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Review

Spatial heterogeneity of occlusive thrombus in acute ischemic stroke: A systematic review

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ABSTRACT

Following the advent of mechanical thrombectomy, occlusive clots in ischemic stroke have been amply characterized using conventional histopathology. Many studies have investigated the compositional variability of thrombi and the consequences of thrombus composition on treatment response. More recent evidence has emerged about the spatial heterogeneity of the clot or the preferential distribution of its components and compact nature. Here we review this emerging body of evidence, discuss its potential clinical implications, and propose the development of adequate characterization techniques.

Introduction

Despite the development of new treatments, ischemic stroke features high mortality rates[1]. The treatment of acute ischemic stroke aims to re-establish blood flow by disintegrating or mechanically removing the offending blood clot. With the advent of mechanical thrombectomy, occlusive clots, also known as thrombi, have been subjected to histological evaluation. This fact sparked numerous research efforts that amply characterized the histological components of the clot and explored their associations with the clinical aspects. Numerous recent reviews reported correlations between clot composition and radiological findings, stroke etiology, and treatment outcomes[2–7]. Clots retrieved from acute ischemic stroke patients have a heterogeneous composition that has been described in terms of the diversity of its components, including fibrin morphologies and various contents of cellular components, which frequently involve red blood cells (RBC), white blood cells (WBC), and platelets; protein expression; or the presence of extracellular DNA. It has been proposed that thrombogenic mechanisms yield a core-shell structure, which is important for designing therapeutic solutions[8]. Evidence has been drawn from clots retrieved from ischemic stroke patients regarding the spatial distribution of thrombus components and variations in thrombus compactness. In this systematic review, we aimed to identify the body of evidence regarding the spatial heterogeneity of ischemic stroke thrombi, significance of thrombus heterogeneity in therapeutic approaches, limitations and advantages of various investigative techniques for characterizing and quantifying thrombus spatial heterogeneity; and propose novel characterization methods.

Methods

This systematic review was performed of the PubMed database according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines to identify all peer-reviewed articles published in English between January 1, 2017, and November 16, 2022, using combinations of the following predetermined search terms: “stroke,” “clot heterogeneity,” “clot microscopy,” “clot microstructure,” “intravital contraction,” “clot retraction,” “ischemic clot ex vivo,” “clot analysis,” “thrombus imaging ex vivo,” “thrombus structure and recanalization,” “thrombus and MRI,” and “thrombus and CT.” Studies were included if they were original articles or case reports describing findings regarding the spatial distribution of thrombus components and/or novel characterization techniques for thrombus heterogeneity. Studies were excluded if they did not report imaging of clots retrieved from patients or the development of novel techniques relevant for the characterization of thrombus

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heterogeneity ex vivo. Therefore, studies reporting mechanistic studies in vitro, those using clot analogs, and animal studies were excluded.

Studies were extracted by a single author, reviewed by two authors independently, and classified according to the imaging methods used for the thrombus heterogeneity characterization. The present review protocol was unregistered. The last date on which the sources were searched was November 15, 2022. The quality of the studies included in the review was evaluated using the Quality Assessment with Diverse Studies criteria[9] (Supplementary material Table S1).

Results

The study selection process is shown in Fig. 1.

Thrombus heterogeneity in histopathological evaluation

Conventional histopathological evaluation is the gold standard for thrombus characterization. RBC were found in various proportions in the clots. An increased RBC content is generally associated with vulnerability to treatment with intravenous thrombolysis or mechanical thrombectomy, while an increased fibrin/platelet content is associated with resistance to treatment. Staessens et al.[10] observed that clots retrieved from acute ischemic stroke (AIS) patients displayed RBC-rich areas with limited complexity, whereas platelet-rich areas are complex, contain dense fibrin structures aligned with von Willebrand factor (vWF), and are abundant in leukocytes and extracellular DNA. These findings indicate that thrombus components are potentially responsible for resistance to thrombolysis. Resistance to thrombectomy was also attributed[11] to vWF and DNA, mostly derived from neutrophil extracellular traps (NETs), when coexisting with microcalcifications at the core of a clot retrieved with 11 thrombectomy passes. Essig et al.[12] confirmed that NETs are almost exclusively located in fibrin-rich areas and indicated that neutrophils tend to encircle fibrin-rich structures. Fitzgerald et al.[13] studied the per-pass histological composition of clots retrieved from patients with large vessel occlusion (LVO) and observed that later passes were associated with significantly higher fibrin and platelet content and smaller clot area. Such findings could indicate that the thrombus structure is most fragile at the interface between RBC-rich regions and fibrin/platelet regions and that, for equivalent compositions, a more heterogeneous structure would be more difficult to retrieve.

Liu et al.[14] recently quantified the heterogeneity of thrombus composition by analyzing histological images divided into grids. This study defined the spatial heterogeneity index (SHI), used artificial neural network models to describe the extent of non-uniformity of RBC distribution, and explored correlations with endovascular treatment (EVT) efficacy. An endeavor of mechanical thrombectomy is to achieve complete recanalization, typically described as Thrombolysis In Cerebral Infarction grade 2c or 3 at the first thrombectomy pass, also known as the first-pass effect (FPE). The authors found that clot heterogeneity can affect the clot's response to thrombectomy devices, with a higher SHI (more heterogeneous structures) associated with lower FPE rates.

Di Meglio[15] used conventional histopathology techniques along with immunofluorescence staining of whole clots and scanning electron microscopy (SEM) to delineate the fibrin-rich and RBC-rich regions in clots retrieved from AIS patients. This study showed that all clots shared a fibrin shell as a common feature that most likely formed through platelet-driven mechanisms. The thrombi cores displayed variable compositions. Importantly, the shell thickness was not significantly different among clots with various etiologies or between clots from patients who received thrombolytic treatment

