal number of clusters based on three complementary techniques: the elbow method, the calculation of the silhouette coefficient, and the calculation of the Davies-Bouldin coefficient.²⁰ For the elbow method, we computed the SSE for each cluster to find the optimal k value. By definition, the SSE decreases with an increasing number of clusters. The elbow-point indicates the optimal trade-off between the SSE and the number of clusters, and is found where the decrease in the SSE becomes linear. The silhouette coefficient is a measure of cohesion within a cluster and separation between the clusters, and ranges between -1 and 1 . The higher the silhouette coefficient, the better the cohesion within the clusters and better separation from the other groups. The Davies-Bouldin coefficient is also a measure of intra-cluster similarity and inter-cluster difference. It is computed as the average ratio between within-cluster and between-cluster distances, and therefore, smaller Davies-Bouldin values indicate better clusters. For each k , we ran the algorithm for 1000 iterations with 100 different initial configurations of the cluster centroids.

Online supplemental table 2 includes definitions of association, correlation, collinearity and unsupervised clustering for additional information.

RESULTS

Our study population consisted of 934 out of 3637 patients from the MR CLEAN Registry (online supplemental figure S1). Included patients had a median age of 72 (IQR 62–80) years. Median National Institutes of Health Stroke Scale (NIHSS) score at baseline was 15 (IQR 11–20). Baseline characteristics of our study cohort and the full MR CLEAN Registry cohort can be found in online supplemental table S3. Patients in our study were more often presented directly to an EVT-capable center (ie, no interhospital transfer), and correspondingly their median onset to groin puncture time was slightly shorter (188 [IQR 139–246] min vs 195 [IQR 150–260] min, $p < 0.01$). NIHSS scores at 24–48 hours were lower in our study population (9 [IQR 3–16] vs 10 [IQR 4–17], $p = 0.02$) compared with the full MR CLEAN Registry cohort.

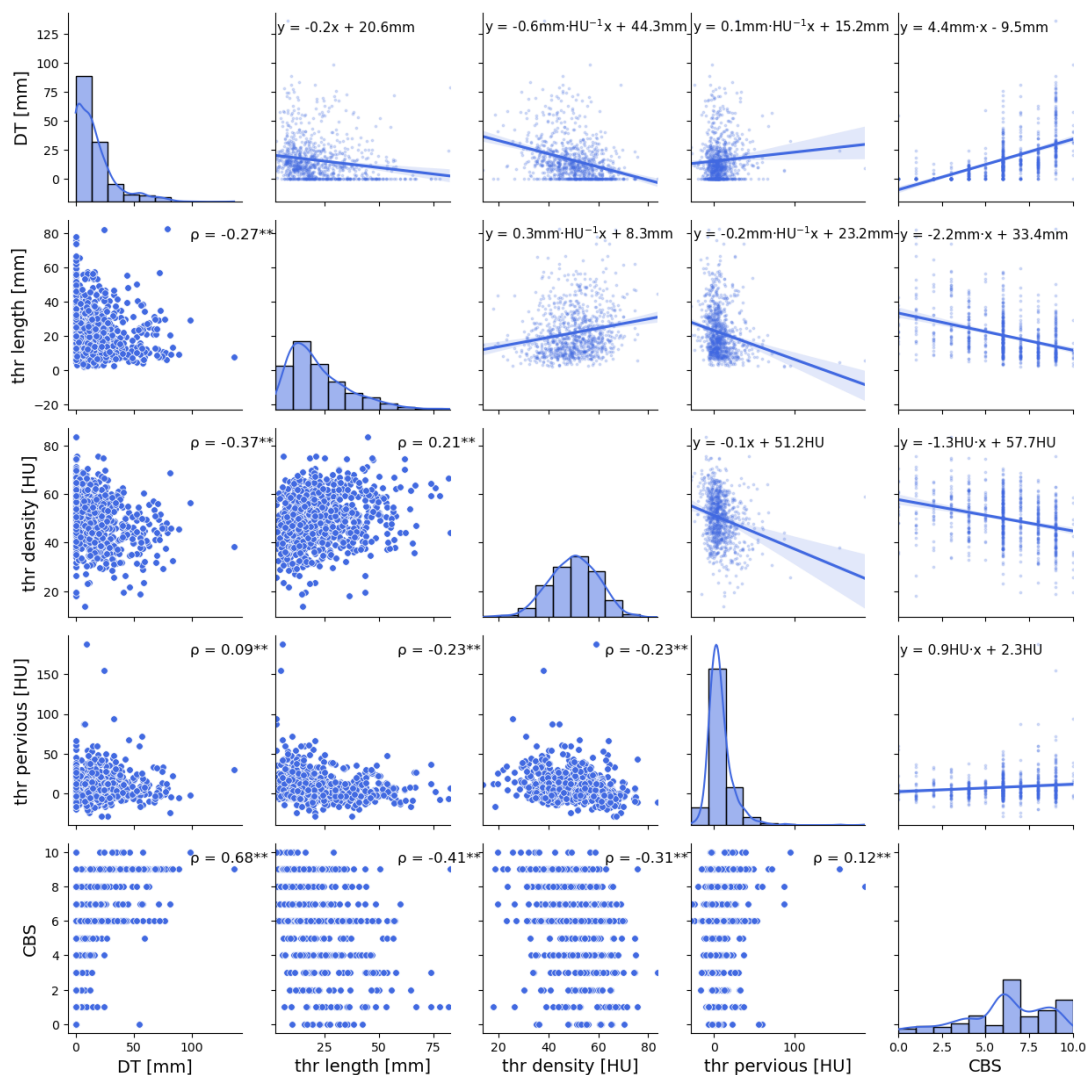


Figure 2 Thrombus imaging characteristics. The diagonal shows the histograms of DT, thrombus length, density, perviousness and CBS. The graphs below the diagonal show bivariate scatter plots, Spearman's correlation coefficient (ρ) and significance level of the correlation, where ** means $p < 0.01$. The plots above the diagonal show bivariate scatter plots with a linear fit. CBS, clot burden score; DT, distance from the internal carotid artery terminus to the thrombus; HU, Hounsfield units; mm, millimeters; thr, thrombus.

