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Inflammation biomarkers in the intracranial blood associate with outcome in ischemic stroke patients

Cyril Dargazanli^{1,2*}, Marine Blaquière¹, Marinette Moynier², Frédéric de Bock¹, Julien Labreuche³, Adrien Ter Shiporst⁴, Imad Derraz², Razvan Radu², Grégory Gasco², Pierre-Henri Lefèvre², Francesca Rapido⁵, Julien Fendeleur⁵, Caroline Arquizan⁴, Romain Bourcier⁶, Philippe Marin¹, Paolo Machi⁷, Federco Cagnazzo^{1,2}, Christophe Hirtz⁸, Vincent Costalat^{1,2*}, Nicola Marchi^{1*}

¹Institut de Génomique Fonctionnelle, Univ. Montpellier, UMR 5203 CNRS - U 1191 INSERM, Montpellier, France, ²Diagnostic and Interventional Neuroradiology Department, Gui de Chauliac Hospital, Montpellier, France, ³Santé Publique: Epidémiologie et Qualité des Soins, CHU Lille, University of Lille, France ⁴Neurology Department, Gui de Chauliac Hospital, Montpellier, France, ⁵Anesthesiology Department, Gui de Chauliac Hospital, Montpellier, France, ⁶Diagnostic and Interventional Neuroradiology Department, CHU de Nantes, France, ⁷Diagnostic and Interventional Neuroradiology Department, Geneva, Switzerland, ⁸Clinical Proteomic Platform, CHU, Montpellier;

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***Equal contribution and corresponding:**

Dr. Cyril Dargazanli. Diagnostic and Interventional Neuroradiology Department, Gui de Chauliac Hospital, Montpellier, France. Cerebrovascular and Glia Research, Institut de Génomique Fonctionnelle, CNRS UMR5203, INSERM U1191, 141 rue de la Cardonille, 34094 Montpellier, Cedex 5, France. Email c-dargazanli@chu-montpellier.fr

Dr. Nicola Marchi. Cerebrovascular and Glia Research, Neuroscience Department, Institut de Génomique Fonctionnelle, University of Montpellier, CNRS UMR5203, INSERM U1191, 141 rue de la Cardonille, 34094 Montpellier, Cedex 5, France. Email nicola.marchi@iqf.cnrs.fr.

Pr. Vincent Costalat. Diagnostic and Interventional Neuroradiology Department, Gui de Chauliac Hospital, Montpellier, France. Cerebrovascular and Glia Research, Institut de Génomique Fonctionnelle, CNRS UMR5203, INSERM U1191, 141 rue de la Cardonille, 34094 Montpellier, Cedex 5, France. Email v-costalat@chu-montpellier.fr

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Abstract

Performing endovascular treatment (EVT) in acute ischemic stroke (AIS) patients allows a port-of-entry for intracranial biological sampling. Here, we tested the hypothesis that specific immune players represent molecular contributors to disease, outcome biomarkers, and potential targets for modifying AIS. We collected post-clot blood samples immediately before EVT and interrogated the pro-inflammatory environment.

We obtained blood samples from $n = 75$ subjects presenting with large vessel occlusion (LVO) of the anterior circulation and undergoing EVT. Intracranial blood samples were obtained by microcatheter aspiration, as positioned for stent deployment. Peripheral blood samples were collected from the femoral artery. Plasma samples were quality-controlled by electrophoresis and analyzed using a Mesoscale multiplex for targeted inflammatory and vascular factors.

We quantified 37 protein biomarkers in our sample cohort. Through multivariate analysis, adjusted for age, intravenous thrombolysis, pre-treatment NIHSS and ASPECT scores, we found that post-clot blood levels of IL-6 were significantly correlated (adjusted p -value < 0.05) with disability assessed by the modified Rankin score (mRS) at 90 days, with medium effect size. Chemokine (C-C) ligand 17 CCL17/TARC levels were inversely correlated with mRS. When examining peripheral blood, these correlations did not reach statistical significance after correction. Intracranial biomarkers IL-6 and SAA levels were specifically associated with a lower likelihood of favorable outcomes, defined as mRS 0-2.

Our findings show a signature of blood inflammatory factors at the cerebrovascular occlusion site. The correlations between these acute-stage biomarkers and mRS outcome support an avenue for add-on immune modulatory strategies in AIS.

Key messages.

What is already known on this topic: examining the intracranial blood environment advances our understanding of acute ischemic stroke (AIS) in patients. This represents an emerging research domain that leverages mechanical thrombectomy.

What this study adds: after multiple comparisons, IL-6 and CCL17/TARC blood levels at the acute phase correlate with mRS outcome at 90 days.