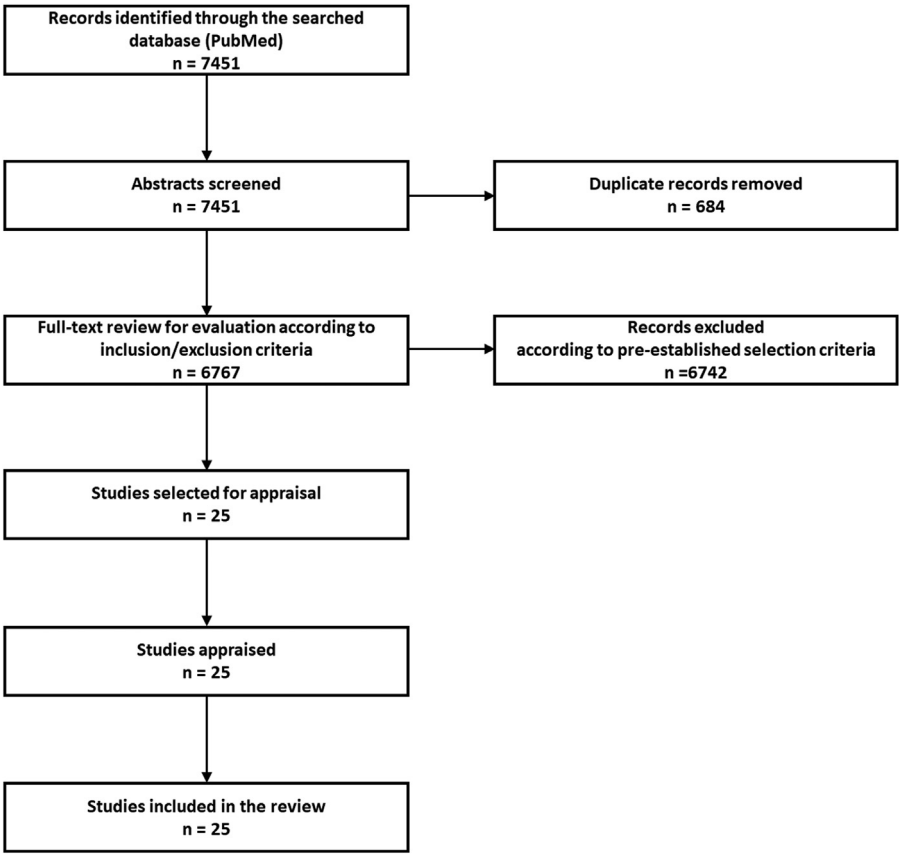

Fig. 1. Chart illustrating the study selection process for this review.

(with intravenous tissue plasminogen activator [IVtPA]) before EVT versus patients who underwent direct EVT. In ex vivo thrombolysis experiments, platelet-rich shells appeared to be resistant to thrombolysis, while the core of the thrombus was preferentially released. Despite these limitations, the shell model accounting for resistance to thrombolysis was challenged by recent histopathological studies. Following an analysis of 1000 clots from patients included in the RESTORE registry, Rossi et al.[16] found that thrombolysis reduced clot size and proportionally reduced all histological clot components, including RBC, fibrin, platelets, collagen, and calcifications. These findings were in agreement with those of another large study that analyzed 1430 clots retrieved from patients enrolled in the Stroke Thromboembolism Registry of Imaging and Pathology[17] and reported no changes in histological composition following tPA treatment. However, changes in thrombus structural integrity nevertheless occur, with IVtPA augmenting the fragility of the clot, because studies show that thrombolytic treatment prior to mechanical thrombectomy increases the risk of embolization.[18,19] In a much smaller study than the one by Di Meglio[15], Mereuta et al.[20] showed that thrombi can deviate from the core-shell model: two of 10 thrombi displayed an RBC-rich core surrounded by fibrin/platelets, while eight of 10 thrombi displayed interspersed RBC-rich and fibrin/platelet regions.

Spatial heterogeneity, which can occur with respect to other thrombus components, has been less investigated to date. In the search for novel biomarkers, Osakada et al.[21] found that clots with different etiologies but similar histological compositions (fibrin, platelets, WBC, or RBC) displayed different expression patterns of the oxidative marker 4-hydroxyl-2-nonenal (HNE). HNE, a well-known modulator of oxidative stress signaling, cell proliferation, transformation, and cell death,[22] is also a proatherogenic compound that contributes to plaque destabilization and rupture[23,24], a known cause of ischemic stroke. Osakada et al.[21] highlighted that the localized distribution of HNE is a distinctive feature of clots from large artery atherosclerosis sources, which helps distinguish them from clots of other etiologies with 100% sensitivity and 82% specificity.

The findings from studies that used histopathological evaluation or electron microscopy to analyze thrombus heterogeneity are summarized in Table 1.

Thrombus heterogeneity observed with electron microscopy

Electron microscopy, scanning electron microscopy (SEM) in particular, provides topographical details and concomitant information about composition that are unavailable on typical histopathological evaluations. Although in coronary arterial thrombi but not thrombi retrieved from AIS patients, Chernysh et al.[25] showed the value of electron microscopy for evaluating RBC shape and compactness, describing the flow-induced preferential orientation of fibrin fibers, and estimating the porosity of fibrin regions. Khismatullin et al.[26] confirmed that, for most cerebral thrombi, the core-shell model is valid. They found that common features among the retrieved clots are a compact core structure composed of tessellated RBC with a polyhedral shape and a fibrin-platelet mesh distributed toward the periphery. These features were induced by intravital contraction or retraction of the clot, a mechanism triggered by platelet activation. However, some thrombi were formed by alternating layers of RBC and fibrin with platelets, a structure that was associated with more favorable outcomes. Thrombi with a high leukocyte content, especially those in which leukocytes were found in clusters, were associated with a fatal outcome. For assessing the extent of intravital contraction in clots retrieved from AIS patients, Khismatullin et al.[27] developed a “contraction ruler” based on in vitro experiments and the quantification of polyhedral/biconcave RBC ratio. The clinical significance of clot contraction gained more attention in recent studies[28,29]. Gao et al.[28] indicated that the percentage of polyhedral RBC in the clot was higher in patients with diabetes mellitus, while the proportion of impervious clots was also higher in this group than in healthy individuals. Non-porous thrombi in SEM were also less pervious according to He et al.[29]; they were also more difficult to retrieve. Compact regions of thrombi tended to evade attachment onto the stent retriever, while porous regions favored attachment onto the stent through wrapping, adhesion, or acting as protrusion sites[30]. At a smaller scale, variations in fibrin morphology and the spatial distribution of platelets and WBC were observed on 3D-SEM (or serial block-face SEM).[20] In a case report, combining SEM and transmission electron microscopy (TEM) observations enabled the visualization of neutrophil accumulation, dense fibrin, and deformed RBC in a thrombolysis-resistant thrombus.[31]

