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Review

Clot Composition Analysis as a Diagnostic Tool to Gain Insight into Ischemic Stroke Etiology: A Systematic Review

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Mechanical thrombectomy renders the occluding clot available for analysis. Insights into thrombus composition could help establish the stroke cause. We aimed to investigate the value of clot composition analysis as a complementary diagnostic tool in determining the etiology of large vessel occlusion (LVO) ischemic strokes (International Prospective Register of Systematic Reviews [PROSPERO] registration # CRD42020199436). Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, we ran searches on Medline (using the PubMed interface) and Web of Science for studies reporting analyses of thrombi retrieved from LVO stroke patients subjected to mechanical thrombectomy (January 1, 2006 to September 21, 2020). The PubMed search was updated weekly up to February 22, 2021. Reference lists of included studies and relevant reviews were hand-searched. From 1,714 identified studies, 134 eligible studies (97 cohort studies, 31 case reports, and six case series) were included in the qualitative synthesis. Physical, histopathological, biological, and microbiological analyses provided information about the gross appearance, mechanical properties, structure, and composition of the thrombi. There were non-unanimous associations of thrombus size, structure, and composition (mainly proportions of fibrin and blood formed elements) with the Trial of Org 10172 in Acute Stroke Treatment (TOAST) etiology and underlying pathologies, and similarities between cryptogenic thrombi and those of known TOAST etiology. Individual thrombus analysis contributed to the diagnosis, mainly in atypical cases. Although cohort studies report an abundance of quantitative rates of main thrombus components, a definite clot signature for accurate diagnosis of stroke etiology is still lacking. Nevertheless, the qualitative examination of the embolus remains an invaluable tool for diagnosing individual cases, particularly regarding atypical stroke causes.

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Introduction

Mechanical thrombectomy has not only become the standard of care in the management of most large vessel occlusion (LVO) strokes, but it also renders the occluding clot available for lab bench analysis.[1] Insights into thrombus composition and properties could help determine its relationships with the clot signs on imaging, stroke cause, resistance to thrombectomy, proce-

dural complications, and outcome measures.[2]

A previous systematic review of studies published between January 2005 and December 2015 on imaging and histologic characteristics of thrombi in acute ischemic stroke (AIS) concluded that the hyperdense artery sign was associated with red blood cell (RBC)-rich thrombi and improved recanalization rates. However, there was no association between the histopathological characteristics of thrombi, stroke etiology, and angiographic outcomes.[3] Another recent scoping systematic review focused on the impact of thrombus composition on the efficacy of mechanical thrombectomy and thrombolysis.[4] However, assessing the value of clot analysis in the diagnosis of stroke etiology and thus guiding secondary prevention strategies seems more challenging.

This systematic review aimed to evaluate the value of clot composition analysis as a complementary diagnostic tool in determining the LVO ischemic stroke etiology. Specifically, we addressed the following research questions: (1) What types of physical, histological, or other biological analyses have been carried out on thrombi retrieved from LVO stroke patients subjected to mechanical thrombectomy?; (2) What kind of information about the structure, and molecular and cellular composition of stroke thrombi has resulted from laboratory analyses?; (3) Could laboratory analyses of clot structure and composition be used as complementary diagnostic tools to determine stroke etiology, and thus reduce the proportion of cryptogenic strokes?; (4) Could specific findings in clot composition be used as ancillary information to diagnose atypical stroke etiologies due to underlying pathologies?

Methods

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Systematic review

This systematic review was carried out according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[5] The protocol was registered in the CRD-NIHR International Prospective Register of Systematic Reviews (PROSPERO) with registration number CRD42020199436.[6]

We searched the published literature reporting the analysis of thrombi retrieved from LVO stroke patients subjected to mechanical thrombectomy and performed a qualitative assessment of the available evidence.

Literature search strategy

We carried out electronic searches on Medline (using the PubMed interface) and Web of Science from January 1, 2006, up to and including September 21, 2020. The search syntax was (stroke AND (thrombus OR thrombi OR clot)) AND (thrombectomy OR endovascular). The search fields were [Title/Abstract] in Medline (PubMed) and [Topic] in Web of Science. There were no language restrictions. The PubMed search was updated weekly through My NCBI up to February 22, 2021. References were added to a Mendeley Reference Manager library dedicated to this review's topic, checked for duplicates, and completed with Mendeley's feedback-delivering personalized suggestions for related articles. Reference lists of included studies and relevant reviews were hand-searched. The electronic database search was supplemented by searching for trial protocols through ClinicalTrials.gov Advanced Search syntax: condition or disease (ischemic stroke) and other terms ((thrombus OR thrombi OR clot) AND (thrombectomy OR composition)). The search was not extended to unpublished studies or other sources of grey literature.

Study selection: eligibility criteria and screening process

The current review considered observational cohort studies, case series and case report studies reporting any kind of physical, histological, or other biological analyses carried out on thrombi retrieved from LVO stroke patients subjected to mechanical thrombectomy. We included studies published as full-length original research articles in any language, provided that the English abstract was available, and abstracts of conference proceedings in English language. In cases of studies with duplicate or overlapping patient populations, only the publication with the most complete dataset was included. Protocol articles, review articles and abstracts later published in full were also excluded.

Titles and abstracts yielded by the search were independently screened against the inclusion criteria by two reviewers. Full reports were obtained for all titles that appeared to meet the inclusion criteria or where there was any uncertainty. Reviewer pairs then screened the full-text reports of potentially eligible studies and decided whether these met the inclusion criteria. Disagreements were resolved through discussion and consensus involving a third reviewer.

Data extraction and synthesis

The following information was collected from the eligible studies and extracted to tables independently by two reviewers: general information (first author name, year of publication, source, and type of study), type(s) of physical, histological, or other biological analyses carried out on thrombi, sample size(s), qualitative and quantitative features about thrombus composi-

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tion, and diagnostic information regarding typical LVO ischemic stroke Trial of Org 10172 in Acute Stroke Treatment (TOAST) etiologies or atypical etiologies with underlying pathologies.

Heterogeneity in study design, outcome measuring, and reporting precluded a meta-analysis of the association between thrombus composition and typical or atypical stroke etiology. Instead, a systematic narrative synthesis is provided with information presented in the text and tables to summarize and explain the characteristics and findings of the included studies.

Results

Search results: study selection and characterization

A detailed study selection flow chart is shown in Figure 1. Briefly, a total of 2,665 records were identified electronically in the Web of Science and Medline (through PubMed) databases up to and including September 21, 2020. After removing duplicate and irrelevant records, and adding relevant records identified through an updated search on PubMed (up to and including February 22, 2021) and other sources, 152 full-text articles and congress abstracts were assessed for eligibility. Subsequent reasoned exclusions rendered 134 studies which were included in the qualitative synthesis (Supplementary Table 1).

A summary of the study characteristics is shown in Table 1.

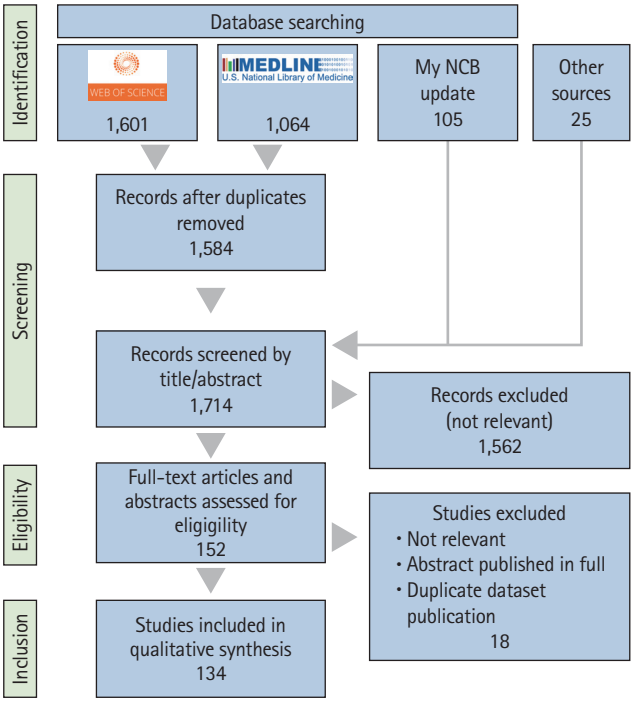

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart of the literature search results and selection of studies at each stage.

Most of them (72.39%) were observational cohort studies with a wide range of cohort sizes, varying from four to 1,022 patients with thrombus samples subjected to analysis (median, 65 [interquartile range, IQR, 37 to 105]). Thirty-one (23.13%) case reports and six (4.48%) case series were also included. In 108 out of the 134 studies, the retrieved thrombi were subjected to one or more types of histopathological examinations. Physical, biological, and/or microbiological analyses were carried out in 62 studies. A relationship between thrombus structure/composition and TOAST or atypical etiologies was reported in 54.48% of the studies. In 46.27% of the studies, the stroke etiology was not taken into consideration, or non-conclusive results were obtained.

The publication chronology of the studies is shown in Figure 2. Although Marder's pioneering study was published 15 years ago,[7] 75.37% of the studies have been published in the last 5 complete years (2016 to 2020). Of note, 80% of the studies assessing thrombus composition by means of biological analyses using biochemical or molecular biology techniques have been published in the last 3 complete years (2018 to 2020). Since 11 studies have been published in 2021 up to and including February 22, around 75 studies are estimated to be published in the present year.

The search for clinical trial protocols identified five ongoing studies in which thrombi and blood samples are being collected for histopathological and/or other biological analyses.[8-12] Concurrently, active multi-institutional registries like RESTORE

Table 1. Study characteristics summary

Variable	No. (%)
All study types	134 (100)
Cohort	97 (72.39)
Case report (1–2 patients)	31 (23.13)
Case series (3–6 patients)	6 (4.48)
Histopathology	108 (80.60)
Conventional	101 (75.37)
Immunohistochemistry	45 (33.58)
Electron microscopy	6 (4.48)
Other analyses	62 (46.27)
Physical (macroscopic, mechanical, etc.)	33 (24.63)
Biological (biochemical, biomolecular, etc.)	15 (11.19)
Microbiological	20 (14.93)
Diagnostic	73 (54.48)
TOAST etiology	32 (23.88)
Atypical etiology/underlying pathology	41 (30.30)
None	62 (46.27)

TOAST, Trial of Org 10172 in Acute Stroke Treatment.