Table 1 Thrombus imaging characteristics per occlusion location (ICA, M1, and M2).

Thrombus imaging characteristics, median (IQR)	ICA, n=202	M1, n=566	M2, n=148	p-value
DT (mm)	0 (0–0)	12 (6–18)	30 (22–55)	<0.01
Thrombus length ^a (mm)	26 (17–43)	18 (12–27)	12 (9–22)	<0.01
Thrombus density (HU)	55 (49–61)	50 (44–57)	44 (38–50)	<0.01
Thrombus perviousness (HU)	3 (-3–12)	5 (-2–12)	6 (-1–18)	0.02
CBS ^b	3 (1–4)	6 (6–7)	9 (9–9)	<0.01

Missing values: ^a1, ^b191.
 CBS, clot burden score; DT, distance from ICA-terminus to the thrombus; HU, Hounsfield units; ICA, internal carotid artery; IQR, interquartile range; M1, middle cerebral artery M1 segment; M2, middle cerebral artery M2 segment; mm, millimeters.

Summary statistics of the thrombus imaging characteristics can be found in online supplemental table S4. The most common occlusion location was the M1 segment (61%), followed by the ICA (22%), and the M2 (16%). The distributions and correlations of thrombus imaging characteristics are visualized in figure 2 and online supplemental table S5. We found that higher CBS was strongly correlated with longer DT ($\rho=0.68$, $p<0.01$), and moderately correlated with shorter thrombus length ($\rho=-0.41$, $p<0.01$). These correlations are individually visualized in online supplemental figure S2. The other correlations, although statistically significant, were weak ($|\rho|<0.40$).

Subgroup analysis: occlusion location

A between-group comparison of the thrombus characteristics per occlusion location can be found in table 1. Thrombus characteristics significantly differed per occlusion location. Thrombi located in the ICA were longer, denser, and less pervious compared with M1 and M2 thrombi. Similarly, M1 thrombi were longer, denser, and less pervious compared with M2 thrombi. More details on the significance level of pairwise comparisons can be found in online supplemental table S6.

Similar to figure 2, the distribution and correlations of these characteristics, color-coded by occlusion location, can be found in online supplemental figure S3 and online supplemental table S7. All found correlations were weak ($|\rho|<0.40$).

Clustering analysis

Both the elbow method and silhouette coefficients indicated that the optimal number of clusters lay around 4 and 6 (figure 3A,B). The low silhouette coefficients, with values all under 0.30, indicate that the clusters cannot be clearly discerned. Davies-Bouldin coefficients indicated that the optimal number of clusters was in the range of 4–10, with two local minima at $k=6$ and $k=9$ (figure 3C). Based on the three methods, we chose to continue our analysis with four clusters. A 3D visualization of the thrombus features of the four clusters is displayed in figure 3D, next to a similar visualization clustered by occlusion location (figure 3E). The cluster centroids can be found in online supplemental table S8. The distribution characteristics for each cluster are also summarized in online supplemental table S8. Cluster 2 consists of longer, denser, and less pervious thrombi, while cluster four includes shorter and less dense thrombi, with higher

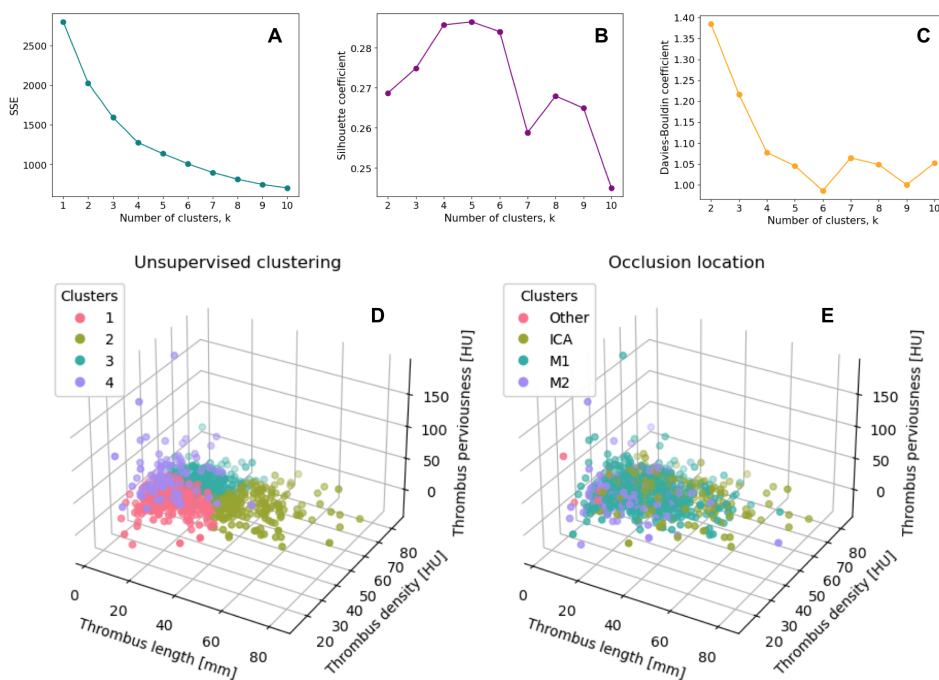


Figure 3 Finding the optimal number of clusters. (A) SSE as a function of the number of clusters. The elbow point is found around 4–6, after which the decrease in SSE becomes linear. (B) Silhouette coefficients as a function of the number of clusters. The highest (and therefore, preferred) silhouette coefficients are found around 4–6. (C) Davies-Bouldin coefficients as a function of the number of clusters. Lower coefficients indicate better clustering. From $k=4$ onwards, Davies-Bouldin coefficients showed similar values, with two local minima at $k=6$ and $k=9$. (D) 3D visualization of thrombus imaging features clustered by 4-means unsupervised clustering. (E) 3D visualization of thrombus imaging features clustered by occlusion location. SSE, sum of the squared error.

perviousness values. Cluster 1 and 3 reflect a mixture of both: shorter thrombi, less pervious, with lower and higher densities, respectively.