Volumetric techniques for evaluating thrombus heterogeneity

To ensure a thorough characterization of thrombus heterogeneity, it is necessary to develop techniques capable of depicting three-dimensional structural information over large-volume regions. Two studies reporting technical developments [32 33] and a pilot study[34] are available to date (Table 2). Optical clearing and immunofluorescence staining were successfully employed to visualize thrombi within millimeter depths, with structures such as deformed RBC and compact cores preserved [32]. High-resolution propagation-based X-ray microtomography proved useful for depicting the spatial distribution and quantification of porosity, RBC content, and extent of regions containing microcalcifications.[33] Through co-registration with SEM images, the study identified the correlation between gray-level organization in microtomography and thrombus structural features, which allowed for segmentation and quantification of relevant volume regions. The micro-computed tomography (CT) evaluation of clots retrieved from patients and fixed in formalin was proposed as an easy-to-use method for evaluating the extent of intravital contraction and calcifications[34] when segmentation is performed based on density distinct thresholds.

CT, computed tomography; pilot study, a study that tests a certain volumetric technique at small scale; technical development study, a study that establishes the rationale for using a new volumetric technique, demonstrates its principle and feasibility, and highlights its practical potential; cCLOT, optical clearing method; RBC, red blood cells

Thrombus heterogeneity in clinical imaging

Many studies agree that clinical imaging can provide qualitative information about thrombus composition, with RBC-rich thrombi being recognizable by the hyperdense artery sign on non-contrast CT (NCCT) and susceptibility vessel sign on magnetic resonance imaging (MRI) scans.[3,35] Although ex vivo characterization provides information regarding the spatial distribution of thrombus components, thrombus heterogeneity in clinical imaging is an underexplored topic. Hashimoto et al. [36] recently reported that NCCT thrombi are heterogeneous, with higher density on the proximal versus distal aspect of the occlusion, and that the difference between the two aspects is positively correlated with thrombus length. Based on these observations and on the inverse correlation between heterogeneity in NCCT and onset-to-imaging time, the authors hypothesized an underlying thrombus growth mechanism consisting of secondary thrombosis at the proximal side of the occlusion. Histopathological studies confirmed that thrombus maturation occurs during prolonged onset-to-treatment time (>4 h) and increases the content of components that make the thrombus less susceptible to treatment (fibrin, vWF, and NETs).[37] Another clinical study failed to confirm an association between the onset-to-imaging time and radiological characteristics of the thrombus.[38] Differences in findings among studies can be attributed to differences in image analysis methods. Tolhuisen et al.[38] averaged the densities obtained in three regions of

Table 1
Summary of studies using histopathological evaluation and/or electron microscopy to investigate thrombus spatial heterogeneity and potential significance of their findings.

Author, year	Number of analyzed thrombi retrieved from AIS patients	Evaluation technique	Analyzed thrombus components and features	Findings about spatial heterogeneity	Significance
Staessens et al., 2020	177	Histopathology	Fibrin, RBC, vWF, platelets, WBC and DNA	Distinct regions: -RBC-rich -platelets/fibrin/vWF rich WBC and DNA at the interface between the regions	Resistance to thrombolysis
Staessens et al., 2021	1	Histopathology	Fibrin, RBC, vWF, platelets, WBC, DNA, calcifications	core of extracellular DNA, vWF, microcalcifications	Resistance to therapy
Essig et al., 2020	37	Histopathology	Fibrin, collagen, RBC, neutrophils, NETs	NETs localized in fibrin-rich areas Neutrophils accumulate at the periphery of fibrin-rich areas Neutrophils are rare and homogeneously distributed in RBC-rich areas	Stroke etiology Resistance to therapy
Fitzgerald et al., 2021	612 fragments (441 patients)	Histopathology	RBC, WBC, fibrin, platelets, collagen	Later passes were associated with significantly higher fibrin and platelets content and smaller clot area	Stroke etiology Therapeutic approach Clot fragmentation
Liu et al., 2021	157	Histopathology	RBC, fibrin, platelets	Clots which required multiple passes to be removed had higher heterogeneity than those with FPE	Clot fragmentation Therapeutic approach
Di Meglio et al., 2019	164 histopathology 30 SEM	Histopathology SEM	Histopathology: fibrinogen, fibrin, vWF, CD42b, plasminogen activator inhibitor 1, protease nexin-1, histone H4 citrulline 3, Shell thickness SEM: shell thickness and compaction; core composition, compactness and organization	Concentration of fibrin, platelets, WBC, VWF, NETs, and RBC in the outermost layer of thrombi Surface shell that covers and seals thrombus surface Variable inner core Thrombus outer shell is more resistant to ex vivo tPA than the thrombus core	Resistance to therapy
Osakada et al., 2021	52	Histopathology	RBC, WBC, fibrin, platelets, CD42b and oxidative/hypoxic stress markers	LAA have a localized pattern of HNE expression, clots with other etiologies have a diffuse pattern or HNE is absent	Stroke etiology
Khismatullin et al., 2022	24 (in addition to 13 venous thrombi and six pulmonary thrombotic emboli)	Histopathology SEM	Polyhedrocyte/biconcave RBC Fibrin, platelets	Quantification of intravital contraction: modifications in shell thickness and core size	Thrombus age, fragmentation, pathophysiology
Chernysh et al., 2020	NA (45 coronary arterial thrombi, compared with 25 venous thrombi and 10 pulmonary emboli)	SEM	Fibrin (individual fibers, bundles, sponge); individual platelets, platelet aggregates, and degranulated platelets; RBC (biconcave, polyhedral, intermediate forms, balloon-like forms, echinocytes); WBC; cellular microvesicles; and space between structures	Polyhedrocites, clot contraction Flow induced, anisotropic directionality of fibrin fibers	Therapeutic approach
Khismatullin et al., 2020	41	SEM	Fibrin (individual fibers, bundles, sponge, debris); balloon-like	intravital contraction: polyhedrocytes core,	Stroke severity Functional outcome

(*continued*)

Table 1 (*Continued*)