How this study might affect research, practice, or policy: this study confirms the role of local inflammation during the acute stroke phase. It supports the continuous investigation of disease-associated biomarkers and the development of add-on therapeutic strategies centered on specific and localized immune modulation.

Introduction

Endovascular treatment (EVT) of acute ischemic stroke (AIS) allows access to intracranial thrombus and its blood environment, which permits an immediate and localized investigation of the cellular and molecular processes of the AIS immune and angiogenic responses[1–4]. Identifying new disease biomarkers at the intracranial level and cross-comparing to peripheral blood is of interest to help develop therapeutic targets as an adjunct strategy to EVT.[5]

Inflammation plays a central role in stroke pathology. In the acute phase, the inflammatory response can initiate i) in the blood with leukocyte activations and cytokines release due to regional cerebrovascular flow cessation [2] or ii) in the brain parenchyma driven by tissue hypoxia, leading to astrocyte or microglial cells reactivity and neuronal death [6,7]. After clot retrieval, reperfusion damage at the downstream microcirculation and the correspondent brain parenchymal territory could be contingent on the pro-inflammatory players accumulating at the occlusion site and present in blood [8]. Only a handful of studies interrogated the clot intracranial environment compared to peripheral blood; they showed some degree of monocyte and neutrophil accumulation, with a specific interest in T-cell chemoattractant CXCL-11 [2]. Neutrophil counts in ischemic post-thrombus blood were associated with stroke outcome [2], thus supporting experimental evidence that immune cells contribute to ischemic brain damage at the acute phase[9]. In another study, intracranial and peripheral blood levels of vascular adhesion molecule 1 (VCAM1) were positively correlated to reduced improvement of NIHSS stroke severity [4]. The platelet-derived neutrophil-activating chemokine C-X-C-motif ligand (CXCL) 4 (PF-4),

sampled intracranially, was associated with the angiographic degree of reperfusion following recanalization and functional outcome at discharge was correlated to myeloperoxidase (MPO) level [10].

Here, we performed a quantitative analysis of a targeted panel (n = 37) of cytokines, chemokines, vascular growth factors, and adhesion molecules in post-clot intracranial blood samples collected during the acute phase. The samples were obtained from a prospective cohort of patients (n = 75) who underwent EVT for AIS caused by large vessel occlusion (LVO). We also studied femoral systemic blood samples collected in the same timeframe. Our objective was to identify players associated with the pathophysiology of AIS. We aimed to explore novel outcome biomarkers and potential therapeutic entry points that could complement EVT of AIS.

Methods

Study design and participants.

Patients aged 18 years and older with suspected AIS secondary to an LVO were recruited without any upper or lower limits of the neurological severity (NIHSS) at baseline. Inclusion criteria were according to the 2019 Update to the 2018 Guidelines for the Early Management of Acute Ischemic Stroke [11]. Patients were required to present imaging evidence of occlusion of the internal carotid artery (ICA), cervical or intracranial parts, the M1 or M2 branches of the middle cerebral artery (MCA), the basilar artery, or a tandem atheromatous occlusion defined by the occlusion of both the cervical carotid and intracranial arteries (carotid artery or MCA). Intravenous thrombolysis (IVT) treatment was allowed and administered according to current guidelines [12]. All patients were included in the Endovascular Treatment in Ischemic Stroke Follow-up Evaluation registry, a French multicenter (n=14), open, observational, and prospective trial (ClinicalTrials.gov Identifier: NCT03776877; <https://ichgcp.net/clinical-trials-registry/NCT03776877>). For blood sampling, we obtained CPP# 2019.10.04_19.07.30.36737. Pre-hospital and in-hospital delays in patient management were calculated in minutes.

Endovascular treatment.

All patients were treated in a dedicated neuroangiography suite under general anesthesia or conscious sedation after evaluation by the anesthesiology team. A

balloon catheter was positioned in the ICA to allow flow arrest during thrombus retrieval. The retriever was delivered through a microcatheter and deployed across the thrombus. A distal aspiration during stent retrieval was performed according to the PROTECT-PLUS technique [13]. A control angiogram was obtained to assess recanalization and reperfusion. This sequence was repeated until a modified treatment in cerebral infarction (mTICI) score of 2b or 2C/3 flow was obtained (defined as successful reperfusion). The 'retrograde' approach (also known as the 'distal-to-proximal' or 'intracranial-first' approach), aiming to recanalize the distal and symptomatic intracranial occlusion before addressing the cervical carotid lesion, was generally chosen for tandem occlusions. The interventional neuroradiologist used another thrombectomy device in case of reperfusion failure (mTICI <2b) with the first retriever. Reperfusion results were reported using the mTICI score. Peri-procedural complications were recorded, including embolization in a new territory, defined as an angiographic occlusion in a previously unaffected vascular territory observed on the angiogram after clot removal, arterial dissection or perforation, vasospasm, and subarachnoid hemorrhage.