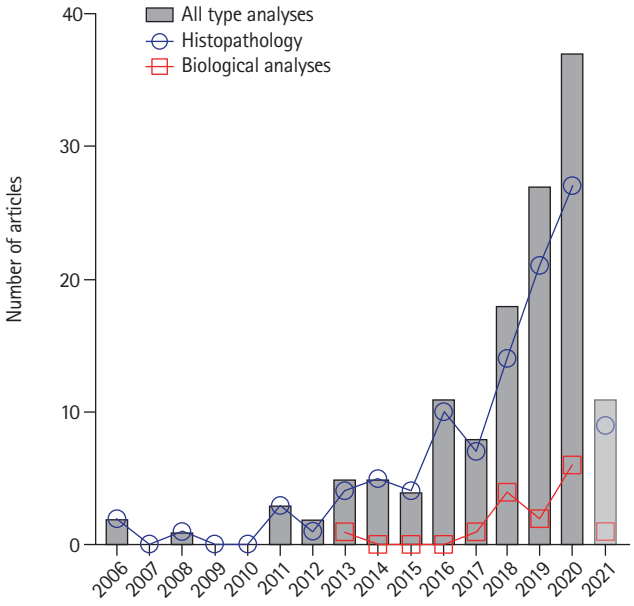

Figure 2. Chronology of study publication. Number of yearly-published studies from January 1, 2006, to February 22, 2021. Faded colors indicate incomplete year 2021.

(National University of Ireland) and Stroke Thromboembolism Registry of Imaging and Pathology, Mayo Clinic (STRIP) are compiling clinical, procedural, imaging, and histopathological data from patients with AIS.

Types of physical, histological, and other biological analyses

Procedures for thrombus retrieval and subsequent analysis have been reviewed.[1,13] Briefly, after retracting the thrombectomy device, the retrieved clot material is gently removed from the device and transferred into saline solution. If clot per-pass analysis is desired, clot material from each pass can be processed separately. The macroscopic appearance and other physical properties of the retrieved thrombus can be freshly examined; otherwise, the clot can be flash-frozen for storage and later biological analyses. For histological analysis, the specimens are fixed, paraffin-embedded, sectioned, and stained depending on the component of interest. Both manual quantifications and color-based segmentation analysis of thrombus components are used.

Supplementary Table 1 summarizes whether physical, histological, or biological analyses were carried out in each of the 134 included studies.[7,14-146]

In 33 studies, thrombi were subjected to some kind of physical analysis. The gross appearance of the retrieved thrombus was reported in 29 studies. In case reports (20 studies) and case series (two studies), the macroscopic aspect of the thrombus was shown mostly through photographs. Mechanical behavior of the thrombi was analyzed in two studies using custom-made platforms and marketed devices, respectively. In one study the thrombi were scanned using high spatial-resolution three-dimensional (3D) T1-weighted magnetic resonance imaging (MRI) to reveal morphological and other physical characteristics. Finally, advanced synchrotron-based imaging techniques were used in one study to map thrombus composition.

Different types of histopathological examinations, including conventional histology, immunohistochemistry, and electron microscopy, were carried out in 108 studies. The most frequently used conventional stains were hematoxylin and eosin (H&E, 86 studies), Martius Scarlet Blue (MSB, 18 studies), Elastica van Gieson (14 studies), Prussian blue (eight studies), Masson's trichrome (five studies), and Von Kossa (four studies). Other seldom-used stains were periodic-acid Schiff, Carstairs' staining, Picro-Mallory, Alcian blue, Luna, Mallory's phosphotungstic acid-hematoxylin, Ladewig trichrome, naphthol AS-D chloroacetate, and Feulgen's reaction. Immunohistochemistry was carried out in 45 studies with specific antibodies against cellular and biomolecular thrombus components. Finally, the thrombi composition and organization were analyzed at the ultrastructural level through electron microscopy in six studies.

Regarding biological analyses, thrombus composition was assessed through different biochemical, biomolecular, and cellular techniques, such as enzymatic assay, enzyme-linked immunosorbent assay (ELISA), transcriptomics (real-time reverse transcription polymerase chain reaction [qRT-PCR]), proteomics, metabolomics, and flow cytometry in 15 studies. Finally, microbiological analysis of the thrombi was performed through classical culture techniques, specific staining for bacteria/fungi and/or metagenomics (qPCR) in 20 studies.

Thrombus gross appearance and mechanical properties

Case reports and case series, usually presenting thrombectomies of LVOs of atypical etiologies, showed mostly photographic images of the thrombus, and described whether single or multiple clots were retrieved, as well as their gross appearance, including size, shape, consistency, visual texture, color, and homo/heterogeneous aspect.[14-34]

Macroscopic analysis of retrieved thrombi was also carried out in some cohort studies and one case series. The clot color was categorized using three different terminologies. Two cohort studies classified the thrombus as "red-black" or "white," showing similar results: 94.2% red-black thrombi versus 5.8% white thrombi;[35] and 91.4% red/black thrombi versus 8.6% white thrombi.[36] Another cohort study categorized the thrombi by visual assessment as pinkish (17.3%), red (53.8%), or dark

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Table 2. Average proportions of fibrin and blood formed elements in stroke thrombi

PLT	FBR	PLT+FBR	RBC	WBC	Reference
59%±138%, 166	41%±25%, 166		38%±25%, 166	4%±5%, 166	56,57,67,72
		52%±19%, 1,025	41%±19%, 1,045	5%±3%, 944	38,81,84,92,122,142-146
27%±16%, 287	38%±37%, 287		41%±91%, 287		54,55,64,65,123
		52%±25%, 441	47%±25%, 441		78,87,101

Values are presented as weighted mean±pooled standard deviation or number.
PLT, platelet; FBR, fibrin; RBC, red blood cell; WBC, white blood cell.

red (28.8%).[37] A fourth cohort study described the thrombi as slightly white, darker, or reddish, but did not report percentages.[38] Regarding thrombus size, other cohort studies focused on the total extracted clot area (ECA) for each case, defined as the sum of the clot areas from all clot fragments within a case.[39] In a cohort of 550 patients, the mean ECA for all cases was 64 mm^2 and the median number of fragments per case was 3.[40] Of note, bridging therapy was associated with the retrieval of significantly smaller clots.[41,42] Median ECA was 0.33 cm^2 (IQR, 0.16 to 0.59) in patients pre-treated with alteplase (recombinant tissue plasminogen activator [rtPA]), versus 0.39 cm^2 (IQR, 0.22 to 0.82) in patients treated with mechanical thrombectomy alone.[41] Finally, in a case series of five patients the median volume of the thrombus, calculated using the ellipsoid formula ($4/3\pi r_1 r_2 r_3$), was 10.47 mm^3.[43]

Only two studies with limited sample sizes evaluated the mechanical behavior of the thrombi. The specimens were mechanically heterogeneous, in line with the histological heterogeneity. Stiffness and elasticity were measured with a dynamic mechanical analyzer, showing that red thromboemboli composed mainly of fibrin (FBR) and RBC were much softer than the calcified and cholesterol-rich material.[44] The tensile strength and response to stress were measured with a quasi-static uniaxial tensile test using a custom-made platform. The ultimate tensile strain of the emboli increased with a higher platelet (PLT) percentage, and the ultimate tensile stress increased with a higher FBR percentage and decreased with a higher RBC percentage.[45]

Thrombus structure and composition: advanced imaging and histopathology

Multiparametric MRI has been used to characterize retrieved cerebral thrombi. Preliminary results showed that T_1-weighted images with the corresponding apparent diffusion coefficient (water mobility) and T_2 maps (relaxation time) could be used to assess thrombus compactness and microstructure, which in turn reflect RBC and PLT/FBR meshwork content.[46] Advanced synchrotron-based imaging techniques, including X-ray fluorescence and Fourier-transform infrared spectroscopy, have been used in freshly retrieved thrombi to map the distribution of biological elements and metabolites, respectively.[47]

Most histopathology studies focused on the presence and relative abundance of FBR, and blood formed elements (RBCs, PLTs, and white blood cells [WBCs]) in the thrombus. Classical H&E staining was almost always used to visualize the general structure of the thrombus. Although H&E staining and machine learning software allowed reproducible quantification of the three major clot components (RBCs, WBCs, and FBR),[48] the more specific MSB staining was used in many studies for the selective quantification of RBCs and FBR.[28,37,39,43,44,49-61] The Picro-Mallory stain was used to assess the maturity of FBR in thrombi, based on its age-dependent differential staining.[62] Immunohistochemical staining procedures allowed the best visualization and reliable separate quantification of RBCs (glycophorin A, also designated CD235a antigen),[63-65] WBCs (CD45 antigen),[66-70] PLTs (CD41, CD42b, and CD61 antigens),[38,45,49,51,53,56,57,60,63,65,66,71-73] and fibrinogen/FBR.[49,53,63,65,66,74] Depending on the discriminatory capability of the staining procedures used, the studies expressed the ratios of clot components according to four different classifications, as shown in Table 2. A number of studies categorized the clots according to the dominant component (usually with a 60% cut-off)[48] as RBC-rich, FBR-rich, PLT-rich, or mixed.[7,50,52,54,58,59,64,75-91] Some studies identified and/or quantified WBC types and subtypes by immunohistochemical staining using specific antibodies against neutrophil elastase (NE),[66,76,92-94] neutrophil myeloperoxidase (MPO),[65,66,76,95] Ly6G (monocyte, granulocyte, and neutrophil),[94] CD3 (T lymphocyte),[66,68-70,90,96] CD4 (T lymphocyte),[52,53] CD14 (monocyte),[66] CD15 (neutrophil, eosinophil, and monocyte),[97] CD20 (B lymphocyte),[66,68-70,96] CD66b (neutrophil),[76,93] and CD68 (monocyte and macrophage).[30,52,67-70,96,98]