Author, year	Number of analyzed thrombi retrieved from AIS patients	Evaluation technique	Analyzed thrombus components and features	Findings about spatial heterogeneity	Significance
			platelets, platelet aggregates; RBC (biconcave, polyhedral, spherocytes, echinocytes); WBC	fibrin-platelet aggregates at the periphery	
Gao et al., 2022	55 (30 with ahDM, 25 non-DM)	SEM	Polyhedrocyte/biconcave RBC Fibrin, platelets	Intravital contraction: polyhedrocites core	Stroke severity CT imaging features – perviousness Functional outcome
He et al., 2022	49	SEM	RBC (biconcave, polyhedral, spherocytes, echinocytes), fibrin (bundle, sponge), porosity	Clot contraction in non-porous thrombi	Vulnerability to thrombectomy CT imaging features – perviousness
Dumitriu LaGrange et al., 2022	8	SEM	Polyhedral/biconcave RBC, RBC aggregates Fibrin, porosity	Integration in stent retrievers differs according to variations in thrombus compactness Core-shell structure for red thrombus RBC aggregates in compact fibrin matrices for intermediate thrombi	Vulnerability to thrombectomy
Mereuta et al., 2021	10	Histopathology 3D-SEM	Histopathology: RBC-rich and fibrin/platelet-rich areas 3D-SEM: RBC packing, fibrin (fibers, sponge, fragments), platelets, degranulated platelets, WBC	Histopatholog: Distribution of RBC-rich and fibrin/platelet-rich areas: core-shell structure vs interspersed 3D-SEM: polyhedral RBC as a marker of clot contraction	Thrombus behavior toward treatment
Li et al., 2020	2	SEM TEM	RBC, WBC, fibrin, platelets Vacuolar particles, mitochondria, cellular content	Density of the inner structures can be different than the density of the surface structures and affected by thrombolysis	Effect of thrombolysis

3D, three-dimensional; ahDM, diabetes mellitus complicated by admission hyperglycemia; CD42b, Cluster of Differentiation 42b; DNA, deoxyribonucleic acid; NETs, neutrophil extracellular traps; non-D, no diabetes mellitus; RBC, red blood cells; TEM, transmission electron microscopy; vWF, von Willebrand factor; WBC, white blood cells; SEM, scanning electron microscopy.

interest for each thrombus – proximal, middle, and distal – on NCCT scans. Hashimoto et al.[36] made a clear distinction between the proximal and distal sides of the occlusion and analyzed them separately.

Quantitative information regarding thrombus imaging heterogeneity was provided by radiomics. Some proof-of-concept studies established associations between quantitative imaging features that describe textural heterogeneity and treatment outcomes,[39–41] between texture features and clot histological composition,[42,43] or between imaging features and stroke pathogenesis[43,44] (Table 3). However, to date, no correlations between thrombus imaging features and the spatial distribution of the actual biological features of thrombi have been reported.

Table 2
Summary of studies reporting volumetric techniques (other than 3D-SEM) for thrombus analysis.

Author, year	Study type	Volumetric technique	Sample preparation	Size of volume regions	Thrombus components analyzed
Höök et al. 2017	Technical development	Light microscopy	cCLOT optical clearing	In-depth, millimeter range	Polyhedral/biconcave RBC; compact core; fibrin
Saghamanesh et al., 2022	Technical development	High-resolution propagation-based X-rays microtomography	Critical point drying	Large fragments of thrombi (cubic millimeters)	RBC individual shape and aggregates microcalcifications porosity
Dumitriu LaGrange et al., 2022	Pilot	Micro-CT attenuation-based	Fresh sample, formalin-fixed or paraffin-embedded	whole thrombus (cubic millimeters)	Compact RBC core

Table 3
Summary of studies reporting heterogeneity features of clinical imaging.

Author, year	Imaging techniques	Method used for thrombus delineation	Method used for image heterogeneity	Findings	Significance
Hashimoto et al., 2022	NCCT, CTA	CTA: Delayed-phase images: site of occlusion indicated by the filling defect of contrast NCCT: establish the proximal and distal ends of the thrombus	Comparing HU density averaged for the proximal and distal halves of thrombus length, each delineated with ROIs manually placed in the axial, coronal, and sagittal planes on NCCT	Occlusive thrombi are heterogeneous. Higher thrombus density on the proximal aspect compared with the distal aspect; a positive correlation with thrombus length and an inverse correlation with time from last-known-well to imaging regardless of stroke subtype	Pathophysiological mechanism in stroke: secondary thrombosis
Qiu et al., 2019	NCCT, CTA	Slice by slice segmentation of axial view on NCCT, after co-registration with CTA for guidance	Radiomics: high-order statistical textural information – single feature vectors calculated by mean value of each feature across all the voxels within the thrombus, on NCCT, CTA and the difference between NCCT and CTA.	thrombus heterogeneity is associated with recanalization with intravenous alteplase	Predicting recanalization with intravenous alteplase
Hofmeister et al., 2020	NCCT, CTA	Slice by slice segmentation of axial view on NCCT, after co-registration with CTA for guidance	Radiomics: high-order statistical textural information from NCCT and filtered NCCT images	First-attempt recanalization was negatively associated with heterogeneity of the clot and coarser texture.	Predicting recanalization with mechanical thrombectomy
Sarioglu et al., 2022	NCCT, CTA	Slice by slice segmentation of axial view on NCCT, after co-registration with CTA for guidance	Radiomics: high-order statistical parameters from NCCT after intensity rescaling	Heterogeneous clot texture associated with FPE failure Homogeneity of homogeneous zones (GLZLM_ZP) as independent predictor of FPE	Predicting recanalization with mechanical thrombectomy
Hanning et al., 2021	NCCT, CTA	Segmentation on CTA. Small-diameter ROIs to capture vessel walls and thrombi; large-diameter ROIs to reflect peri-vascular tissue responses. ROIs extracted from NCCT after co-registration with CTA.	Radiomics: texture features from CTA and NCCT	Texture features more important for differentiating RBC-rich than fibrin-rich thrombi	Predicting clot histological composition
Jiang et al., 2022	NCCT, CTA	Manual thrombus segmentation on CTA was guided by co-registration of CTA with DSA. Thrombus segmentation on NCCT defined based on CTA segmentation	Radiomics: texture features from CTA and NCCT	Highly significant correlations between texture features and thrombus composition, potentially its compactness	Predicting thrombus composition and stroke etiology (cardio-embolic vs. non-cardioembolic)
Chung et al., 2019	GRE, 3T MRI	Single axial slice ROIs indicating the blooming artifact	Features for machine learning are the intensity profiles perpendicular to the centerline of ROIs	Intensity profiles differ for clots related to AF compared to clots not related to AF	Predictor of atrial fibrillation as stroke cause

AF, atrial fibrillation; CTA, computed tomography angiography; DSA, digital subtraction angiography; FPE, first-pass effect; GLZLM_ZP, gray-level zone length matrix, zone percentage; GRE, gradient-echo sequence; HU, Hounsfield units; MRI, magnetic resonance imaging; NCCT, non-contrast computed angiography; ROIs, regions of interest.