Measures and main outcome.

Follow-up imaging was performed between 16 and 30 h after EVT to assess intracranial hemorrhage. Symptomatic intracranial hemorrhage was defined as any hemorrhage occurring within 24 h and associated with an increase of ≥ 4 points in the NIHSS score or confirmed as the cause of death [14]. Patient assessment using the modified Rankin Score (mRS) was carried out during a telephone visit within 3 months (+/- 15 days) following the stroke by a trained study team member certified in

mRS evaluation, independent of the management of the patient. A favorable functional outcome was defined as a mRS ≤ 2 or equal to pre-stroke mRS.

Imaging.

Infarct volume was automatically measured with RAPID software (iSchemiaView, Redwood City, CA, USA) on either computed tomography (CT) perfusion imaging or magnetic resonance imaging (MRI) with a diffusion-weighted-imaging (DWI) sequence. When baseline imaging was performed in a remote center where RAPID software was not implemented, baseline infarct volume was semi-manually delineated using the GE PACS console (GE Medical Systems, Prospect, IL, USA). Perfusion mismatch corresponding to a chosen ratio between core volume and perfusion deficit volume was assessed when available, i.e., for patients who had perfusion MRI or CT at baseline. Perfusion mismatch was defined with RAPID software using a mismatch ratio of at least 1.2 and a minimal penumbra volume of 10 mL, in addition to a maximum core and severely hypoperfused tissue volume (DWI lesion and $T_{max} \geq 8$ sec volume < 100 mL, respectively), according to DEFUSE criteria [15].

Blood sampling.

A blood sample was obtained at the femoral puncture site immediately after groin access (peripheral blood). After the thrombus was crossed with either a 0.021in (Headway 21, Microvention, Tustin, CA, USA, or Trevo Pro 18, Stryker) or, less frequently, a 0.027in (Headway 27; Microvention) inner lumen diameter microcatheter

mounted on a 0.014in microwire, a 1 or 3 cc syringe was connected to the hub of the microcatheter. Immediately before deploying the retriever device, the dead space volume was aspirated and discarded (0.027in = 0.7ml; 0.021in = 0.5ml dead space). Subsequently, a sample of 0.5 ml of ischemic blood was obtained, and the EVT procedure was initiated. When central sampling was not possible, the patient was excluded from the study, and the EVT procedure was continued without any delay. All samples were immediately transferred into an EDTA tube inverted manually approximately five times to ensure the anticoagulant was evenly dispersed. A maximum time window of 24 h (4°C) after initial sampling was allowed for sample processing as the feasibility study showed no impact on the protein content. Samples were centrifuged, and plasma was stored at -80° C. For quality control of protein integrity, on randomly chosen intracranial and peripheral samples (n=10), we performed SDS-gel electrophoresis coupled with High-Select™ Top14 Abundant Protein Depletion Mini Spin Columns (rA36370 Thermo Scientific).

Statistical analysis.

Data are presented as the mean (standard deviation [SD]) or median (interquartile range [IQR]) for quantitative variables according to the normality of distribution and numbers (percentage) for categorical variables. The normality of distribution was assessed using histograms and the Shapiro-Wilk test. For biomarkers, values under the limit of detection were considered as zero values in analyses. Due to the high skewness of distributions for most of the biological parameters, we compared the intracranial versus peripheral values using Wilcoxon's signed rank tests and assessed the correlation between intracranial and peripheral biomarkers values using Spearman's rank correlation coefficients; 95% confidence

intervals (CIs) were estimated using the Fisher z transformation. As the main analysis, we first investigated the association of the overall mRs distribution at 90-days with intracranial biomarkers levels before and after adjustment for pre-treatment NIHSS and ASPECT scores using Spearman's rank correlation analysis. To account for multiple testing, a false discovery rate (FDR) controlling procedure was applied and adjusted correlation coefficients were considered significant if the FDR-adjusted p-value was inferior or equal to 0.05. As a secondary analysis, we evaluated the association of overall mRs distribution at 90-day with peripheral biomarkers values using the same method. We further calculated the odds ratios (ORs) of favorable outcomes (defined as 90-day mRS 0-2 or equal to pre-stroke) for biomarkers (intracranial or peripheral values) with adjusted correlation coefficients with 90-day mRs distribution greater than 0.3 in absolute values. ORs were calculated per one-standard-deviation increase in log-transformed biomarkers values using logistic regression models before and after adjusting for pre-treatment NIHSS and ASPECT scores. Finally, we investigated the correlation between biomarkers and infarct volume at 24-hours using the same method described to study the correlation with 90-day mRs. Data were analyzed using the SAS software package, release 9.4 (SAS Institute, Cary, NC, USA).