DISCUSSION

In our analysis of thrombus imaging characteristics in EVT-treated acute ischemic stroke patients, we found that all assessed thrombus imaging characteristics were significantly intercorrelated. Higher CBS was strongly correlated with longer DT and was moderately correlated with shorter thrombus length. These correlations became weak when stratifying per occlusion location. Longer, denser, and less pervious thrombi were mostly found in more proximal occlusion locations. Our clustering analysis suggests that thrombus imaging characteristics form a continuum, and that no typical thrombus types can be identified similar to the TOAST classification.

To our knowledge, this is the largest dataset of thrombus imaging characteristics of acute ischemic stroke patients. Previous studies have focused on the association of thrombus imaging characteristics with treatment and patient outcome, and have partially assessed associations between thrombus characteristics.²¹ Other studies have related thrombus imaging characteristics to their histopathology,²² etiology,²³ and biomechanical characteristics.²⁴ The statistical models used to study these associations between thrombus characteristics and outcomes do not commonly account for the collinearity between these characteristics. This collinearity can lead to unreliable or unrepresentative model results, or a reduction in statistical significance. In a multivariable regression, collinearity hinders the relationship of the independent variable with the dependent one, since the independent variables change simultaneously. This implies that, although the overall predictions are unaffected, the regression coefficients, corresponding standard errors and significance can be 'wrongly' estimated.²⁵ Moreover, in case of collinearity, the predictive value can be assigned to one of the parameters whereas the relation with outcome could also be affected by another collinear parameter.

Although the results of our study do not imply a direct change in current clinical practice, they illustrate the importance of studying collinearity when building predictive models – which are used to draw conclusions of treatment effects and outcomes consequence of current clinical practice.

In our study, we found that the correlations between most thrombus characteristics are significant but weak. This means there is still a large variety of other thrombus characteristics that are given a single thrombus variable. This was also reflected in our clustering analysis. When clustering by occlusion location, we found that even if the distributions of length, density, and perviousness significantly differed between the groups, some overlap still existed. With unsupervised clustering, thrombi were classified based on their length, density and perviousness. However, we did not observe clearly distinct thrombus archetypes, but rather found that thrombus imaging characteristics form a continuum which hinders their clustering.

In current clinical practice, occlusion location (among other baseline characteristics) determines EVT eligibility. Stroke guidelines indicate that patients with proximal occlusions are eligible for EVT,⁴ whereas the eligibility of (more distal) medium vessel occlusion stroke is currently being researched. The definition of, eg, M1, M2, and M3 segments can be challenging and highly dependent on the observer.²⁶ DT better defines the distality of the occlusion.²⁷ However, DT does not take anatomical landmarks such as bifurcations into account (which are used to determine occlusion location) and can be critical for the chance of

EVT success. In our results, we saw that for the same DT, the patient could either have an M1 or M2 occlusion. Unfortunately, the number of M3 occlusions in our database was insufficient to further study this relation.

We found that thrombus imaging characteristics significantly differ per occlusion location and that some of these characteristics are moderately and strongly correlated with CBS. Currently, the manual assessment of thrombus characteristics is time-consuming and requires thin-slice CT imaging.¹ On the other hand, assessment of occlusion location and CBS is commonly done in clinical practice/studies, is less time-consuming than manual thrombus measurements, and does not require thin-slice images.¹⁶ Correlations between occlusion-location/CBS and DT/length/density/perviousness offer the possibility of uncomplicated characterization of the thrombus, which could ultimately lead to a reduction of time-consuming assessments. With the enhanced development of automated measurements and thrombus segmentations, quantitative characterization of the thrombus might be optimized.²⁸

Finally, in-silico models that simulate stroke onset, treatment, and outcome require, among others, an accurate and quantitative representation of the thrombus.²⁹ Combining thrombus imaging, histological and biomechanical properties can help to capture the whole spectrum of thrombus characteristics needed for such in-silico modeling. Once the correlations between these characteristics are well-established, typical thrombi can be sampled from such distributions and can be used to study treatment success in different scenarios. Our study contributes to the quantification of such correlations.

Limitations

Our study had some limitations. We only included patients with available thin-slice imaging, which led to a high exclusion rate, and therefore, to selection bias (less intra-hospital transfers, shorter onset-to-groin times, and lower NIHSS scores at 24–48 hours compared with the whole MR CLEAN Registry population).

Occlusion location and thrombus measurements were not assessed by the same group of observers (MR CLEAN Registry core lab for occlusion location and CBS vs trained observers for DT, thrombus length, density, and perviousness) nor in the same CT modality (CTA only by MR CLEAN Registry core lab vs NCCT and CTA by trained observers). This might create some incongruencies between occlusion location and DT, especially close to the ICA-terminus. For example: on CTA, lack of contrast in distal ICA-terminus can be scored as an M1 occlusion (expected DT ≠ 0), while when looking at both NCCT and CTA simultaneously, it is scored as an ICA-T occlusion (expected DT = 0). In addition, because occlusion location was defined as the most proximal occluded (intracranial) vessel segment on CTA, patients with multiple occlusions might have been classified as one occlusion location, while the thrombus characteristics were measured in a more distal occluded segment where the NCCT hyperdense artery sign was visible.

The measured thrombus length was based on the absence of contrast as seen in single-phase CTA, supported by the NCCT hyperdense artery sign, if present. Measurements on multiphase CTA might more accurately capture the true thrombus length.³⁰

CONCLUSION

Thrombus imaging characteristics are significantly intercorrelated and some of them show moderate and strong correlations. The collinearity of these thrombus characteristics should be considered when multiple thrombus characteristics are

included in future predictive modeling studies. The observed weak correlations reflect that there is still a large variety of characteristics for a given single thrombus variable: thrombus imaging characteristics form a continuum, which does not allow for grouping of typical thrombus archetypes based on these features. The continuous spectrum and specific variability of thrombus imaging characteristics should be considered when developing novel treatment approaches.

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Patient consent for publication Not applicable.