Discussion

Clinical significance of thrombus heterogeneity and future directions

Variability in the spatial heterogeneity of thrombi can be clinically significant in various ways. Studies to date[45,29] have reported that thrombi with a compact core are less pervious. However, the presence of a compact core and the extent of intravital contraction may have an ambivalent effect on thrombus permeability. While an increased extent of intravital contraction is expected to increase thrombus compactness and decrease perviousness, the retraction process could potentially decrease thrombus diameter and allow flow to permeate the thrombus surface and vessel wall.[46] Perviousness measures obtained for entire thrombus segmentation are associated with favorable functional outcomes.[47] It is important to understand the effect of intravital contraction on stroke severity and treatment outcomes.

Table 4
Comparison of various investigation techniques evaluating thrombus spatial heterogeneity.

Technique	Advantages	Disadvantages
Histopathology	Well established methods – gold standard technique Probing a variety of structural elements (new and aged fibrin, cellular content, proteins, DNA) Long specimen shelf life	Thin 2D sections, which might not be relevant for the entire specimen Specimens more distorted compared to other preparation techniques Single and predetermined specimen orientation for slicing
SEM	Topographical information, evaluation of thrombus composition and organization: the number and the shapes of the cells, fibrin morphologies and orientation, porosity Information can be gathered at several levels of magnitude, and in various directions across the sample upon sectioning	Many observations are necessary, across multiple cuts in different directions, to obtain a information about a heterogeneous specimen Qualitative or semi-quantitative method unless a very large number of observations are performed
3D-SEM (SBFSEM)	Provides information about relative spatial distribution of thrombus components with high level of detail	Analyzes a small volume of sample ($< 10^{-4}$ mm^3) Sample is destroyed during observations
TEM	Provides details about cellular content	Analyzes only small sample regions
Volumetric techniques: optical	Microscopes available at many institutions	Sample preparation can be a delicate process Limitations related with the optical path in thrombus Image reconstruction to the level of whole thrombus might not be possible
X-rays based volumetric techniques	Analysis of a large volume of thrombus or whole thrombus Provides information about compactness, calcifications, about the shape and organization of red blood cells Quantitative information, which is relevant for comparing volumes of interest within thrombus and will help define new variables, useful for studying associations with stroke etiology, thrombus clinical imaging features, thrombus fragmentation or treatment outcome	Tradeoff between field of view and resolution – need to optimize number of observations Equipment for high-resolution imaging can be expensive

3D-SEM, three-dimensional scanning electron microscopy; DNA, deoxyribonucleic acid; SBFSEM, serial block-face scanning electron microscopy; TEM, transmission electron microscopy.

Thrombus spatial heterogeneity is related to mechanical thrombectomy efficacy, with evidence suggesting that more heterogeneous structures are difficult to retrieve. However, the effect of heterogeneity on thrombolytic treatment is unclear, as histopathological evaluations do not confirm a preferential response of thrombus components to treatment. The degree of variability of RBC-rich thrombi has not yet been elucidated in terms of extent of compact core regions consisting of tessellated RBC versus porous regions. Thrombi can display longitudinally multiple regions of compact RBC alternating with loosely packed regions along the thrombus length.[30] Thrombus embolization following mechanical thrombectomy, an undesired event with unfavorable effects on functional recovery,[19] is associated with RBC-rich composition[48 19] and thrombus length.[19] Further explorations are needed to elucidate how thrombus spatial heterogeneity in terms of distribution and extent of compact core regions affects its fragmentation behavior as well as how variations in compactness relate to other thrombus characteristics such as thrombus length that determine treatment efficacy.

Clinical imaging has not yet been explored for its full potential to describe the spatial heterogeneity of thrombi. Decreasing the CT slice thickness[49,50] and disposing of automatic segmentation[51 52] might increase the ability of clinical imaging to depict clot heterogeneity. High-resolution and ultra-high-resolution photon counting detectors are finding their way to clinical CT scanners and bring a beneficial tradeoff between patient dose and image quality[53,54]. Dual-energy CT and post-processing methods using virtual monoenergetic imaging algorithms could also lead to new developments in this direction.[55–57] Radiomic techniques encounter technical challenges[58,59] that prevent their introduction in the near foreseeable future in clinical practice for the characterization of thrombus spatial heterogeneity. Among the commonly recognized limitations are the lack of standardization in the process of selecting radiomic features and model training, risk of bias due to the lack of adequate dataset size and imbalanced data, overfitting tendency, and poor generalizability across various centers and scanners. Depictions of thrombus spatial heterogeneity may benefit from the future development of MRI techniques. Multimodality imaging can help increase MRI sensitivity for LVO detection, for example, by using the bright vessel sign on arterial spin labeling in combination with magnetic resonance angiography or digital subtraction angiography.[60] Recent developments in vessel wall imaging techniques have improved thrombus detection and delineation.[61,62] T1-weighted cube imaging has advantages such as high-resolution, isotropic imaging, and blood signal suppression.[61] Three-dimensional proton density-weighted variable refocusing flip angle pulse and turbo spin-echo sequences with volume isotropic turbo spin-echo acquisition enables distinguishing the blood vessels and the occluded lesion from the brain parenchyma and cerebrospinal fluid based on their different intensities.[62] These approaches, along with quantitative T2* mapping,[63] can help establish correlations between clinical imaging features and the spatial distribution of thrombus biological features, for example, by helping differentiate the compositions of the proximal and distal regions.

A major setback in understanding thrombus heterogeneity and defining its clinical implications is the lack of characterization techniques that can provide quantitative results. A comparison of various techniques used to date is presented in Table 4. Conventional histopathology and SEM offer sectional views from which parameters such as porosity or compact core extent are averaged and extrapolated for the entire thrombus volume. Volumetric techniques for ex vivo examination, such as CT techniques with improved resolution, can help overcome the current drawbacks and provide meaningful quantitative variables for thrombus heterogeneity characterization.