Neutrophils were the predominant leukocyte subset in stroke thrombi.[66,93] Neutrophil extracellular traps (NETs) have been identified as part of the clot scaffold by using antibodies against NETosis biomarkers (citrullinated histones) and histochemical staining of extracellular DNA.[63,65,66,76,93-95,99] NETs were visualized in almost all (79.1% to 100%) of the analyzed thrombi in different studies,[63,65,66,76,93,95] in amounts ranging

from 0.21% to 13.45% of total thrombus area,[93] and 1.1%±2.7% on average.[76] NET signals were observed as being confined within cells, filopodia-like structures, or web-like structures,[65] especially in the outer thrombus layers,[63,95] and almost exclusively within FBR-rich areas.[76] Inside the NETs, citrullinated histones were co-localized with inflammasome proteins (caspase-1 and apoptosis-associated speck-like protein containing a caspase-recruitment domain [ASC]),[99] granular neutrophil proteins (MPO) and extracellular DNA released from neutrophils.[93,95] The addition of histone-DNA complexes to FBR resulted in thicker fibers accompanied by increased rigidity, which contributed to the structural complexity and stabilization of the thrombi.[76] Of interest, monocytes could also form extracellular traps, but to a lesser extent than neutrophils.[66,94]

Coagulation system proteins other than fibrinogen/FBR were immunohistochemically identified by using specific antibodies against von Willebrand factor (VWF),[52-56,60,63,74,97,100] and two PLT-derived direct inhibitors of tissue plasminogen activator (tPA): plasminogen activator inhibitor-1[63,74] and protease nexin-1.[63] The proportion of VWF varied from 0.1% to 94.3% of the total clot area,[60] with mean values between 11.8% and 29.8% in different studies,[56,60,100] and higher content in thrombi retrieved after unsuccessful intravenous thrombolysis.[74] VWF levels were correlated with those of FBR and PLTs.[97] White FBR-rich thrombi showed higher percentages of VWF+ areas co-localized with regions of FBR/collagen.[52] Similarly, PLT-rich areas were characterized by dense FBR structures aligned with VWF.[53]

The presence of other thrombus components has been assessed using specific staining procedures. Some cohort studies used standard protocols including H&E and one or more of the following staining procedures to visualize elastic collagen fibers (Elastica van Gieson), hemosiderin/iron (Prussian blue), calcifications (Von Kossa), and collagen (Masson's trichro me).[67-70,78,81,86,88,89,91,96,101] However, positive results showing the occasional presence of intimal collagen fibers, cholesterol clefts, and smooth muscle cells (immunohistochemically stained for α-smooth muscle actin) were reported only in three studies assessing thrombectomy-induced wall damage, which identified vascular wall components or atheromatous gruel in a low proportion (2.6% to 20%) of the retrieved thrombi.[78,91,101] Case reports also used these specific stainings to identify elastic fibers, hemosiderin granules, collagen fibrous stalks, calcium deposits, and myofibroblast-like cells, usually in atypical thrombi.[15,23,30,43,102]

The presence of foreign bodies in thrombi has also been evaluated. Delaminated polymer coating particulates were readily detected in 33% of H&E-stained preparations.[103] Thread or sheet-like structures were found in 25% of thrombi scanned by electron microscopy.[104]

Classical descriptions of thrombus histological patterns based on light microscopy categorized their organized structure as layered (PLT bands arranged in layers), serpentine (PLT bands arranged in a serpentine way), or erythrocytic (with RBCs and nucleated cells interspersed).[7,52,75,105,106] The molecular and cellular organization revealed distinct features between clots as well as among different regions within a clot. Thrombi were composed of two main area types: RBC-rich and PLT-rich areas. RBC-rich areas had limited complexity and consisted of RBCs entangled in a meshwork of thin FBR. Conversely, PLT-rich areas were characterized by dense FBR structures aligned with VWF and abundant amounts of WBCs and DNA that accumulate around.[53] Similarly, serial block-face scanning electron microscopy (SEM) showed a thrombus 3D ultrastructure that varied greatly depending on the region analyzed. RBC-rich areas were composed mainly of tightly packed RBCs deformed into polyhedrocytes with scant FBR fibers interwoven between cells. The regions with mixed composition showed thick FBR fibers along with PLTs, WBCs, and RBC clusters. FBR-rich areas contained dense FBR masses with sparse RBCs.[56] High-resolution SEM and transmission electron microscopy revealed a dense, sealed, rtPA-resistant external shell encapsulating a loose RBC-rich core. Shell components were densely compacted and agglomerated and formed a continuous layer, in which individual cells could hardly be detected. This was in stark contrast to the clearly identifiable RBCs, FBR fibers, and aggregated PLTs in the inner core.[63,107] Other ultrastructural studies showed morphologic features consistent with the presence of NETs, calcified deposits and cholesterol crystals in the clots.[44,99]

Microbial pathogens in thrombi

Regarding septic cerebral emboli, Marder's pioneering study reported one case of mycotic embolus.[7] However, both real-time and standard PCR demonstrated no expression of bacterial 16S rDNA in any of the 20 clot samples. Gram staining results also showed no evidence of bacteria.[108] Contrastingly, bacteria were detected in Gram-stained clots of four out of 65 patients (6.2%).[109] A larger cohort of 75 patients showed DNA signatures of oral streptococcal bacteria in 84% of the retrieved thombi.[110] Metagenomics analysis also showed the presence of bacterial DNA in all four thrombi originated from symptomatic carotid plaques.[111]

Thrombus composition: molecular biology, biochemical assays, and flow cytometry

Clot homogenates subjected to qRT-PCR showed the expression

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of inflammatory cytokines (interleukin 1β [IL-1β], IL-6, IL-8, IL-18, tumor necrosis factor α [TNF-α], and monocyte chemoattractant protein-1 [MCP-1]), matrix metalloproteinases-2, -9,[112] and endothelial CD31.[75] Four-plex assay showed the expression of cytokines (IL-1β and IL-18) and other inflammasome signaling proteins such as caspase-1 and ASC. Moreover, the presence of nucleotide-binding oligomerization domain (NOD)-like receptor protein-1 and absent in melanoma-2, two receptors that interact with caspase-1 and ASC to form an inflammasome complex, was shown by immunoblot analysis.[99]

Proteomic analysis has been used to characterize the protein cargo of thrombi (thousands) and commonly present proteins (hundreds). Functional bioinformatics analyses revealed protein clusters related to inflammation;[113] immunological functions, blood cell dependent functions, and peripheral vascular processes;[114] metabolic processes, inflammatory track, and cell proliferation, activation, or motility;[115] primary hemostasis, integrin and kinase signaling linked to integrins, glycolysis, and acute phase reactants.[116] Nevertheless, metabolomics analysis suggested clot sorbitol content as a surrogate marker reflecting blood glucose level at stroke onset.[117]

PLT, RBC, and WBC content of AIS thrombi could be estimated through biochemical quantification of glycoprotein (GP) VI (immunoassay), heme (formic acid-based colorimetric assay), and DNA (dsDNA fluorescence assay kit) in thrombus homogenates.[118] Hemoglobin (ELISA) and heme content were highly correlated with RBC content determined by flow cytometry.[119] Different lymphocyte profiles were identified in cell suspensions of clots subjected to flow cytometry.[120]

The presence of NETs was confirmed in intact thrombus samples subjected to endonuclease treatment to release NE activity,[95] and by incubating with DNase-I to produce *ex vivo* thrombolysis.[71] Thrombin elution pattern assessed by measuring secreted thrombin activity along serial washings has been suggested as a biomarker of clot content.[121]

Relationship between thrombus features and stroke etiology

Analysis of thrombus size, structure and composition in patient cohorts found associations with TOAST etiology (large-artery atherosclerosis [LAA; TOAST 1]; cardioembolism [CE; TOAST 2]; stroke of other determined cause [ODC; TOAST 4]; cryptogenic [CRY] stroke of undetermined etiology [TOAST 5]), embolic stroke of undetermined source (ESUS), or underlying pathologies (Supplementary Table 2). LAA thrombi showed a larger ECA[39,40] and higher number of fragments.[40] Eight studies, reporting results from 1,183 thrombi (median 73), showed higher RBC proportions in thrombi from LAA or non-CE (LAA+ODC) sources.[39,57,64,82,96,122-124] Contrastingly, three studies (119 thrombi, median 37) showed higher RBC proportions in CE thrombi.[72,84,125] Seven studies (1,061 thrombi, median 58) showed higher FBR proportions in CE,[39,57,76,82,96,123,124] while in two studies (82 thrombi, median 41) the FBR proportion was higher in LAA.[72,125] CE thrombi also showed a higher FP (FBR+PLTs) proportion in one study (137 thrombi).[122] PLT proportion was higher in LAA in two studies (1,127 thrombi, median 563.5),[50,59] but higher in CE in another two studies (697 thrombi, median 348.5).[39,67] Further support for higher PLT proportions in non-CE thrombi came from higher GP VI content in thrombus homogenates.[118] LAA thrombi showed mostly peripheral PLT distribution patterns (PDPs), while mostly clustering PDP was observed in CE.[51] Non-CE thrombi showed higher RBC/PLT ratio,[73] in line with lower FP/RBC ratio.[126] Results from the large multicentric STRIP registry (1,350 thrombi), published during the preparation of this manuscript, showed that LAA thrombi had a higher mean RBC density (46%±23% vs. 42%±22%, P=0.01) and a lower PLT density (24%±18% vs. 27%±18%, P=0.03) than CE thrombi.[147] Regarding WBCs, three studies (358 thrombi, median 137) showed higher proportions in CE,[89,96,122] while one study (37 thrombi) reported higher proportions in LAA.[84] Another study supported higher WBC proportions in CE, as estimated by DNA content in thrombus homogenates.[118] When WBC subtypes were analyzed, CE thrombi showed higher contents of neutrophils,[76] NETs,[66,93] and netting neutrophils,[66] as well as more macrophages.[67] As for lymphocytes, CE thrombi contained more suppressor-cytotoxic T-cells,[120] while LAA thrombi showed higher T-cell,[90] helper T-cell, and natural killer (NK)-cell contents.[120] Finally, higher IL-1β expression was measured in LAA thrombi,[112] while coagulation factor XIII was associated with CE.[115]