Results

Patients' characteristics.

Between March 2021 and March 2022, 380 patients with AIS were treated by EVT in our comprehensive stroke center. One hundred ten patients were eligible for study inclusion (Figure 1, flowchart). Patients were excluded due to LVO recanalization on baseline DSA (n = 10); patients treated with direct aspiration first pass technique (ADAPT) technique (n = 92); microcatheter aspiration not attempted (n = 168; Figure 1). The aspiration of ischemic blood was attempted in 110 patients and successful in 75 individuals. Successful reperfusion (defined as mTICI 2b or 3) was achieved in 70 patients (93.3%) after the median time from symptom onset of 337 min (IQR 220–414). Symptomatic intracranial hemorrhage was observed in five (6.9%) patients and the median infarct core at day 1 was 17 (6-69) mL. Baseline and treatment characteristics and outcomes are reported in Table 1. The mean age was 75± 11 years, and 60% were men. Median NIHSS and pre-procedural ASPECT scores at presentation were 17 (IQR 11–21) and 8 (IQR 6–9), respectively. Twenty-nine patients (38.7%) received intravenous rt-PA treatment before the EVT. The median time from symptom onset to groin puncture was 287 min (IQR 182–380). On the initial angiogram, 43 (57.3%) patients showed MCA occlusion, 18 (24%) an ICA-T, 12 (16%) a tandem occlusion, 1 (1.3%) a basilar occlusion, and 1 (1.3%) a cervical carotid occlusion.

Intracranial and peripheral blood biomarkers quantification.

Figure 2 A-B illustrates examples of intracranial blood sampling and quality control utilizing SDS gel-electrophoresis of plasma samples, with or without larger protein depletion, to identify distinct bands by coomassie blue across various molecular weights, thereby excluding degradation (Figure 2 C-C1). The median levels for the 37 analytes assessed from intracranial and peripheral blood samples are reported in Supplemental Table 1.

Blood biomarkers and functional outcomes at 90-days

After adjustment for main potential confounders (age, intravenous thrombolysis, pre-treatment NIHSS and ASPECT scores) and for multiple comparisons, two intracranial biomarkers were significantly associated with mRs 90-day (Figure 3A). Higher values of IL-6 ($r=0.36$; 95%CI, 0.13 to 0.55, adjusted FDR p-value=0.039), was correlated with higher mRs scores, whereas higher value of chemokine (C-C) ligand 17 (CCL17/TARC) was correlated with lower mRs scores ($r=-0.37$; 95%CI, -0.56 to -0.15; adjusted FDR p-value=0.039). We observed a similar non-significant positive correlation with serum amyloid A (SAA; $r=0.30$; 95%CI, 0.07 to 0.50; adjusted FDR p-value=0.13).

In peripheral blood samples, no correlation between biomarkers and mRS 90-days attained statistical significance following adjustments for primary confounding

variables and multiple comparisons (Figure 3B). We nevertheless report the following trend values for IL-6 ($r=0.30$; 95%CI, 0.06 to 0.50; adjusted FDR $p=0.25$), and bFGF ($r=0.30$; 95%CI, 0.06 to 0.50; adjusted FDR $p=0.25$).

As shown in Table 2, higher intracranial levels of IL-6 were associated with a lower likelihood (mRS 90-days >2) of favorable outcome (adjusted OR per SD increase, 0.51; 95%CI, 0.7 to 0.95), while high intracranial CCL17/TARC levels corresponded to favorable outcome (adjusted OR per SD increase, 2.07; 95%CI, 1.14 to 3.76). The association were attenuated with peripheral values. As a corollary, between intracranial and peripheral samples a fair positive correlation (>0.8) was observed for SAA ($r=0.93$), IP-10 ($r=0.81$), IL-17A ($r=0.84$), IL-12/IL-23p40 ($r=0.88$), IL-8($r=0.83$), IL-4 ($r=0.83$), IL-10 ($r=0.92$), and IFN- γ ($r=0.85$).