Ethics approval These patients are part of the MR CLEAN Registry, a multicenter prospective observational registry of all patients undergoing EVT for acute ischemic stroke in the Netherlands. This registry was approved by the central medical ethics committee of the Erasmus Medical Center Rotterdam, which served as the review board of all participating centers (MEC-2014-235). The requirement for written informed consent was waived, but all patients or legal representatives were provided with oral and written information on the registry, and had the opportunity to withdraw consent to use their data via an opt-out form, conforming to the European Union General Data Protection Regulation.

Provenance and peer review Not commissioned; externally peer reviewed.

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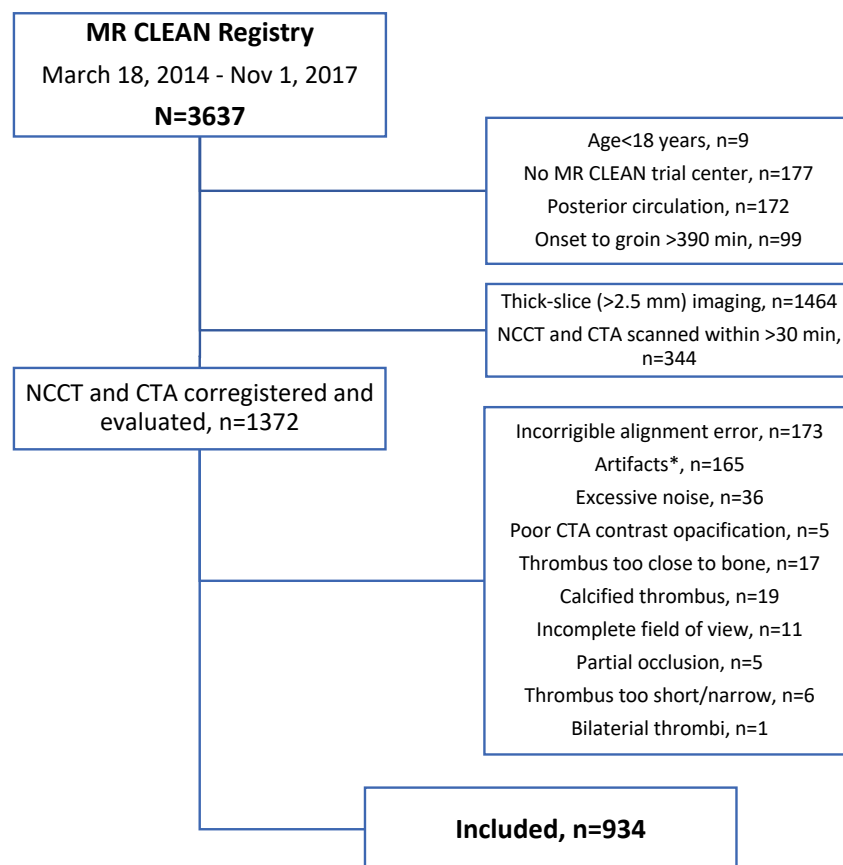
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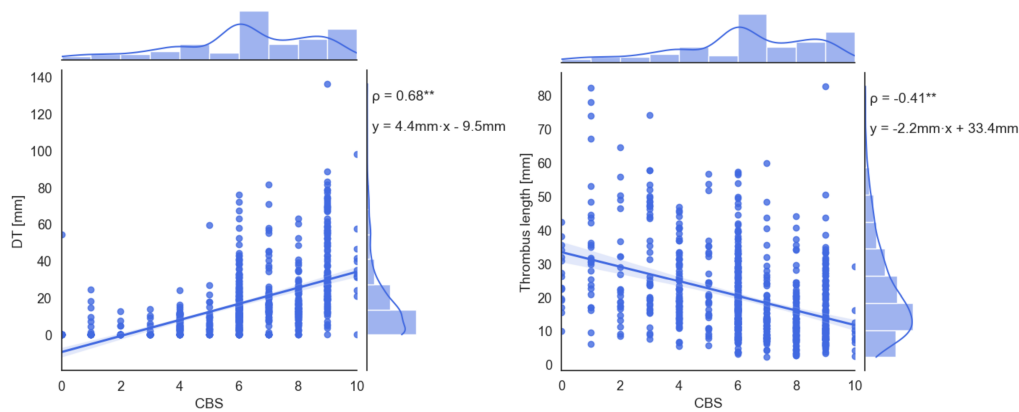
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SUPPLEMENTAL MATERIAL