A limitation of our systematic review stems from not including unpublished data.

Conclusions

There is a sufficient body of evidence acquired through ex vivo investigations to support the fact that thrombi in vivo frequently adopt a core-shell configuration. The degree to which thrombi with

similar compositions vary in terms of compactness and the spatial distribution of their components remains unknown. More exploratory studies are necessary to determine how thrombus heterogeneity influences thrombus response to treatment, incorporation into thrombectomy devices, and correlation with frequency of embolization. Therefore, it is important to develop quantitative volumetric ex vivo characterization techniques that can help provide insight into the variability of thrombus heterogeneity and its clinical significance.

Understanding the heterogeneous nature of thrombi is essential for developing new devices and therapeutic strategies to improve functional outcomes in the treatment of patients with AIS.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.neurad.2023.01.004.

References

1. Zhang R, Wang Y, Fang J, Yu M, Wang Y, Liu G. Worldwide 1-month case fatality of ischaemic stroke and the temporal trend. *Stroke Vasc Neurol*. 2020;5(4):353–360. https://doi.org/10.1136/svn-2020-000371.
2. Alkarithi G, Duval C, Shi Y, Macrae FL, Ariëns RAS. Thrombus structural composition in cardiovascular disease. *Arterioscler Thromb Vasc Biol*. 2021;41(9):2370–2383. https://doi.org/10.1161/ATVBAHA.120.315754.
3. Cahalane R, Boodt N, Akyildiz AC, et al. A review on the association of thrombus composition with mechanical and radiological imaging characteristics in acute ischemic stroke. *J Biomech*. 2021;129: 110816. https://doi.org/10.1016/j.jbiomech.2021.110816.
4. Juega, J., Palacio-Garcia, C., Rodriguez, M., et al. ITACAT study group. Monocyte-to-lymphocyte ratio in clot analysis as a marker of cardioembolic stroke etiology. *Transl Stroke Res*. Published online September 29, 2021. doi:10.1007/s12975-021-00946-w.
5. Staessens S, De Meyer SF. Thrombus heterogeneity in ischemic stroke. *Platelets*. 2021;32(3):331–339. https://doi.org/10.1080/09537104.2020.1748586.
6. Staessens S, François O, Brinjikji W, et al. Studying stroke thrombus composition after thrombectomy: what can we learn? *Stroke*. 2021;52(11):3718–3727. https://doi.org/10.1161/STROKEAHA.121.034289.
7. Desilles JP, Di Meglio L, Delvoye F, et al. Composition and organization of acute ischemic stroke thrombus: a wealth of information for future thrombolytic strategies. *Front Neurol*. 2022;13: 870331. https://doi.org/10.3389/fneur.2022.870331.
8. Kadri OE, Surblyte M, Chandran VD, Voronov RS. Is the endothelial cell responsible for the thrombus core and shell architecture? *Med Hypotheses*. 2019;129: 109244. https://doi.org/10.1016/j.mehy.2019.109244.
9. Harrison R, Jones B, Gardner P, Lawton R. Quality assessment with diverse studies (QuADS): an appraisal tool for methodological and reporting quality in systematic reviews of mixed- or multi-method studies. *BMC Health Serv Res*. 2021;21(1):144. https://doi.org/10.1186/s12913-021-06122-y.
10. Staessens S, Denorme F, Francois O, et al. Structural analysis of ischemic stroke thrombi: histological indications for therapy resistance. *Haematologica*. 2020;105(2):498–507. https://doi.org/10.3324/haematol.2019.219881.
11. Staessens S, François O, Desender L, et al. Detailed histological analysis of a thrombectomy-resistant ischemic stroke thrombus: a case report. *Thromb J*. 2021;19(1):11. https://doi.org/10.1186/s12959-021-00262-1.
12. Essig F, Kollikowski AM, Pham M, et al. Immunohistological analysis of neutrophils and neutrophil extracellular traps in human thrombemboli causing acute ischemic stroke. *Int J Mol Sci*. 2020;21(19):7387. https://doi.org/10.3390/ijms21197387.
13. Fitzgerald S, Rossi R, Mereuta OM, et al. Per-pass analysis of acute ischemic stroke clots: impact of stroke etiology on extracted clot area and histological composition. *J NeuroInterventional Surg*. 2021;13(12):1111–1116. https://doi.org/10.1136/neurintsurg-2020-016966.
14. Liu Y, Brinjikji W, Abbasi M, et al. Quantification of clot spatial heterogeneity and its impact on thrombectomy. *J NeuroInterventional Surg*. 2021. https://doi.org/10.1136/neurintsurg-2021-018183. Published online December 15:neurintsurg-2021-018183.
15. Di Meglio L, Desilles JP, Ollivier V, et al. Acute ischemic stroke thrombi have an outer shell that impairs fibrinolysis. *Neurology*. 2019;93(18):e1686–e1698. https://doi.org/10.1212/WNL.0000000000008395.
16. Rossi R, Molina S, Mereuta OM, et al. Does prior administration of rtPA influence acute ischemic stroke clot composition? Findings from the analysis of clots retrieved with mechanical thrombectomy from the RESTORE registry. *J Neurol*. 2022;269(4):1913–1920. https://doi.org/10.1007/s00415-021-10758-5.
17. Brinjikji W, Abbasi M, Mereuta OM, et al. Histological composition of retrieved emboli in acute ischemic stroke is independent of pre-thrombectomy alteplase use. *J Stroke Cerebrovasc Dis*. 2022;31(4): 106376. https://doi.org/10.1016/j.jstrokecerebrovasdis.2022.106376.
18. Mohammaden MH, Stapleton CJ, Brunozzi D, et al. Risk factors for distal clot migration during mechanical thrombectomy of anterior circulation large vessel occlusion. *Cerebrovasc Dis*. 2020;49(2):185–191. https://doi.org/10.1159/000507341.
19. Wong GJ, Yoo B, Liebeskind D, et al. Frequency, determinants, and outcomes of emboli to distal and new territories related to mechanical thrombectomy for acute ischemic stroke. *Stroke*. 2021;52(7):2241–2249. https://doi.org/10.1161/STROKEAHA.120.033377.