Some studies aimed to compare the features of CRY thrombi with those of known TOAST etiology. Most of them found similar proportions of RBCs,[39,57,64,96,122-124,127] FBR, PLTs (or both together),[57,76,96,122-124,127] and WBCs between CRY and CE thrombi.[96,122,127] CRY and CE thrombi also shared smaller ECA and number of retrieved fragments,[39,40] clustering PDP,[51] higher neutrophil counts,[76] and NETosis,[66] temporal profile of eluted thrombin activity,[121] and low expression of IL-1β.[112] ESUS and CE thrombi showed similar RBC/PLTs ratios.[73] Conversely, a few studies found similar proportions of RBCs,[84] PLTs,[50] and WBCs[84,89] in CRY and LAA thrombi. Finally, ESUS and LAA thrombi shared low macrophage proportions.[67]

Regardless of TOAST etiology, some thrombus features have been associated with the patient's age and underlying pathologies. The clots from elderly subjects had higher FBR proportions compared to younger patients.[128] As for gross appearance, white thrombi were much more frequent in the context of pa-

thologies like active cancer (AC) or infective endocarditis (IE).[36,38] Furthermore, thrombi showed lower RBC proportions, and higher FBR and/or PLT proportions, with underlying AC[38,65] or diabetes mellitus (DM).[55] Other composition characteristics were lower WBC proportion in AC,[38] and higher NETosis in DM.[94] Direct presence of tumor cells in the thrombus was rare in AC.[129] Contrastingly, bacteria or fungi were present with underlying IE[7,109] or other systemic infections.[109]

Thrombus analysis in individual cases was a valuable complementary tool in diagnosing the stroke etiology, especially atypical ones (Supplementary Table 3). Proteomic analysis determined a common origin for tandem occlusions after traumatic carotid thrombosis and embolization.[130] Macroscopic and thorough histopathological examination confirmed the occlusion by an intracranial atherosclerotic plaque, revealing the atherothrombotic etiology.[19] Identification of cocci or bacilli in the thrombus confirmed septic emboli in cases of bacterial IE,[14,18,31,32,102,131-135] including a rare case of Whipple's endocarditis.[26] Similarly, the presence of *Candida* confirmed septic embolus in a case of fungal IE.[136] Observation of fungal hyphae confirmed cases of septic embolus[137] and angioinvasive mucormycosis secondary to sinusitis.[138] Identification of papillary fronds or myxomatous tissue helped diagnose embolization secondary to cardiac papillary fibroelastoma[15,16,20] and myxoma,[29] respectively. Visual assessment and histopathology identified valve tissue,[24] calcifications,[27,30] chordae tendineae,[21] and aortic wall tissue[23] as embolism sources, which detached spontaneously or periprocedurally during valve replacement surgery. Immunohistopathology contributed to diagnose the embolism of a Libman-Sacks vegetation in systemic lupus erythematosus-associated endocarditis.[22] Thrombus calcification, cholesterol crystal cleft and foamy cells confirmed the aortogenic embolic stroke due to atheromatous lesion in the aortic arch.[139,140] Embolizations of carotid free-floating and carotid web thrombi were determined by assessing the clot aspect and blood cell content.[28,141] Clot visual appearance and high FBR content indicated thromboembolism secondary to coronavirus disease 2019-associated hypercoagulability[34] and cancer-related Trousseau syndrome.[33] Finally, thrombus examination confirmed periprocedural catheter-related thromboembolism during valve implantation[17] and inadvertent embolization of foreign bodies during aneurysm treatment.[25]

Discussion

In response to the four research questions posed in this study, our results show that:

(1) Thrombi were subjected to physical analyses (macroscopic appearance, mechanical behavior, MRI, and synchrotron-based imaging), histopathological analyses (conventional histology, immunohistochemistry, and electron microscopy), biological analyses (biochemical, biomolecular, and cellular techniques, such as enzymatic assay, ELISA, transcriptomics, proteomics, metabolomics, and flow cytometry), and microbiological analyses (culture, histological staining, and metagenomics).

(2) The information obtained about thrombi included gross appearance (size, shape, consistency, visual texture, color, and homo/heterogeneous aspect), mechanical properties (stiffness, elasticity, tensile strength, and response to stress), structure (compactness, molecular and cellular organization, and ultrastructure), and composition (FBR, other coagulation factors, blood formed elements, NETs, vessel wall and plaque components, microbial pathogens, inflammatory mediators, protein cargo, metabolites, elements, and even foreign bodies).

(3) There were associations of thrombus size, structure and composition (mainly proportions of FBR and blood formed elements) with TOAST etiology and underlying pathologies, and similarities between cryptogenic thrombi and those of known TOAST etiology.

(4) Individual thrombus analysis proved to be a valuable complementary tool in the diagnosis of stroke etiology, particularly in atypical cases.

The first endovascular device clearance by the U.S. Food and Drug Administration occurred in 2004,[148] and the first report describing a systematic histological analysis of thrombi retrieved from cerebral arteries was published in 2006.[7] Since then, a total of 134 eligible studies have been identified in this systematic review. Of note, there has been a surge of reports in the last 5 years, just after the publication of Brinjikji's systematic review.[3] Although most studies carried out histopathological analyses, studies reporting biochemical or biomolecular analyses have been increasing in the last 3 years. Almost 10,000 thrombi/emboli retrieved from LVO stroke patients have been analyzed predominantly in cohort studies but also in case reports.

Physical, histopathological and biological analyses provided a considerable amount of information about the gross appearance, mechanical properties, structure, and composition of thrombi in patient cohorts. However, assessments of possible associations of thrombus features with stroke causes have been carried out mainly based on thrombus size, color, and proportions of FBR and blood formed elements. LAA thrombi were larger and more fragmented. Regarding composition, non-unanimous evidence supported higher RBC contents in

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thrombi from LAA or non-CE (LAA+ODC) sources, and higher FBR contents in thrombi of CE origin. WBC content, neutrophil count, and NETosis were also higher in CE thrombi. As for the PLT proportion, controversial histopathological evidence did not support a clear association with the CE or non-CE cause, although non-CE thrombi showed higher GP VI content (PLT marker), and peripheral PDP. Cryptogenic mechanisms account for 10% to 40% of all ischemic strokes.[149] Most evidence pointed to similar features in CRY and CE thrombi, mainly regarding proportions of RBCs, FBR, PLTs, and WBCs, and smaller size and number of retrieved fragments, clustering PDP, and higher NETosis. This could aid the etiologic investigation and reduce the percentage of CRY strokes. Of note, the large multicentric STRIP registry found statistically significant but clinically insignificant differences between clots of CE and LAA etiologies.[147] Although LAA clots had a higher mean RBC density and a lower mean PLT density than CE clots, the receiver operating characteristics analysis showed that identification of a reliable threshold with a high area under the curve for differentiating clots of these two etiologies based on composition analysis alone was not possible; this suggests that conventional histological analyses of the cellular composition do not provide insights into stroke etiology in cryptogenic cases. Regardless of TOAST etiology, a few studies found associations of thrombus color and composition with underlying pathologies like cancer, diabetes, and IE.

Macroscopic, histopathological, biomolecular, and microbiological analyses of thrombi were useful in the diagnosis of the stroke cause in individual cases. Thrombus features confirmed cases of typical intracranial and carotid thromboembolism, and atypical free-floating and web thrombi. Histopathology also contributed to diagnose embolisms due to cardiac tumor fragments, sterile endocarditis vegetations, aortogenic lesions, and tissue fragments detached during valve replacement surgery. Microbiological analysis revealed septic emboli secondary to IE, allowing appropriate antibiotic therapy. Finally, thromboembolisms due to secondary hypercoagulable states were diagnosed based on clot features.

Current methods of assessing thrombus features vary widely. Despite a consensus statement on the analyses of thrombi in AIS[1] and recommendations for thrombus handling and procedures,[13] the results in this review show a lack of standardization in the research and reporting of thrombus characteristics and parameters. Heterogeneity in study designs, outcome measuring, and reporting precluded a meta-analysis of the association between thrombus composition and stroke etiology. Hopefully, ongoing multi-institutional registers, larger cohorts, and homogenous protocols will overcome this limitation. Although traditional histopathological techniques seem of limited value, the application of pathophysiological classifications show promise in differentiating between CE and LAA emboli.[150] Nevertheless, focusing on immunohistochemical analysis and more advanced techniques could help increase the knowledge on the composition and structure of thrombi. Advanced analysis of microRNA signatures,[151] proteomic analysis,[152] and combined "omic" analysis (proteome and metabolome)[153] are promising molecular approaches to elucidate the composition of emboli. Moreover, ultrastructural analysis using high-resolution SEM shows the finely organized clot components.[56] The role of all these advanced techniques in identifying stroke etiology could be further explored.

Conclusions

Although cohort studies report an abundance of quantitative rates of main thrombus components, a definite clot signature for accurate diagnosis of stroke etiology is still lacking. Nevertheless, qualitative examination of the embolus remains an invaluable tool in the diagnostic work-up of individual cases, particularly regarding atypical stroke causes. Beyond conventional histopathological and immunohistochemical clot analyses, future studies should emphasize the analysis of biomolecular composition and structural organization to provide insights about reliable links between clot features and stroke etiology.

Supplementary materials

Supplementary materials related to this article can be found online at https://doi.org/10.5853/jos.2021.02306.

Disclosure

The authors have no financial conflicts of interest.