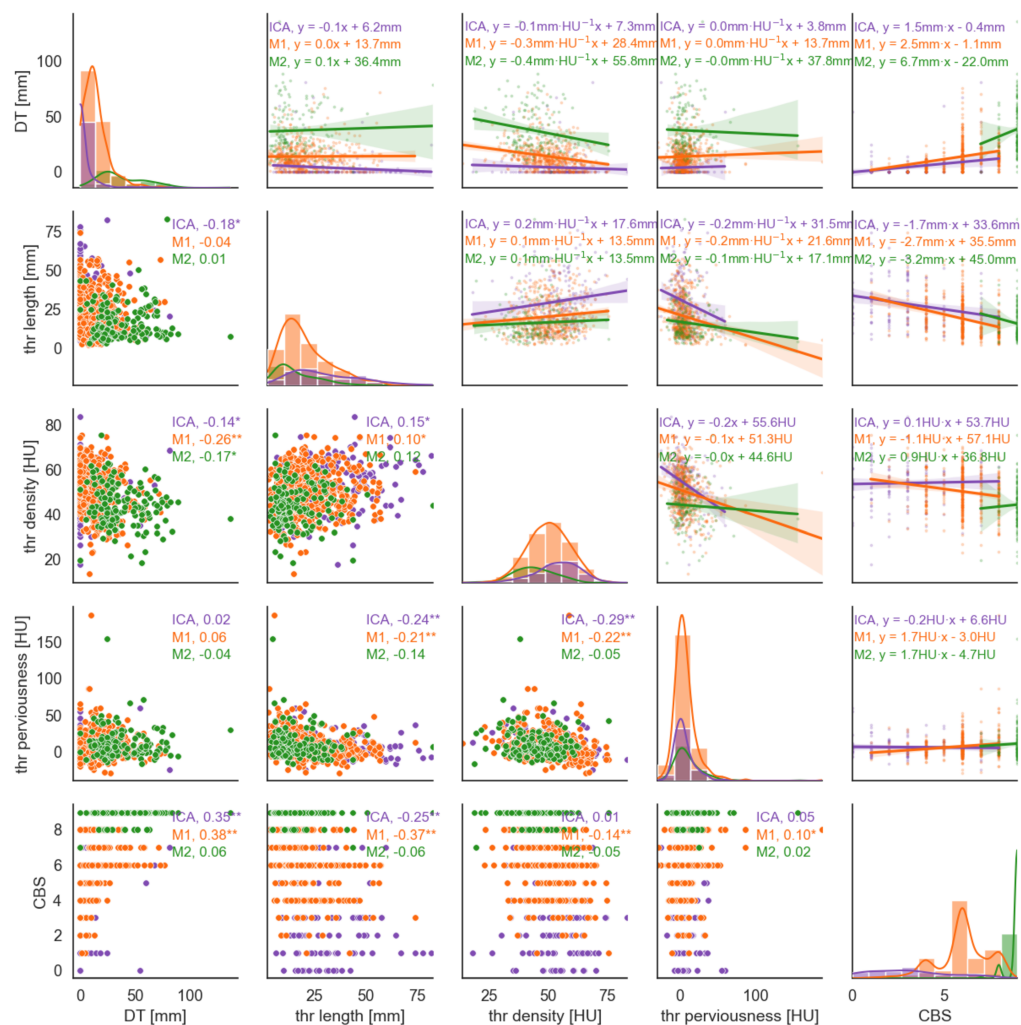
FIGURES



Supplemental Figure S1. Patient inclusion flowchart. *movement, metal and beam hardening artefacts. CTA, computed tomography angiography; MR CLEAN, Multicenter Randomized CLinical trial of Endovascular treatment for Acute ischemic stroke in the Netherlands; NCCT, non-contrast computed tomography.



Supplemental Figure S2. Bivariate scatter plots, individual distributions and Spearman's correlation coefficient of thrombus characteristics with a moderate ($0.40 \leq |\rho| < 0.60$) or strong ($|\rho| \geq 0.60$) correlation. A) DT [mm] against CBS displaying a strong correlation $\rho=0.68$ with $p<0.01$. B) Thrombus length [mm] against CBS displaying a moderate correlation $\rho=-0.41$ with $p<0.01$. Each graph also includes a linear fit to illustrate the relationship between the variables. *CBS*, clot burden score; *DT*, distance from the internal carotid artery terminus to the thrombus; *mm*, millimeters; *thr*, thrombus.



Supplemental Figure S3. Thrombus imaging characteristics for ICA, M1 and M2 occlusions. The diagonal shows the histograms of DT, thrombus length, density, perviousness and CBS. The graphs below the diagonal show the bivariate scatter plots, Spearman's correlation coefficient (ρ) and significance level of the correlation, where * indicates $p < 0.05$ and ** indicates $p < 0.01$. The graphs above the diagonal show bivariate scatter plots with a linear fit. All graphs are color-coded by occlusion location: ICA occlusions in purple, M1 occlusions in orange, and M2 occlusions in green. CBS, clot burden score; DT, distance from the internal carotid artery terminus to the thrombus; HU, Hounsfield units; mm, millimeters; thr, thrombus.

TABLES**Supplemental Table S1. Image acquisition parameters.**

Scanner manufacturer	Scanner model		no./total (%)		
GE MEDICAL SYSTEMS	BrightSpeed		3/935 (0)		
	Discovery CT		17/935 (2)		
	LightSpeed 16		1/935 (0)		
	LightSpeed VCT		12/935 (1)		
	Optima CT660		15/935 (2)		
	Revolution CT		2/935 (0)		
PHILIPS	Brilliance 16		2/935 (0)		
	Brilliance 40		8/935 (1)		
	Brilliance 64		18/935 (2)		
	iCT 256		204/935 (22)		
	Ingenuity CT		20/935 (2)		
	IQon – Spectral CT		2/935 (0)		
	Mx8000 IDT 16		1/935 (0)		
SIEMENS	Sensation 16		1/935 (0)		
	Sensation 64		83/935 (9)		
	SOMATOM Def. AS		45/935 (5)		
	SOMATOM Def. AS+		20/935 (2)		
	SOMATOM Def. Edge		80/935 (9)		
	SOMATOM Def. Flash		182/935 (20)		
	SOMATOM Force		62/935 (7)		
TOSHIBA	Aquilion		31/935 (3)		
	Aquilion ONE		112/935 (12)		
	Aquilion PRIME		14/935 (1)		
Imaging protocol	NCCT		CTA		
Convolution kernel	EC	Hr38s	A	FC03	IMR1
	FC21	Hr40s	B	FC05	J30f
	FC26	Hr59h	B20f	FC08	J40f
	FC30	IMR1	B26f	FC12	J45s
	FC41	J30f	B30f	FC41	Q30f
	FC64	J30s	B31f	FC43	Q33f
	H20f	J40f	B40f	H20f	Q40f
	H30f	J40s	B46f	H30f	Qr40d
	H30s	J45s	Bv36d	H41f	SOFT
	H31f	J70h	Bv40d	H70h	STANDARD
	H31s	SE	Bv44d	Hf38d	UA

	H41s	SOFT	C	I26f	UB
	H60f	STANDARD	D	I30f	UC
	H60s	UA	D24f	I31f	
	H70h	UB	D45f	I40f	
	Hf38s	UC	DETAIL	I46f	
Imaging parameters – median (IQR)	NCCT		CTA		
Peak Voltage [kV]	120 (100-120)		100 (100-120)		
Tube3 current [mA]	240 (175-320) ^α		314 (188-446) ^β		
Exposure [mA·s]	301 (240-394) ^γ		156 (73 – 221) ^δ		
Exposure time [s]	1000 (750-1049) ^ε		500 (381-500) ^ζ		

Missing values: ^α9, ^β3, ^γ9, ^δ5, ^ε18, and ^ζ11.

CTA, computed tomography angiography; Def, definition; IQR, interquartile range; kV, kilovoltage; mA, milliamperes; NCCT, non-contrast computed tomography; s, seconds.