20. Mereuta OM, Fitzgerald S, Christensen TA, et al. High-resolution scanning electron microscopy for the analysis of three-dimensional ultrastructure of clots in acute ischemic stroke. *J NeuroInterventional Surg*. 2021;13(10):906–911. https://doi.org/10.1136/neurintsurg-2020-016709.
21. Osakada Y, Yamashita T, Morihara R, et al. 4-Hydroxyl-2-Nonenal localized expression pattern in retrieved clots is associated with large artery atherosclerosis in stroke patients. *J Stroke Cerebrovasc Dis*. 2021;30(3): 105583. https://doi.org/10.1016/j.jstrokecerebrovasdis.2020.105583.
22. Dalleau S, Baradat M, Guéraud F, Huc L. Cell death and diseases related to oxidative stress:4-hydroxynonenal (HNE) in the balance. *Cell Death Differ*. 2013;20(12):1615–1630. https://doi.org/10.1038/cdd.2013.138.
23. Gargiulo S, Testa G, Gamba P, Staurenghi E, Poli G, Leonarduzzi G. Oxysterols and 4-hydroxy-2-nonenal contribute to atherosclerotic plaque destabilization. *Free Radic Biol Med*. 2017;111:140–150. https://doi.org/10.1016/j.freeradbiomed.2016.12.037.
24. Nègre-Salvayre A, Garoby-Salom S, Swiader A, Rouahi M, Pucelle M, Salvayre R. Proatherogenic effects of 4-hydroxynonenal. *Free Radic Biol Med*. 2017;111:127–139. https://doi.org/10.1016/j.freeradbiomed.2016.12.038.
25. Chernysh IN, Nagaswami C, Kosolapova S, et al. The distinctive structure and composition of arterial and venous thrombi and pulmonary emboli. *Sci Rep*. 2020;10(1):5112. https://doi.org/10.1038/s41598-020-59526-x.
26. Khismatullin RR, Nagaswami C, Shakirova AZ, et al. Quantitative morphology of cerebral thrombi related to intravital contraction and clinical features of ischemic stroke. *Stroke*. 2020;51(12):3640–3650. https://doi.org/10.1161/STROKEAHA.120.031559.
27. Khismatullin RR, Abdullayeva S, Peshkova AD, et al. Extent of intravital contraction of arterial and venous thrombi and pulmonary emboli. *Blood Adv*. 2022;6(6):1708–1718. https://doi.org/10.1182/bloodadvances.2021005801.
28. Gao Q, Qi P, Wang J, et al. Effects of diabetes mellitus complicated by admission hyperglycemia on clot histological composition and ultrastructure in patients with acute ischemic stroke. *BMC Neurol*. 2022;22(1):130. https://doi.org/10.1186/s12883-022-02660-y.
29. He Y, Xu J, Zhong W, et al. Non-porous thrombi are less pervious and easier to be retrieved: implication from scanning electron microscopy study. *J NeuroInterventional Surg*. 2022. https://doi.org/10.1136/neurintsurg-2022-018689. Published online March 28neurintsurg-2022-018689.
30. Dumitriu LaGrange D, Bernava G, Reymond P, et al. A high resolution scanning electron microscopy analysis of intracranial thrombi embedded along the stent retrievers. *Sci Rep*. 2022;12(1):8027. https://doi.org/10.1038/s41598-022-11830-4.
31. Li Y, Wang H, Zhao L, et al. A case report of thrombolysis resistance: thrombus ultrastructure in an ischemic stroke patient. *BMC Neurol*. 2020;20(1):135. https://doi.org/10.1186/s12883-020-01706-3.
32. Höök P, Brito-Robinson T, Kim O, et al. Whole blood clot optical clearing for nondestructive 3D imaging and quantitative analysis. *Biomed Opt Express*. 2017;8(8):3671. https://doi.org/10.1364/BOE.8.003671.
33. Saghamanesh S, Dumitriu LaGrange D, Reymond P, et al. Non contrast enhanced volumetric histology of blood clots through high resolution propagation-based X-ray microtomography. *Sci Rep*. 2022;12(1):2778. https://doi.org/10.1038/s41598-022-06623-8.
34. Dumitriu LaGrange D, Braunersreuther V, Wanke I, et al. MicroCT can characterize clots retrieved with mechanical thrombectomy from acute ischemic stroke patients—a preliminary report. *Front Neurol*. 2022;13: 824091. https://doi.org/10.3389/fneur.2022.824091.
35. Brinjikji W, Duffy S, Burrows A, et al. Correlation of imaging and histopathology of thrombi in acute ischemic stroke with etiology and outcome: a systematic review. *J Neurointerventional Surg*. 2017;9(6):529–534. https://doi.org/10.1136/neurintsurg-2016-012391.
36. Hashimoto T, Kunieda T, Honda T, et al. Heterogeneity between proximal and distal aspects of occlusive thrombi on pretreatment imaging in acute ischemic stroke. *Neuroradiol J*. 2022;35(3):378–387. https://doi.org/10.1177/19714009211049713.
37. Abbasi M, Arturo Larco J, Mereuta MO, et al. Diverse thrombus composition in thrombectomy stroke patients with longer time to recanalization. *Thromb Res*. 2022;209:99–104. https://doi.org/10.1016/j.thromres.2021.11.018.
38. Tolhuisen ML, Kappelhof M, Dutra BG, et al. The MR CLEAN registry investigators. Influence of onset to imaging time on radiological thrombus characteristics in acute ischemic stroke. *Front Neurol*. 2021;12: 693427. https://doi.org/10.3389/fneur.2021.693427.
39. Qiu W, Kuang H, Nair J, et al. Radiomics-based intracranial thrombus features on CT and CTA predict recanalization with intravenous alteplase in patients with acute ischemic stroke. *Am J Neuroradiol*. 2019;40(1):39–44. https://doi.org/10.3174/ajnr.A5918.
40. Hofmeister J, Bernava G, Rosi A, et al. Clot-based radiomics predict a mechanical thrombectomy strategy for successful recanalization in acute ischemic stroke. *Stroke*. 2020;51(8):2488–2494. https://doi.org/10.1161/STROKEAHA.120.030334.
41. Sarioglu O, Sarioglu FC, Capar AE, Sokmez DF, Mete BD, Belet U. Clot-based radiomics features predict first pass effect in acute ischemic stroke. *Interv Neuroradiol*. 2022;28(2):160–168. https://doi.org/10.1177/15910199211019176.
42. Hanning U, Sporns PB, Psychogios MN, et al. Imaging-based prediction of histological clot composition from admission CT imaging. *J NeuroInterventional Surg*. 2021;13(11):1053–1057. https://doi.org/10.1136/neurintsurg-2020-016774.
43. Jiang J, Wei J, Zhu Y, et al. Clot-based radiomics model for cardioembolic stroke prediction with CT imaging before recanalization: a multicenter study. *Eur Radiol*.