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Supplementary Table 1. Studies included in the qualitative synthesis

Study	Type	Sample size	Physical analysis	Histopathology	Biological analysis	Microbiology	Diagnostic
Abdel-Wahed et al. (2019) Am J Med[14]	Case report	1	Yes	Yes	No	Yes	Atypical etiology; Underlying pathology
Ahn et al. (2016) Int J Stroke[57]	Cohort	36	No	Yes	No	No	TOAST
Almekhlafi et al. (2008) Ann Neurol[43]	Case series	5	Yes	Yes	No	No	None
Ambrosioni et al. (2018) Clin Infect Dis[131]	Case series	6	No	No	No	Yes	Atypical etiology; Underlying pathology
Anuwatworn et al. (2015) JACC Cardiovasc Interv[24]	Case report	1	Yes	Yes	No	No	Atypical etiology; Underlying pathology
Baek et al. (2018) Ann Clin Transl Neurol[112]	Cohort	82	No	No	Yes	No	TOAST
Bain et al. (2011) J Neuroimaging[132]	Case report	1	No	Yes	No	Yes	Atypical etiology; Underlying pathology
Benson et al. (2020) J NeuroIntervent Surg[58]	Cohort	57	No	Yes	No	No	None
Berndt et al. (2018) World Neurosurg[126]	Cohort	137	No	Yes	No	No	TOAST
Berndt et al. (2018) Stroke[146]	Cohort	32	No	Yes	No	No	None
Berndt et al. (2021) Clin Neuroradiol[145]	Cohort	59	No	Yes	No	No	None
Bhaskar et al. (2019) Eur J Neurol[127]	Cohort	85	No	Yes	No	No	TOAST
Bhaskar et al. (2019) Can J Neurol Sci[102]	Case series	4	No	Yes	No	Yes	Atypical etiology; Underlying pathology
Biraschi et al. (2016) J Stroke Cerebrovasc Dis[15]	Case report	1	Yes	Yes	No	No	Atypical etiology; Underlying pathology
Boeckh-Behrens et al. (2016) Clin Neuroradiol[89]	Cohort	34	No	Yes	No	No	TOAST
Boeckh-Behrens et al. (2016) Stroke[122]	Cohort	137	No	Yes	No	No	TOAST
Bourcier et al. (2020) J Stroke Cerebrovasc Dis[35]	Cohort	139	Yes	No	No	No	None
Brinjikji et al. (2020) Stroke[59]	Cohort	1,022	No	Yes	No	No	TOAST
Chapot et al. (2006) Am J Neuroradiol[25]	Case report	1	Yes	Yes	No	No	Atypical etiology
Chen et al. (2021) Front Pharmacol[99]	Cohort	30	No	Yes	Yes	No	None
Choi et al. (2018) Stroke[37]	Cohort	52	Yes	Yes	No	No	None
Chueh et al. (2011) Am J Neuroradiol[44]	Cohort	4	Yes	Yes	No	No	None
Cline et al. (2013) J NeuroIntervent Surg[106]	Cohort	16	No	Yes	No	No	None
Darcourt et al. (2021) Int J Stroke[87]	Cohort	102	No	Yes	No	No	None
Dargazanli et al. (2016) PLoS ONE[90]	Cohort	54	No	Yes	No	No	TOAST
Dargazanli et al. (2020) Front Neurol[115]	Cohort	60	No	No	Yes	No	TOAST
Deng et al. (2020) Neurosci Lett[94]	Cohort	46	No	Yes	No	No	Underlying pathology
Di Meglio et al. (2020) Eur J Neurol[119]	Cohort	84	No	No	Yes	No	None
Di Meglio et al. (2020) Stroke[118]	Cohort	250	No	No	Yes	No	TOAST
Di Meglio et al. (2019) Neurology[63]	Cohort	199	No	Yes	No	No	None
Distefano et al. (2019) J Stroke Cerebrovasc Dis[133]	Case report	1	No	Yes	No	Yes	Atypical etiology; Underlying pathology
Donnerstag et al. (2021) Intervent Neuroradiol[91]	Cohort	302	No	Yes	No	No	None
Douglas et al. (2020) J NeuroIntervent Surg[60]	Cohort	91	No	Yes	No	No	None
Ducroux et al. (2018) Stroke[95]	Cohort	108	No	Yes	Yes	No	None
Duffy et al. (2019) Stroke[61]	Cohort	106	No	Yes	No	No	None
Elijovich et al. (2018) Stroke[75]	Cohort	25	No	Yes	Yes	No	None
Elodie et al. (2019) J Neurol Sci[26]	Case report	1	Yes	No	No	Yes	Atypical etiology; Underlying pathology
Essig et al. (2020) Int J Mol Sci[76]	Cohort	37	No	Yes	No	No	TOAST
Fassa et al. (2014) Circ Cardiovasc Interv[27]	Case report	1	Yes	Yes	No	No	Atypical etiology

Supplementary Table 1. Continued

Study	Type	Sample size	Physical analysis	Histopathology	Biological analysis	Microbiology	Diagnostic
Fitzgerald et al. (2019) Stroke[50]	Cohort	105	No	Yes	No	No	TOAST
Fitzgerald et al. (2021) J Stroke Cerebrovasc Dis[40]	Cohort	550	Yes	No	No	No	TOAST
Fitzgerald et al. (2019) PLoS ONE[48]	Cohort	50	No	Yes	No	No	None
Fitzgerald et al. (2020) J NeuroIntervent Surg[39]	Cohort	612	Yes	Yes	No	No	TOAST
Fitzgerald et al. (2019) J NeuroIntervent Surg[49]	Cohort	85	No	Yes	No	No	None
Fitzpatrick et al. (2018) J NeuroIntervent Surg[28]	Case series	3	Yes	Yes	No	No	Atypical etiology
Fu et al. (2020) Stroke[38]	Cohort	152	Yes	Yes	No	No	Atypical etiology; Underlying pathology
Funatsu et al. (2019) J NeuroIntervent Surg[101]	Cohort	150	No	Yes	No	No	None
Garcia-Ptacek et al. (2014) J NeuroIntervent Surg[29]	Case report	2	Yes	Yes	No	No	Atypical etiology; Underlying pathology
Genchi et al. (2020) J Neurol Sci[30]	Case report	1	Yes	Yes	No	No	Atypical etiology
Goebel et al. (2020) Am J Neuroradiol[67]	Cohort	85	No	Yes	No	No	TOAST
Gong et al. (2019) Cell Transplant[125]	Cohort	45	No	Yes	No	No	TOAST
Gurkas et al. (2019) Stroke[77]	Cohort	111	No	Yes	No	No	None
Hanning et al. (2021) J NeuroIntervent Surg[88]	Cohort	112	No	Yes	No	No	None
Hashimoto et al. (2016) Stroke[78]	Cohort	83	No	Yes	No	No	None
Hernández-Fernández et al. (2017) Cardiovasc Intervent Radiol[109]	Cohort	65	No	Yes	No	Yes	Atypical etiology; Underlying pathology
Hinman et al. (2013) Front Neurol[130]	Case report	1	No	Yes	Yes	No	Atypical etiology
Horie et al. (2019) World Neurosurg[42]	Cohort	65	No	Yes	No	No	None
Hund et al. (2019) J Cereb Blood Flow Metab[104]	Cohort	281	No	Yes	No	No	None
Itsekson Hayosh et al. (2020) J NeuroIntervent Surg[121]	Cohort	68	No	No	Yes	No	TOAST
Juega et al. (2019) Stroke[120]	Cohort	40	No	No	Yes	No	TOAST
Kaesmacher et al. (2017) Am J Neuroradiol[92]	Cohort	85	No	Yes	No	No	None
Kan et al. (2012) World Neurosurg[31]	Case report	1	Yes	No	No	Yes	Atypical etiology; Underlying pathology
Katano et al. (2020) Clin Neurol[137]	Case report	1	No	Yes	No	Yes	Atypical etiology; Underlying pathology
Khashim et al. (2019) Interv Neuroradiol[108]	Cohort	20	No	No	No	Yes	None
Khismatullin et al. (2020) BioNanoScience[62]	Case series	3	No	Yes	No	No	None
Kim et al. (2020) J Clin Neurosci[51]	Cohort	52	No	Yes	No	No	TOAST
Kim et al. (2014) Neurointervention[32]	Case report	1	Yes	Yes	No	Yes	Atypical etiology; Underlying pathology
Kim et al. (2015) Am J Neuroradiol[72]	Cohort	37	No	Yes	No	No	TOAST
Koneru et al. (2021) J NeuroIntervent Surg[141]	Case series	3	No	Yes	No	No	Atypical etiology; Underlying pathology
Krajíčková et al. (2018) Circ J[98]	Cohort	80	No	Yes	No	No	None
Laridan et al. (2017) Ann Neurol[93]	Cohort	68	No	Yes	No	No	TOAST
Li et al. (2020) BMC Neurology[107]	Case report	2	No	Yes	No	No	None
Liao et al. (2020) Front Neurol[123]	Cohort	88	No	Yes	No	No	TOAST
Liebeskind et al. (2011) Stroke[79]	Cohort	50	No	Yes	No	No	None
Liu et al. (2020) J Neurosurg[45]	Cohort	16	Yes	Yes	No	No	None
Liu et al. (2020) Thromb Res[80]	Cohort	84	No	Yes	No	No	None
López-Cancio et al. (2013) Cerebrovasc Dis[74]	Cohort	15	No	Yes	No	No	None
Lopez-Pedrera et al. (2020) Res Pract Thromb Haemost[116]	Cohort	50	No	No	Yes	No	None

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Supplementary Table 1. Continued