Supplemental Table S2: Glossary.

Word	Definition
Association	(between two variables) defines the relationship or dependency between two variables. The associations can be linear, logarithmic, exponential, etc. Association does not imply causation.
Correlation	(between two variables) is a specific type of association which implies that the relation between two variables follows a monotonically decreasing or increasing function. If two variables are correlated, they are associated, but the opposite is not true: two associated variables do not have to be correlated.
Collinearity	(between two variables) the two variables are linearly correlated, i.e.: the monotonically decreasing/increasing function is a linear function.
Unsupervised clustering	machine learning algorithm used to group (i.e., cluster) items based on specific item's features without any prior information/rules.

Supplemental Table S3. Baseline characteristics of the MR CLEAN Registry population (N=3637) and our study population (n=934).

Baseline characteristics	MR CLEAN Registry, N=3279	Current study population, n=934	p-value
Age [years] – median (IQR)	72 (61-80)	72 (62-80)	0.89
Sex, female – no./total (%)	1696/3279 (52)	489/934 (52)	0.73
Medical history – no./total (%)			
Previous stroke	546/3252 (17)	158/925 (17)	0.84
Diabetes mellitus	532/3255 (16)	153/925 (17)	0.89
Hypertension	1688/3213 (53)	447/913 (49)	0.06
Atrial fibrillation	772/3236 (24)	236/920 (26)	0.26
Pre-stroke mRS – no./total (%)			0.86
0	2170/3207 (68)	619/913 (68)	
1	424/3207 (13)	112/913 (12)	
2	241/3207 (7)	72/913 (8)	
≥3	372/3207 (12)	110/913 (12)	
Systolic blood pressure ^α [mmHg] – median (IQR)	150 (131-165)	149 (132-165)	0.50
Diastolic blood pressure ^β [mmHg] – median (IQR)	80 (71-91)	80 (71-90)	0.84
NIHSS _{BL} ^γ – median (IQR)	16 (11-20)	15 (11-20)	0.87
ASPECTS _{BL} ^δ – median (IQR)	9 (7-10)	9 (8-10)	0.38
CS – no./total (%)			0.09
0	187/3072 (6)	61/914 (7)	
1	1100/3072 (36)	341/914 (37)	
2	1190/3072 (39)	369/914 (40)	
3	595/3072 (19)	143/914 (16)	
Treatment and workflow			
IVT – no./total (%)	2450/3275 (75)	680/933 (73)	0.14
Transferred patients – no./total (%)	1783/3278 (54)	343/933 (37)	<0.01
Stroke onset* to first hospital presentation ^e [min] – median (IQR)	57 (39-105)	60 (40-108)	0.23
Hospital presentation to IVT ^ζ [min] – median (IQR)	24 (18-33)	23 (17-31)	0.13
Stroke onset* to groin puncture ^η [min] – median (IQR)	195 (150-260)	188 (139-246)	<0.01
Procedure time ^θ – median (IQR)	59 (38-84)	55 (37-81)	0.13
Retrieval attempts – median (IQR)			0.18
1	954/3279 ()	305/934 ()	
2	575/3279 ()	168/934 ()	
3	398/3279 ()	96/934 ()	
≥4	495/3279 ()	130/934 ()	

eTICI 2B-3 – no./total (%)	1965/3191 (62)	588/912 (65)	0.11
Outcome			
NIHSS FU [†] – median (IQR)	10 (4-17)	9 (3-16)	0.02
mRS ^κ – median (IQR)	3 (2-6)	3 (2-6)	0.18
siCH – no./total (%)	193/3279 (6)	51/934 (6)	0.62

Missing values: ^α110, ^β120, ^γ71, ^δ112, ^ε770, ^ζ1746, ^η21, ^θ394, ^ι405 and ^κ278.

*Time of stroke onset was defined as the time of witnessed symptom onset or, if unknown, as the time the patient was last seen well.

ΔNIHSS, change in NIHSS score at 24-48h compared to baseline; ASPECTS, Alberta Stroke Programme Early CT Score; BL, baseline; CBS, clot burden score; CS, collateral score; CT, computed tomography; ENT, embolization to new (previously unaffected) vascular territory; eTICI, expanded thrombolysis in cerebral infarction; EVT, endovascular treatment; IQR, interquartile range; IVT, intravenous treatment with alteplase; mRS, modified Rankin Scale; NIHSS, National Institute of Health Stroke Scale; siCH, symptomatic intracranial hemorrhage.

Supplemental Table S4. Thrombus imaging characteristics.

Thrombus imaging characteristics	Study population, n=934
Occlusion location – no./total (%)	
ICA	202/925(22)
M1	566/925 (61)
M2	148/925 (16)
Other (M3, anterior)	9/925 (1)
DT [mm] – median (IQR)	11 (2-22)
Thrombus length ^α [mm] – median (IQR)	18 (11-29)
Thrombus density [HU]– median (IQR)	50 (44-57)
Thrombus perviousness [HU] – median (IQR)	5 (-2-13)
CBS ^β – median (IQR)	6 (5-8)

Missing values: ^α1, ^β194,

CBS, clot burden score; DT, distance from ICA-T to the thrombus; HU, Hounsfield units; ICA-T, internal carotid artery T-junction; IQR, interquartile range; mm, millimeters.

Supplemental Table S5. Correlation and regression analyses. Below the diagonal: Spearman's correlation coefficient (ρ) and significance level of the correlation. Above the diagonal: linear regression coefficients

Thrombus imaging chars.		DT [mm]	Thrombus length ^α [mm]	Thrombus density [HU]	Thrombus perviousness [HU]	CBS ^β
		Regression models of the form: $y = ax + b$, where (a, b) =				
DT [mm]			(-0.2, 20.6)	(-0.6, 44.3)	(0.1, 15.2)	(4.4, -9.5)
Thrombus length ^α [mm]	$\rho =$	-0.27**		(0.3, 8.3)	(-0.2, 23.2)	(-2.2, 33.4)
Thrombus density [HU]		-0.37**	0.21**		(-0.1, 51.2)	(-1.3, 57.7)
Thrombus perviousness [HU]		0.09**	-0.23**	-0.23**		(0.9, 2.3)
CBS ^β		0.68**	-0.41**	-0.32**	0.12**	

**significance level of Spearman's correlation coefficient is $p < 0.01$.

Missing values: ^α1, ^β194.