2022;6. https://doi.org/10.1007/s00330-022-09116-4. Published online September.
44. Chung J, Kim Y, Cha J, et al. Characterization of clot composition in acute cerebral infarct using machine learning techniques. *Ann Clin Transl Neurol*. 2019;6(4):739–747. https://doi.org/10.1002/acn3.751.
45. Gao Q, Hu S, Yang X, Wang J, Lu J, Wang D. Histologic differences between in situ and embolized carotid web thrombi: a case report. *BMC Neurol*. 2021;21(1):398. https://doi.org/10.1186/s12883-021-02428-w.
46. Tutwiler V, Peshkova AD, Andrianova IA, Khasanova DR, Weisel JW, Litvinov RI. Contraction of blood clots is impaired in acute ischemic stroke. *Arterioscler Thromb Vasc Biol*. 2017;37(2):271–279. https://doi.org/10.1161/ATVBAHA.116.308622.
47. Santos EMM, Arrarte Terreros N, Kappelhof M, et al. Associations of thrombus perviousness derived from entire thrombus segmentation with functional outcome in patients with acute ischemic stroke. *J Biomech*. 2021;128: 110700. https://doi.org/10.1016/j.jbiomech.2021.110700.
48. Ye G, Qi P, Chen K, et al. Risk of secondary embolism events during mechanical thrombectomy for acute ischemic stroke: a single-center study based on histological analysis. *Clin Neurol Neurosurg*. 2020;193: 105749. https://doi.org/10.1016/j.clineuro.2020.105749.
49. Rosskopf J, Kloth C, Dreyhaupt J, Braun M, Schmitz BL, Graeter T. Thin slices and maximum intensity projection reconstructions increase sensitivity to hyperdense middle cerebral artery sign in acute ischemic stroke. *Cerebrovasc Dis*. 2020;49(4):437–441. https://doi.org/10.1159/000509378.
50. Hund H, Boodt N, Arrarte Terreros N, et al. MR CLEAN registry investigators. quantitative thrombus characteristics on thin-slice computed tomography improve prediction of thrombus histopathology: results of the MR CLEAN registry. *Eur Radiol*. 2022. https://doi.org/10.1007/s00330-022-08762-y. Published online April 30,.
51. Tolhuisen ML, Ponomareva E, Boers AMM, et al. A convolutional neural network for anterior intra-arterial thrombus detection and segmentation on non-contrast computed tomography of patients with acute ischemic stroke. *Appl Sci*. 2020;10(14):4861. https://doi.org/10.3390/app10144861.
52. Mojtahedi M, Kappelhof M, Ponomareva E, et al. Fully automated thrombus segmentation on CT images of patients with acute ischemic stroke. *Diagnostics*. 2022;12(3):698. https://doi.org/10.3390/diagnostics12030698.
53. Rajendran K, Petersilka M, Henning A, et al. Full field-of-view, high-resolution, photon-counting detector CT: technical assessment and initial patient experience. *Phys Med Biol*. 2021;66(20). https://doi.org/10.1088/1361-6560/ac155e.
54. Rajendran K, Tao S, Abdurakhimova D, Leng S, McCollough C. Ultra-high resolution photon-counting detector CT reconstruction using spectral prior image constrained compressed-sensing (UHR-SPICCS). *Proc SPIE– Int Soc Opt Eng*. 2018;10573: 1057318. https://doi.org/10.1117/12.2294628.
55. D'Angelo T, Cicero G, Mazziotti S, et al. Dual energy computed tomography virtual monoenergetic imaging: technique and clinical applications. *Br J Radiol*. 2019 20180546. https://doi.org/10.1259/bjr.20180546. Published online April 9.
56. Ding YH, Abbasi M, Michalak G, et al. Characterization of thrombus composition with multimodality CT-based imaging: an in-vitro study. *J NeuroInterventional Surg*. 2021;13(8):738–740. https://doi.org/10.1136/neurintsurg-2020-016799.
57. Mangesius S, Janjic T, Steiger R, et al. Dual-energy computed tomography in acute ischemic stroke: state-of-the-art. *Eur Radiol*. 2021;31(6):4138–4147. https://doi.org/10.1007/s00330-020-07543-9.
58. van Timmeren JE, Cester D, Tanadini-Lang S, Alkadhi H, Baessler B. Radiomics in medical imaging—"how-to" guide and critical reflection. *Insights Imaging*. 2020;11(1):91. https://doi.org/10.1186/s13244-020-00887-2.
59. Fusco R, Granata V, Grazzini G, et al. Radiomics in medical imaging: pitfalls and challenges in clinical management. *Jpn J Radiol*. 2022;28. https://doi.org/10.1007/s11604-022-01271-4. Published online March.
60. McCullough-Hicks ME, Yu Y, Mlynash M, Albers GW, Zaharchuk G. The bright vessel sign on arterial spin labeling MRI for heralding and localizing large vessel occlusions. *J Neuroimaging*. 2021;31(5):925–930. https://doi.org/10.1111/jon.12888.
61. Zhang C, Dou W, Jiang S, Dong D, Wang X. High-resolution vessel wall MR imaging in diagnosis and length measurement of cerebral arterial thrombosis: a feasibility study. *J Magn Reson Imaging*. 2022;56(4):1267–1274. https://doi.org/10.1002/jmri.28170.
62. Kumagai K, Hayashi M, Ueda T, et al. Three-dimensional vessel wall MRI to characterize thrombus prior to endovascular thrombectomy for large vessel occlusion stroke. *J Neuroimaging*. 2022;32(6):1070–1074. https://doi.org/10.1111/jon.13050.
63. Gilbert A, Detraz L, Alexandre PL, et al. Magnetic resonance imaging quantitative $T2^*$ mapping to predict the red blood cell content of in vivo thrombi retrieved from patients with large vessel occlusions in acute ischemic stroke. *Interv Neuroradiol J Peritherapeutic Neuroradiol Surg Proced Relat Neurosci*. 2022;28(5):523–530. https://doi.org/10.1177/15910199211042473.