Study	Type	Sample size	Physical analysis	Histopathology	Biological analysis	Microbiology	Diagnostic
Maegerlein et al. (2018) Intervent Neuroradiol[81]	Cohort	64	No	Yes	No	No	None
Maekawa et al. (2018) Cerebrovasc Dis Extra[82]	Cohort	43	No	Yes	No	No	TOAST
Marder et al. (2006) Stroke[7]	Cohort	25	No	Yes	No	Yes	Atypical etiology; None; Underlying pathology
Matsumoto et al. (2016) J Stroke Cerebrovasc Dis[33]	Case report	2	Yes	Yes	No	No	Atypical etiology; Underlying pathology
Matsumoto et al. (2020) J Stroke Cerebrovasc Dis[139]	Case report	1	No	Yes	No	No	Atypical etiology
Meher et al. (2018) Int J Stroke[47]	Cohort	19	Yes	Yes	No	No	None
Mehta et al. (2019) J NeuroIntervent Surg[103]	Cohort	92	No	Yes	No	No	None
Meng et al. (2020) Oxid Med Cell Longev[128]	Cohort	147	No	Yes	No	No	None
Mereuta et al. (2020) Stroke[100]	Cohort	79	No	Yes	No	No	TOAST
Mereuta et al. (2021) J NeuroIntervent Surg[56]	Cohort	10	No	Yes	No	No	None
Mönch et al. (2021) Clin Neuroradiol[142]	Cohort	69	No	Yes	No	No	None
Muñoz et al. (2018) Int J Mol Sci[114]	Cohort	4	No	No	Yes	No	None
Nakanishi et al. (2018) Clin Neurol[134]	Case report	1	No	No	No	Yes	Atypical etiology; Underlying pathology
Niesten et al. (2014) PLoS ONE[64]	Cohort	22	No	Yes	No	No	TOAST
Nouh et al. (2020) BMC Neurology[73]	Cohort	33	No	Yes	No	No	TOAST
Novotny et al. (2020) Neurology[66]	Cohort	71	No	Yes	No	No	TOAST
Park et al. (2019) Ann Neurol[65]	Cohort	48	No	Yes	No	No	Atypical etiology; Underlying pathology
Patel et al. (2021) Am J Neuroradiol[144]	Cohort	40	No	Yes	No	No	None
Patrakka et al. (2019) J Am Heart Assoc[110]	Cohort	75	No	No	No	Yes	None
Peña-Martínez et al. (2019) Stroke[71]	Cohort	10	No	Yes	Yes	No	None
Pisano et al. (2020) J Stroke Cerebrovasc Dis[34]	Case report	1	Yes	No	No	No	Atypical etiology; Underlying pathology
Prochazka et al. (2018) Med Sci Monit[97]	Cohort	90	No	Yes	No	No	None
Qureshi et al. (2016) J Vasc Intervent Neurol[83]	Cohort	18	No	Yes	No	No	None
Rao et al. (2017) Front Neurol[113]	Cohort	20	No	No	Yes	No	None
Rossi et al. (2021) J Thromb Thrombolysis[41]	Cohort	550	Yes	No	No	No	None
Salam et al. (2018) J Stroke Cerebrovasc Dis[16]	Case report	1	Yes	Yes	No	No	Atypical etiology; Underlying pathology
Salinas et al. (2013) JACC Cardiovasc Interv[17]	Case report	1	Yes	No	No	No	Atypical etiology
Sallustio et al. (2015) J Neurol Disord Stroke[105]	Cohort	28	No	Yes	No	No	None
Scharf et al. (2017) J NeuroIntervent Surg[18]	Case report	1	Yes	Yes	No	Yes	Atypical etiology; Underlying pathology
Scharf et al. (2016) Neurocrit Care[138]	Case report	1	No	No	No	Yes	Atypical etiology; Underlying pathology
Schuhmann et al. (2016) Int J Mol Sci[52]	Cohort	37	No	Yes	No	No	None
Semerano et al. (2019) J Neurol[19]	Case report	1	Yes	Yes	No	No	TOAST
Sgreccia et al. (2020) J Neuroradiol[136]	Case report	1	No	No	No	Yes	Atypical etiology; Underlying pathology
Sgreccia et al. (2019) J NeuroIntervent Surg[36]	Cohort	255	Yes	No	No	No	Atypical etiology; Underlying pathology
Shin et al. (2018) PLoS ONE[84]	Cohort	93	No	Yes	No	No	TOAST
Simons et al. (2015) J Neuroradiol[85]	Cohort	40	No	Yes	No	No	None
Singh et al. (2013) Stroke[86]	Cohort	48	No	Yes	No	No	None

Supplementary Table 1. Continued

Study	Type	Sample size	Physical analysis	Histopathology	Biological analysis	Microbiology	Diagnostic
Sporns et al. (2021) J NeuroIntervent Surg[68]	Cohort	163	No	Yes	No	No	None
Sporns et al. (2017) Stroke[96]	Cohort	187	No	Yes	No	No	TOAST
Sporns et al. (2017) Cerebrovasc Dis[70]	Cohort	180	No	Yes	No	No	None
Sporns et al. (2019) Stroke[69]	Cohort	163	No	Yes	No	No	None
Staessens et al. (2020) Haematologica[53]	Cohort	177	No	Yes	No	No	None
Suissa et al. (2020) Metabolites[117]	Cohort	41	No	No	Yes	No	None
Sukumaran et al. (2012) Neurol India[135]	Case report	1	No	Yes	No	Yes	Atypical etiology; Underlying pathology
Tejada et al. (2014) J NeuroIntervent Surg[20]	Case report	1	Yes	Yes	No	No	Atypical etiology; Underlying pathology
Thomas et al. (2017) J NeuroIntervent Surg[21]	Case report	1	Yes	Yes	No	No	Atypical etiology; Underlying pathology
Usui et al. (2019) Intern Med[140]	Case report	1	No	Yes	No	No	Atypical etiology
Vajpeyee et al. (2021) Neurointervention[111]	Cohort	4	No	No	No	Yes	TOAST
Valente et al. (2019) J Clin Neurosci[22]	Case report	1	Yes	Yes	No	Yes	Atypical etiology; Underlying pathology
Vidmar et al. (2019) Radiol Oncol[46]	Cohort	17	Yes	No	No	No	None
Wei et al. (2021) Radiology[143]	Cohort	77	No	Yes	No	No	None
Wollenweber et al. (2016) Neurology[23]	Case report	1	Yes	Yes	No	No	Atypical etiology
Wolpert et al. (2020) Eur J Neurol[129]	Cohort	32	No	Yes	No	No	Atypical etiology; Underlying pathology
Xue et al. (2018) Natl Med J China[124]	Cohort	58	No	Yes	No	No	TOAST
Ye et al. (2020) Interv Neuroradiol[55]	Cohort	52	No	Yes	No	No	Underlying pathology
Ye et al. (2020) Clin Neurol Neurosurg[54]	Cohort	54	No	Yes	No	No	None

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Supplementary Table 2. Cohort studies: thrombus composition and TOAST stroke etiology or underlying pathology

Study	Sample size (clots/ patients)	Analysis	Thrombus composition items	Etiology groups (patients)	Association composition-etiology or underlying pathology
Ahn et al. (2016) Int J Stroke[57]	36	Histopathology	RBC and FBR proportions	LAA (8), CE (22), and CRY (6)	RBCs most abundant (56.9%±12.2%) in LAA, higher than CE. FBR most abundant (39.5%±13.5%) in CE, higher than LAA. Similar composition in CRY and CE
Baek et al. (2018) Ann Clin Transl Neurol[112]	82	Biomolecular RTqPCR	Expression of inflammatory mediators	LAA (9), CE (51), and CRY (22)	Higher IL-1β expression in LAA than in both CE and CRY. Similar expression in CRY and CE
Berndt et al. (2018) World Neurosurg[126]	137	Histopathology	FP/RBC ratio	LAA (22), CE (67), ODC (11), and CRY (36)	Higher FP/RBC in CE+CRY than non-CE (LAA+ODC)
Bhaskar et al. (2019) Eur J Neurol[127]	85	Histopathology	RBC, FP, and WBC proportions	CE, non-CE, and CRY	RBC (26%), FP (61%) and WBC (11%) proportions in CE similar to RBC (28%), FP (64%) and WBC (9%) proportions in CRY. Different proportions in non-CE
Boeckh-Behrens et al. (2016) Clin Neuroradiol[89]	34	Histopathology	RBC, FP, and WBC proportions	LAA (3), CE (16), ODC (6), and CRY (9)	Higher proportion of WBCs in CE than in LAA or CRY
Boeckh-Behrens et al. (2016) Stroke[122]	137	Histopathology	RBC, FP, and WBC proportions	LAA (22), CE (67), ODC (11), and CRY (36)	Different composition in CE (RBC 38.3%±20.0%, FP 52.6%±18.6% and WBC 9.1%±6.4%) and in non-CE (LAA+ODE) (RBC 52.7%±25.2%, FP 40.9%±23.3%, and WBC 6.5%±3.8%). Similar composition in CRY (RBC 42.0%±21.4%, FP 50.8%±20.8%, and WBC 7.1%±4.5%) and in CE
Brinjikji et al. (2020) Stroke[59]	1,022	Histopathology	PLT proportion	LAA and CE	Higher PLT content in LAA (PLT-rich clots [55.0%], PLT-area [22.1%]) than in CE (PLT-rich clots [21.2%], PLT-area [13.9%])
Dargazanli et al. (2016) PLOS One[90]	54	Histopathology	CD3+ T-cell count	LAA (10), CE (25), and other causes (ODC+CRY, 19)	Higher T-cell count in LAA (53.60±28.78) than in both CE (20.08±15.66) or other causes (21.77±18.31)
Dargazanli et al. (2020) Front Neurol[115]	60	Biomolecular Proteomics	Relative protein	LAA (28) and CE (32)	Coagulation factor XIII associated with CE
Deng et al. (2020) Neurosci Lett[94]	46	Histopathology	NETs (H3Cit) proportion	NG (28), AHG (9), and DM (9)	Higher NETs proportion in both AHG and DM than in NG
Di Meglio et al. (2020) Stroke[118]	250	Biochemical	GP (glycoprotein) VI, heme, and DNA contents	CE (142), non-CE (33), and ESUS (75)	CE richer in DNA (35.8 ng/mg), i.e., more leukocytes, and poorer in GP VI (0.104 ng/mg), i.e., less PLTs, than non-CE (DNA 13.8 ng/mg; GP VI 0.117 ng/mg)
Essig et al. (2020) Int J Mol Sci[76]	37	Histopathology	Neutrophil count and FBR proportion	CE (21), non-CE (7), and CRY (9)	Higher neutrophil counts in both CE (799.1±477.6 cells/mm^2) and CRY (734.1±329.1 cells/mm^2) compared to non-CE (376±128.5 cells/mm^2). Higher FBR proportion in both CE (46.1%±29.9%) and CRY (46.6%±21.8%) compared to non-CE (25.9%±12.1%)
Fitzgerald et al. (2019) Stroke[50]	105	Histopathology	RBC, WBC, FBR, and PLTs+other proportions	LAA (20), CE (52), ODC (12), and CRY (21)	Higher PLT content in LAA (PLT-rich clots [55.0%], PLT-area [22.1%±18.6%]) than in CE (PLT-rich clots [21.2%], PLT-area [13.9%±14.3%]). More PLT-rich clots in both LAA (55.0%) and CRY (50.0%) than in CE (21.2%)
Fitzgerald et al. (2020) J NeuroInterv Surg[39]	612 / 441	Histopathology	ECA; RBC, WBC, FBR, PLTs+other, and collagen proportions	LAA (115), CE (209), ODC (16), and CRY (101)	Larger ECA in LAA (54.96 mm^2) than in CE (33.64 mm^2), ODC (39.60 mm^2), and CRY (32.28 mm^2). Higher RBC proportion in LAA (48.89%) than in CE (35.57%), ODC (42.82%), and CRY (39.08%). Highest proportion of both FBR (33.3%) and PLTs+other (28.53%) in CE
Fitzgerald et al. (2021) J Stroke Cerebrovasc Dis[40]	550	Histopathology	ECA. Number of clot fragments	LAA (110), CE (197), ODC (33), and CRY (143). Excluded (67)	Larger ECA in LAA (109 mm^2) than in CE (52 mm^2), ODC (52 mm^2), and CRY (47 mm^2). Greater number of fragments in LAA (5.36) than in CE (3.72), ODC (3.73), and CRY (3.52)