CBS, clot burden score; DT, distance from ICA-T to the thrombus; HU, Hounsfield units; ICA-T, internal carotid artery T-junction; IQR, interquartile range; mm, millimeters.

Supplemental Table S6. Thrombus imaging characteristics per occlusion location (ICA, M1, and M2): distance from ICA-terminus to the thrombus, thrombus length, thrombus density, thrombus perviousness and clot burden score. The diagonal shows the median with IQR. The lower diagonal shows the p-value of the corresponding pairwise Mann-Whitney-U test with Bonferroni corrections for multiple tests.

DT [mm] – median (IQR)			
Occlusion location	ICA, n=202	M1, n=566	M2, n=148
ICA, n=202	0 (0-0)		
M1, n=566	p<0.01	12 (6-18)	
M2, n=148	p<0.01	p<0.01	30 (22-55)
Thrombus length^α [mm] – median (IQR)			
Occlusion location	ICA, n=202	M1, n=566	M2, n=148
ICA, n=202	26 (17-43)		
M1, n=566	p<0.01	18 (12-27)	
M2, n=148	p<0.01	p<0.01	12 (9-22)
Thrombus density [HU] – median (IQR)			
Occlusion location	ICA, n=202	M1, n=566	M2, n=148
ICA, n=202	55 (49-61)		
M1, n=566	p<0.01	50 (44-57)	
M2, n=148	p<0.01	p<0.01	43.5 (38-50)
Thrombus perviousness [HU] – median (IQR)			
Occlusion location	ICA, n=202	M1, n=566	M2, n=148
ICA, n=202	3 (-3-12)		
M1, n=566	p=0.40	5 (-2-12)	
M2, n=148	p=0.02	p=0.18	6 (-1-18)
CBS^β – median (IQR)			
Occlusion location	ICA, n=202	M1, n=566	M2, n=148
ICA, n=202	3 (1-4)		
M1, n=566	p<0.01	6 (6-7)	
M2, n=148	p<0.01	p<0.01	9 (9-9)

Missing values: ^α1, ^β191,

CBS, clot burden score; DT, distance from ICA-T to the thrombus; HU, Hounsfield units; IQR, interquartile range.

Supplemental Table S7. Correlation and regression analyses. Below the diagonal: Spearman's correlation coefficient (ρ) and significance level of the correlation. Above the diagonal: linear regression coefficients.

Thr. ima. chars.	DT [mm]	Thr. length ^α [mm]	Thr. density [HU]	Thr. pervious. [HU]	CBS ^β	Occl. loc.
	Regression models of the form: $y = ax + b$, where (a, b) =					
DT [mm]		(-0.1, 6.2)	(-0.1, 7.3)	(0.0, 3.8)	(1.5, -0.4)	ICA
		(0.0, 13.7)	(-0.3, 28.4)	(0.0, 13.7)	(2.5, -1.1)	M1
		(0.1, 36.4)	(-0.4, 55.8)	(0.0, 37.8)	(6.8, -22.0)	M2
Thr. length ^α [mm]	-0.18*		(0.2, 17.6)	(-0.2, 31.5)	(-1.7, 33.6)	ICA
	-0.04		(0.1, 13.5)	(-0.2, 21.6)	(-2.7, 35.5)	M1
	0.01		(0.1, 13.5)	(-0.1, 17.1)	(-3.2, 45.0)	M2
Thr. density [HU]	-0.14*	0.15*		(-0.2, 55.6)	(0.1, 53.7)	ICA
	-0.26**	0.10*		(-0.1, 51.3)	(-1.1, 57.1)	M1
	-0.17*	0.12		(-0.0, 44.6)	(0.9, 36.8)	M2
	ρ					
Thr. pervious. [HU]	0.02	-0.24**	-0.29**		(-0.2, 6.6)	ICA
	0.06	-0.21**	-0.22**		(1.7, -3.0)	M1
	-0.04	-0.14	-0.05		(1.7, -4.7)	M2
CBS ^β	0.35**	-0.25**	0.01	0.05		ICA
	0.38**	-0.37**	-0.14**	0.10*		M1
	0.06	-0.06	-0.05	0.02		M2

Significance level of Spearman's correlation coefficient, * $p < 0.05$, ** $p < 0.01$

Missing values: ^α1, ^β194.

CBS, clot burden score; DT, distance from ICA-T to the thrombus; HU, Hounsfield units; ICA-T, internal carotid artery T-junction; mm, millimeters; thr, thrombus.

Supplemental Table S8. Cluster centroids and summary statistics for each cluster.

Cluster centroids	Cluster 1, n=313	Cluster 2, n=185	Cluster 3, n=347	Cluster 4, n=88
Thrombus length [mm]	15	44	18	14
Thrombus density [HU]	42	53	58	43
Thrombus perviousness [HU]	6	2	3	42
Median (IQR)	Cluster 1, n=313	Cluster 2, n=185	Cluster 3, n=347	Cluster 4, n=88
Thrombus length [mm]	14 (9-20)	42 (36-49)	18 (12-23)	12 (8-18)
Thrombus density [HU]	43 (39-47)	52 (47-59)	57 (54-61)	44 (37-50)
Thrombus perviousness [HU]	5 (0-13)	2 (-5-7)	3 (-2-9)	36 (28-46)

HU, Hounsfield units; IQR, interquartile range; mm, millimeters.

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13	Other financial or non-financial interests	<input checked="" type="checkbox"/> None <table border="1" style="width: 100%; border-collapse: collapse;"> <tr><td style="height: 20px;"> </td><td style="width: 50%;"> </td></tr> <tr><td style="height: 20px;"> </td><td> </td></tr> <tr><td style="height: 20px;"> </td><td> </td></tr> </table>							
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ICMJE DISCLOSURE FORM

Date: 5/2/2022

Your Name: Charles BLM Majoie

Manuscript Title: Thrombus imaging characteristics: similarities and interdependence within acute ischemic stroke

Manuscript Number (if known): [Click or tap here to enter text.](#)

In the interest of transparency, we ask you to disclose all relationships/activities/interests listed below that are related to the content of your manuscript. "Related" means any relation with for-profit or not-for-profit third parties whose interests may be affected by the content of the manuscript. Disclosure represents a commitment to transparency and does not necessarily indicate a bias. If you are in doubt about whether to list a relationship/activity/interest, it is preferable that you do so.