Supplementary Table 2. Continued

Study	Sample size (clots/ patients)	Analysis	Thrombus composition items	Etiology groups (patients)	Association composition-etiology or underlying pathology
Fu et al. (2020) Stroke[38]	152	Macroscopic. Histopathology	RBC, FP, and WBC proportions	AC (19), LAA (26), and CE (107)	White gross appearance in AC vs. darker/reddish in LAA or CE. Higher FP proportion in AC (85.7%) than in LAA (42.5%) or CE (43.9%). Lower RBC proportion in AC (8.1%) than in LAA (51.7%) or CE (52.2%). Lower WBC proportion in AC (1.9%) than in LAA (3.1%) or CE (3.7%)
Goebel et al. (2020) Am J Neuroradiol[67]	85	Histopathology	RBC, WBC (macrophages, lymphocytes, granulocytes), FBR, and PLTs proportions	LAA (16), CE (51), ODC (1), and ESUS (17)	Higher proportion (range) of macrophages in CE (0.9% [0.1%–3.3%]) than in LAA (0.3% [0.1%–3.8%]) or ESUS (0.4% [0.0%–5.2%]). Higher proportion of PLTs in CE (19.1% [3.6%–81.1%]) than LAA (10.3% [2.3%–25.1%])
Gong et al. (2019) Cell Transplant[125]	45	Histopathology	RBC and FBR proportions	LAA (9) and CE (36)	Higher RBC proportion in CE (69%) than in LAA (55.5%). Lower FBR proportion in CE (31%) than in LAA (44.5%)
Hernández-Fernández et al. (2017) Cardiovasc Intervent Radiol[109]	65	Histopathology. Bacteriological	Distribution of RBCs, PLTs, and WBCs. Bacteria presence	CE (38), non-CE or CRY (27)	Gram-positive bacteria in four thrombi: infective endocarditis (2), urinary tract infection (1), and pneumonia (1)
Itsekson Hayosh et al. (2020) J NeuroInterv Surg[121]	68	Biochemical	Eluted thrombin activity (ETA)	LAA (15), CE (18), ODC (18), and CRY (17)	Temporal profile of ETA similar in CRY and CE, and different from LAA
Juega et al. (2019) Stroke[120]	40	Flow cytometry	Leukocyte populations	LAA, CE, and ODC	Higher proportion of CD4 T lymphocytes in LAA (24.85%) than in CE (15.83%). Higher proportion of natural killer (NK) cells in LAA (21.08%) than in CE (17.04%). Lower proportion of CD8 T lymphocytes in LAA (13.56%) than in CE (20.24%)
Kim et al. (2020) J Clin Neurosci[51]	52	Histopathology	RBC, FBR, and PLTs proportions. PLT distribution pattern (PDP)	LAA (10), CE (31), and CRY (11)	Mostly peripheral PDP in LAA (70%). Mostly clustering PDP in CE (77.4%). Similar PDPs in CE and CRY.
Kim et al. (2015) Am J Neuroradiol[72]	37	Histopathology	RBC, FBR, PLTs, and WBC proportions	LAA (8), CE (22), and CRY (7)	Higher RBC proportion in CE (37.8%) than in LAA (16.9%). Lower FBR proportion in CE (32.3%) than in LAA (48.5%)
Laridan et al. (2017) Ann Neurol[93]	68	Histopathology	NETs (H3Cit) proportion	LAA (7), CE (40), ODC (6), and CRY (15)	Nearly double amount of NETs in CE (3.07%±2.21%) than non-CE (LAA+ODC; 1.57%±1.23%)
Liao et al (2020) Front Neurol[123]	88	Histopathology	RBC, FBR, and PLTs proportions. WBC count	LAA (25), CE (46), ODC (6), and CRY (11)	Higher RBC proportion (range) in LAA (53.44% [49.91%–56.97%]) than in CE (35.70% [32.04%–39.36%]) or CRY (38.18% [31.01%–45.35%]). Higher FBR proportion in both CE (35.91% [31.44%–40.39%]) and CRY (39.73% [27.97%–51.49%]) than in LAA (22.96% [17.81%–28.11%]) or ODC (26.33% [12.31%–40.36%]).
Maekawa et al. (2018) Cerebrovasc Dis Extra[82]	43	Histopathology	RBC, FBR, and WBC proportions	LAA (5), CE (30), ODC (1), and CRY (7)	Lower RBC proportion in CE (29.5%±26.2%) than in non-CE (49.6%±26.1%). Higher FBR proportion in CE (66.2%±25.8%) than in non-CE (46.4%±25.5%).
Marder et al. (2006) Stroke[7]	25	Histopathology	Distribution of RBCs, PLTs, and WBCs. Fungi presence	LAA (4), CE (16), ODC (3), and CRY (2)	One mycotic thrombus. Aortic valve infective endocarditis
Mereuta et al. (2020) Stroke[100]	79	Histopathology	VWF proportion	LAA (13), CE (39), ODC (12), and CRY (15)	Higher VWF proportion in CRY when compared to CE

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Supplementary Table 2. Continued

Study	Sample size (clots/ patients)	Analysis	Thrombus composition items	Etiology groups (patients)	Association composition-etiology or underlying pathology
Niesten et al. (2014) PLOS One[64]	22	Histopathology	RBC, FBR, and PLTs proportions	LAA (8), CE (6), ODC (3), and CRY (5)	Higher RBC proportion (range) in both LAA (50% [35%–90%]) and ODC (35% [20%–40%]), than in CE (35% [5%–45%]) or CRY (25% [2%–40%])
Nouh et al. (2020) BMC Neurology[73]	33	Histopathology	RBC and PLTs proportions. RBC/PLTs ratio	LAA (9), CE (14), ODC (4), and ESUS (6)	RBC/PLTs ratio in ESUS (0.36±0.33) similar to CE (0.78±0.65), and different from LAA (1.73±2.38) or ODC (1.44±0.70)
Novotny et al. (2020) Neurology[66]	71	Histopathology	FBR and PLTs proportions. WBC subtypes counts. NETs and H3Cit counts	LAA (15), CE (35), and CRY (21)	Lower NETs count and netting neutrophils rate in LAA than in CE or CRY.
Park et al. (2019) Ann Neurol[65]	48	Histopathology	RBC, FBR, and PLTs proportions. Neutrophil and NETs counts	Control (16), AC (16), and IC (16)	Higher PLT proportion in AC (43.2%) than in IC (12.9%) or control (14.1%). Lower RBC proportion in AC (3.4%) than in IC (43.5%) or control (40.7%)
Sgreccia et al. (2019) J NeuroIntervent Surg[36]	255	Macroscopic	Visual aspect: red/black or white	LAA (53), CE (127), ODC (13), CRY (45), and atypical (17)	Atypical etiologies (AC, IE, etc.) more frequent in white clots (27.3%) than red/black clots (4.7%)
Shin et al. (2018) PLOS One[84]	93 / 37	Histopathology	RBC, FP, and WBC proportions	LAA (7), CE (22), and CRY (8)	Higher RBC proportion in CE (38%) than in LAA (23%) or CRY (26%). Lower WBC proportion in CE (3%) than in LAA (6%) or CRY (5%)
Sporns et al. (2017) Stroke[96]	187	Histopathology	RBC, FBR, and WBC proportions	LAA (35), CE (77), ODC (11), and CRY (64)	Composition (range) in both CE (RBC 28.0% [11.0%–53.0%], FBR 60.0% [40.0%–80.0%], and WBC 8.0% [5.0%–12.5%]) and CRY (RBC 26.0% [10.5%–43.5%], FBR 63.5% [45.5%–77.8%], and WBC 10.0% [5.0%–14.5%]) different from non-CE (LAA+ODE) (RBC 42.0% [20.9%–71.8%], FBR 51.5% [19.5%–68.5%], and WBC 5.0% [4.0%–10.0%]). Similar composition in and in CE and CRY
Wolpert et al. (2020) Eur J Neurol[129]	32	Histopathology	Tumor cell presence	AC: LAA (4), CE (8), ODC (1), and CRY (19)	Tumor cells in one out of 32 with AC (3.1%)
Xue et al. (2018) Natl Med J China[124]	58	Histopathology	RBC and FBR proportions	LAA (17), CE (31), and CRY (10)	Higher RBC proportion in LAA (58%) than in CE (46%), and higher FBR proportion in CE (54%) than in LAA (42%). Similar composition in CE (RBC 46%, FBR 54%) and CRY (RBC 47%, FBR 53%)
Ye et al. (2020) Interv Neuroradiol[55]	52	Histopathology	RBC, FBR, and PLT proportions. VWF content	LAA (12), CE (34), and CRY (6). NG (26) and DM (26)	Lower RBC proportion in DM (26.0%) than in NG (42.9%). Higher FBR proportion in DM (44.2%) than in NG (28.3%)

TOAST, Trial of Org 10172 in Acute Stroke Treatment; RBC, red blood cell; FBR, fibrin; LAA, large-artery atherosclerosis (TOAST 1); CE, cardioembolism (TOAST 2); CRY, cryptogenic stroke of undetermined etiology (TOAST 5); RTqPCR, reverse transcriptase quantitative polymerase chain reaction; IL-1β, interleukin-1β; FP, fibrin+platelet; ODC, stroke of other determined cause (TOAST 4); WBC, white blood cell; PLT, platelet; NET, neutrophil extracellular trap; NG, normoglycemia; H3Cit, citrullinated histone H3; AHG, acute hyperglycemia; DM, diabetes mellitus; ESUS, embolic stroke of undetermined cause; ECA, extracted clot area; AC, active cancer; VWF, von Willebrand factor; IC, inactive cancer; IE, infective endocarditis.

Supplementary Table 3. Case reports: thrombus/embolus composition and stroke etiologies or underlying pathologies

Study	Case(s) presentation	Thrombus/ embolus analysis	Thrombus/ embolus composition	Further diagnostic work-up	Stroke cause/ underlying pathology
Abdel-Wahed et al. (2019) Am J Med[14]	A 72-year-old woman. Bioprosthetic aortic valve. Suspected urosepsis	Histopathology	Gram-positive cocci in chains	*Streptococcus viridans* in blood cultures. *Klebsiella* in urine cultures. Vegetations on the prosthetic aortic valve	Septic embolus. Infective endocarditis
Ambrosioni et al. (2018) Clin Infect Dis[131]	Six cases. Pre- or post-stroke infective endocarditis. Prosthetic or native valves	Molecular biology	*Streptococcus*	*Staphylococcus* or *Streptococcus* in blood cultures in four cases	Septic embolus. Infective endocarditis
Anuwatworn et al. (2015) JACC Cardiovasc Interv[24]	A 78-year-old man. Transcatheter aortic valve replacement (TAVR)	Macroscopic. Histopathology	Heart valve tissue	Aortic valve echo-dense mobile mass disappearing after TAVR	Cardiac valve tissue embolization
Bain et al. (2011) J Neuroimaging[132]	A 24-year-old female. Left ventricular assist device (LVAD). Epidermidis, septicemia and persistent infections of the LVAD	Histopathology	Gram-positive cocci and bacilli		Septic embolus. Infective endocarditis
Bhaskar et al. (2019) Can J Neurol Sci[102]	Four cases. Prosthetic valves. History of infective endocarditis in two cases	Histopathology	Fibrinoid material with clusters of bacterial cocci	Prosthetic valve vegetations. *Enterococcus faecalis* and *Staphylococcus aureus* in blood culture in two cases	Septic embolus. Infective endocarditis
Biraschi et al. (2016) J Stroke Cerebrovasc Dis[15]	A 75-year-old man. Hypertension and atrial fibrillation	Macroscopic. Histopathology	White-pink hard tissue-like material. Papillary fronds, endothelium and elastic fibers		Cardiac embolic papillary fibroelastoma
Chapot et al. (2006) Am J Neuroradiol[25]	A 43-year-old woman. Endovascular intracranial aneurysm treatment	Macroscopic. Histopathology	Cotton-like synthetic fibers		Inadvertent embolization of foreign bodies
Distefano et al. (2019) J Stroke Cerebrovasc Dis[133]	A 75-year-old man. Hypertension. Suspected infection	Histopathology	Necrotic material and bacterial colonies	Vegetation in aortic valve. *E nterococcus faecalis* in blood cultures	Septic embolus. Infective endocarditis
Elodie et al. (2019) J Neurol Sci[26]	A 70-year-old woman. Mitral stenosis and atrial fibrillation	Macroscopic. Histopathology. Molecular biology	*Tropheryma whipplei* DNA sequences	Negative blood culture. Cleared previous suspected small aortic vegetation	Septic embolus. Whipple's endocarditis
Fassa et al. (2014) Circ Cardiovasc Interv[27]	A 90-year-old woman. TAVR	Macroscopic. Histopathology	Calcific		Detached aortic valve or aortic wall calcification
Fitzpatrick et al. (2018) J NeuroInterv Surg[28]	Three cases. History of deep vein thrombosis	Macroscopic. Histopathology	Elongated, pale-colored clot	Carotid free-floating thrombus (FFT)	FFT embolism
Garcia-Ptacek et al. (2014) J NeuroInterv Surg[29]	Two cases. Concomitant peripheral thrombi	Histopathology	Myxomatous material	Echocardiographic mobile mass	Cardiac myxoma embolism
Genchi et al. (2020) J Neurol Sci[30]	An 84-year-old man. Atrial fibrillation and uncomplicated carotid plaques	Macroscopic. Histopathology	Reddish tissue with white inclusions. Organized calcified thrombus	Hyperechoic aortic valve calcification	Dislodged aortic valve calcification
Hinman et al. (2013) Front Neurol[130]	A 28-year-old male. Retinal surgery and post-operative neck compression	Macroscopic. Proteomics	96% Common proteins	Tandem cervical and intracranial occlusions	Traumatic carotid thrombosis and embolization
Kan et al. (2012) World Neurosurg[31]	A 78-year-old woman. Mitral valve prolapse. Suspected infection	Macroscopic. Bacteriological	Positive for viridans streptococci	Large, mobile vegetation on the aortic valve. Positive blood cultures	Septic embolus. Infective endocarditis
Katano et al. (2020) Clin Neurol[137]	An 88-year-old man	Histopathology	*Aspergillus* fungus	Paranasal sinus invasion	Septic embolus. Sinusitis

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Supplementary Table 3. Continued

Study	Case(s) presentation	Thrombus/ embolus analysis	Thrombus/ embolus composition	Further diagnostic work-up	Stroke cause/ underlying pathology
Kim et al. (2014) Neurointervention[32]	A 40-year-old woman. Fever, malaise and systolic murmur	Histopathology	Gram-positive cocci	Mitral valve vegetation. Blood cultures positive for *Streptococcus mitis*	Septic embolus. Infective endocarditis
Koneru et al. (2021) J NeuroInterv Surg[141]	Three cases. Aged 41–55 years. Few to no vascular risk factors	Histopathology	Fresh appearance. Usual RBC, FBR, and WBC contents	Ipsilateral angiographic carotid web (CaW). No superimposed thrombus	Embolization from CaW
Matsumoto et al. (2016) J Stroke Cerebrovasc Dis[33]	Two cases. Active cancer	Macroscopic. Histopathology	White and solid. Fibrin >90%. No tumor cells	Cancer-related hypercoagulation	Trousseau syndrome-related thromboembolism
Matsumoto et al. (2020) J Stroke Cerebrovasc Dis[139]	A 67-year-old man. History of dyslipidemia, taking statins, and smoking	Histopathology	Small calcification and a cholesterol crystal cleft	Atheromatous lesion at the aortic arch	Aortogenic embolic stroke
Nakanishi et al. (2018) Clin Neurol[134]	An 80-year-old woman. Suspected infection	Macroscopic. Histopathology	White thrombus. Gram-positive cocci	Negative blood culture and echocardiogram	Septic embolus. Infective endocarditis
Pisano et al. (2020) J Stroke Cerebrovasc Dis[34]	A 33-year-old. Exposure to a COVID-19 positive relative	Macroscopic	Over 50 mm in length	SARS-CoV-2 positive. Hypercoagulability	Thromboembolism secondary to COVID-19
Salam et al. (2018) J Stroke Cerebrovasc Dis[16]	A 25-year-old woman. No vascular risk factors.	Macroscopic. Histopathology	Pale, white soft material. Papillary neoplasm with hyalinized cores lined by endothelium		Cardiac embolic papillary fibroelastoma
Salinas et al. (2013) JACC Cardiovasc Interv[17]	An 88-year-old woman. Transcatheter aortic valve implantation (TAVI)	Macroscopic	Red-dark	Ventricular echo-dense mobile mass disappearing after TAVI	Catheter-related thromboembolism
Scharf et al. (2016) Neurocrit Care[138]	A 56-year-old man. Immunocompromised	Histopathology	Fungal hyphae of *Zygomycetes* species	Fungal culture of sphenoid sinus biopsy positive for *Rhizomucor* species	Angioinvasive mucormycosis
Scharf et al. (2017) J NeuroIntervent Surg[18]	A middle-aged adult. Mitral valve replacement and infective endocarditis	Macroscopic. Histopathology	Firm and rigid texture. Hyaline and coccal forms	Vegetation on both the mitral valve prosthesis and native aortic valve	Septic embolus. Infective endocarditis
Semerano et al. (2019) J Neurol[19]	An 86-year-old man. Arterial hypertension	Macroscopic. Histopathology	Solid, yellow with red spot. Intima layer, foamy macrophages, lymphocytes, extracellular matrix, smooth muscle cells, cholesterol clefts, focal hemorrhage and outer fibrin cap	Angiographic focal truncal-type occlusion with distal anterograde repermeability in the cerebral vessel	Intracranial atherosclerotic plaque
Sgreccia et al. (2020) J Neuroradiol[136]	A 31-year-old male. Suspected infection. Aortic murmur	Macroscopic. Molecular biology	White-colored. *Candida parapsilosis*	Vegetation in bicuspid aortic valve. *C. parapsilosis* confirmed in blood culture	Septic embolus. Fungal endocarditis
Sukumaran et al. (2012) Neurol India[135]	A 33-year-old male. Fever and malaise. Cardiac murmur	Histopathology	Clusters of gram-positive cocci	Mobile mass in mitral valve. Alpha hemolytic streptococci in blood culture	Septic embolus. Infective endocarditis
Tejada et al. (2014) J NeuroIntervent Surg[20]	A 64-year-old woman. No vascular risk factors	Macroscopic. Histopathology	White soft aspect. Branching papillary lesions, lined by endothelium		Cardiac embolic papillary fibroelastoma

Supplementary Table 3. Continued

Study	Case(s) presentation	Thrombus/ embolus analysis	Thrombus/ embolus composition	Further diagnostic work-up	Stroke cause/ underlying pathology
Thomas et al. (2017) J NeuroInterv Surg[21]	A 69-year-old woman. Mitral valve replacement surgery	Macroscopic. Histopathology	Tan rubbery object. Collagen, elastin and endothelial cells		Cardiac chordae tendineae embolization
Usui et al. (2019) Intern Med[140]	A 91-year-old man. Hypertension and atrial fibrillation	Histopathology	Foamy cells	Atherosclerotic lesion with ulceration in the aortic arch	Aortogenic embolic stroke
Valente et al. (2019) J Clin Neurosci[22]	A 38-year-old male. Systemic lupus erythematosus	Macroscopic. Histopathology	Firm, pale to tan. Collagenous. IgG, C1q, C3, and IgA positive	Antiphospholipid syndrome	Embolism of a Libman-Sacks vegetation
Wollenweber et al. (2016) Neurology[23]	An 80-year-old woman. TAVI	Macroscopic. Histopathology	Solid tissue. Endothelialized, atherosclerotic arterial vessel wall		Aortic wall fragment embolization

RBC, red blood cell; FBR, fibrin; WBC, white blood cell; COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; Ig, immunoglobulin.

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