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5	Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events	<input checked="" type="checkbox"/> None <table border="1" style="width: 100%; height: 30px;"> <tr><td> </td><td> </td></tr> <tr><td> </td><td> </td></tr> <tr><td> </td><td> </td></tr> </table>							
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8	Patents planned, issued or pending	<input checked="" type="checkbox"/> None <table border="1" style="width: 100%; height: 30px;"> <tr><td> </td><td> </td></tr> <tr><td> </td><td> </td></tr> <tr><td> </td><td> </td></tr> </table>							
9	Participation on a Data Safety Monitoring Board or Advisory Board	<input checked="" type="checkbox"/> None <table border="1" style="width: 100%; height: 30px;"> <tr><td> </td><td> </td></tr> <tr><td> </td><td> </td></tr> <tr><td> </td><td> </td></tr> </table>							
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12	Receipt of equipment, materials, drugs, medical writing, gifts or other services	<input checked="" type="checkbox"/> None	
13	Other financial or non-financial interests	<input checked="" type="checkbox"/> None	
<p>Please place an "X" next to the following statement to indicate your agreement:</p> <p><input checked="" type="checkbox"/> I certify that I have answered every question and have not altered the wording of any of the questions on this form.</p>			

ICMJE DISCLOSURE FORM

Date: 5/2/2022

Your Name: Diederik WJ Dippel

Manuscript Title: Thrombus imaging characteristics: similarities and interdependence within acute ischemic stroke

Manuscript Number (if known): [Click or tap here to enter text.](#)

In the interest of transparency, we ask you to disclose all relationships/activities/interests listed below that are related to the content of your manuscript. "Related" means any relation with for-profit or not-for-profit third parties whose interests may be affected by the content of the manuscript. Disclosure represents a commitment to transparency and does not necessarily indicate a bias. If you are in doubt about whether to list a relationship/activity/interest, it is preferable that you do so.

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12	Receipt of equipment, materials, drugs, medical writing, gifts or other services	<input checked="" type="checkbox"/> None	
13	Other financial or non-financial interests	<input checked="" type="checkbox"/> None	
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ICMJE DISCLOSURE FORM

Date: 5/2/2022

Your Name: Ed van Bavel

Manuscript Title: Thrombus imaging characteristics: similarities and interdependence within acute ischemic stroke

Manuscript Number (if known): [Click or tap here to enter text.](#)

In the interest of transparency, we ask you to disclose all relationships/activities/interests listed below that are related to the content of your manuscript. "Related" means any relation with for-profit or not-for-profit third parties whose interests may be affected by the content of the manuscript. Disclosure represents a commitment to transparency and does not necessarily indicate a bias. If you are in doubt about whether to list a relationship/activity/interest, it is preferable that you do so.

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ICMJE DISCLOSURE FORM

Date: 5/2/2022

Your Name: Henk A. Marquering

Manuscript Title: Thrombus imaging characteristics: similarities and interdependence within acute ischemic stroke

Manuscript Number (if known): [Click or tap here to enter text.](#)

In the interest of transparency, we ask you to disclose all relationships/activities/interests listed below that are related to the content of your manuscript. "Related" means any relation with for-profit or not-for-profit third parties whose interests may be affected by the content of the manuscript. Disclosure represents a commitment to transparency and does not necessarily indicate a bias. If you are in doubt about whether to list a relationship/activity/interest, it is preferable that you do so.

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		H.A. Marquering reports co-founder and shareholder of Nicolab, a company that focuses on the use of artificial intelligence for medical image analysis.	
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ICMJE DISCLOSURE FORM

Date: 5/2/2022

Your Name: Heitor CBR Alves

Manuscript Title: Thrombus imaging characteristics: similarities and interdependence within acute ischemic stroke

Manuscript Number (if known): [Click or tap here to enter text.](#)

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ICMJE DISCLOSURE FORM

Date: 5/2/2022

Your Name: Josje Brouwer

Manuscript Title: Thrombus imaging characteristics: similarities and interdependence within acute ischemic stroke

Manuscript Number (if known): [Click or tap here to enter text.](#)

In the interest of transparency, we ask you to disclose all relationships/activities/interests listed below that are related to the content of your manuscript. "Related" means any relation with for-profit or not-for-profit third parties whose interests may be affected by the content of the manuscript. Disclosure represents a commitment to transparency and does not necessarily indicate a bias. If you are in doubt about whether to list a relationship/activity/interest, it is preferable that you do so.

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ICMJE DISCLOSURE FORM

Date: 5/2/2022

Your Name: Jan W. Hoving

Manuscript Title: Thrombus imaging characteristics: similarities and interdependence within acute ischemic stroke

Manuscript Number (if known): [Click or tap here to enter text.](#)

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ICMJE DISCLOSURE FORM

Date: 5/2/2022

Your Name: Katinka R. Kranendonk

Manuscript Title: Thrombus imaging characteristics: similarities and interdependence within acute ischemic stroke

Manuscript Number (if known): [Click or tap here to enter text.](#)

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ICMJE DISCLOSURE FORM

Date: 5/2/2022

Your Name: Ludo FM Beenen

Manuscript Title: Thrombus imaging characteristics: similarities and interdependence within acute ischemic stroke

Manuscript Number (if known): [Click or tap here to enter text.](#)

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Manuscript Title: Thrombus imaging characteristics: similarities and interdependence within acute ischemic stroke

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Date: 5/2/2022

Your Name: Nerea Arrarte Terreros

Manuscript Title: Thrombus imaging characteristics: similarities and interdependence within acute ischemic stroke

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Manuscript Title: Thrombus imaging characteristics: similarities and interdependence within acute ischemic stroke

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Date: 5/2/2022

Your Name: Wim H van Zwam

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