Blood biomarkers and Infarct volume

None of the correlation coefficients between biomarkers and infarct volume assessed prior to treatment (see Supplemental Figure 1) and at 24-hours (Figure 4) were significant after adjustment by age, intravenous thrombolysis, pre-treatment NIHSS and ASPECT scores, and multiple comparisons. Only a coefficient greater than 0.3 in absolute value was found between infarct volume at 24-hours and intracranial values of ICAM-1 ($r, -0.33$; 95%CI, -0.54 to -0.09) (Figure 4A). No correlations were found when the change in infarct volume (infarct growth at 24-hours) were analyzed (see Supplemental Figure 2).

Discussion

We analyzed the correlation between a targeted panel of neuroinflammatory and vascular-angiogenesis biomarkers, clinical and imaging readouts in a cohort of consecutive AIS patients treated by EVT, using the largest cohort of blood samples obtained to date in the intracranial ischemic arterial bed beyond the LVO. Multivariate analysis showed that IL-6 and CCL17/TARC were associated with outcomes, here mRS 90-days. Our results integrate emerging evidence based on the analysis of intracranial and peripheral blood samples during the acute phase of the vascular occlusion [1,2,4,5]. We reported inflammatory activations, possibly intersecting with angiogenic mechanisms, detectable when the pathology unfolds, disclosing an opportunity for biomarkers and therapeutic entry points.

Inflammatory players and AIS.

Inflammation in response to the ischemic insult has beneficial and detrimental effects, with diverse clusters of blood and brain parenchymal immune cells involved. Here, we quantified IL-6 levels in intracranial blood samples correlating with mRS outcomes. Previous research indicated that IL-6 is involved in AIS, as measured in peripheral blood clinically and experimentally [16–20]. IL-6 is released by circulating leukocytes and resident brain microglial cells or astrocytes during disease [6,21,22]. Importantly, circulating IL-6 can stimulate the production of acute-phase proteins or inflammatory signals from the liver [23,24], contributing to the systemic response. Our data converged with this knowledge as levels of SAA were increased (although non-

significant) with negative mRS outcome, unveiling a brain-peripheral inflammatory communication during AIS.

Elevated serum IL-6 levels were implicated in a higher risk of AIS [25], and its peripheral levels were considered to be an adverse prognostic factor in AIS patients [25], associated with imaging parameters (mean volume of DWI lesion at admission, perfusion deficit, final infarct size [18]). A recent study showed that an elevated peripheral blood level of IL-6 is a biomarker of futile reperfusion in patients undergoing EVT [16]. A lower level of IL-6 at baseline was associated with a first-pass effect, defined as complete or near-complete reperfusion achieved after a single thrombectomy attempt, predictive of a favorable outcome in AIS patients [17]. Significantly, pre-clinical studies have suggested a dual role for IL-6, such as its potential to enhance post-stroke angiogenesis while concurrently reducing ischemic damage [23–27]. Due to its multifaceted roles, targeting IL-6 in human AIS using monoclonal antibodies is debated. Two ongoing clinical trials are assessing the prognostic implications of IL-6 levels in ischemic stroke (NCT05004389; NCT03297827). In our study, a higher level of central CCL17/TARC was associated with lower mRS at 3 months. Another study showed an inverse correlation between stroke severity (NIHSS score) and CCL17 level one day after AIS [28]. A plethora of functions has emerged for the chemokine CCL17, as it can amplify the immune response to enable beneficial or detrimental effects, possibly depending on disease specificity and stage [29]. In a particular instance, CCL17 was observed to activate macrophages/microglia in a pre-clinical model of intracerebral hemorrhage. This activation led to a reduction in associated brain edema, improvement in neurological deficits, and a decrease in neuroinflammation and neuronal apoptosis [30]. Further studies in human AIS are necessary to clarify the origins and impacts of CCL17

during both the acute and post-acute phases. It is also important to explore its interaction between specific cytokines, such as IL-6.

Going beyond mechanical thrombectomy: conclusions.

Our data and emerging literature suggest that an immuno-modulatory strategy delivered to the site of cerebrovascular occlusion could represent a next-generation treatment complementing EVT. However, there is a need for a precise temporal and quantitative definition of the equilibriums between pro- and anti-inflammatory pathways that occur during AIS and cerebrovascular reperfusion. These mechanisms could extend regionally, from the occlusion to the penumbra brain tissue, where damage needs to be reversed or halted. The biomarkers identified in our study are coherent with a regional involvement of parenchymal astrocytes and microglial cells, immune effectors in the brain responding to the initial ischemic insults. The lack of blood flow and shear forces in the intravascular compartment also enables leukocyte interaction at the endothelium, perpetuating inflammation [7]. Here, we acknowledge that the mRS at 90 days, the traditional measure in AIS trials, remains an imperfect outcome. Recovery after AIS depends on several factors, including patient demographics, comorbidities, stroke interventions such as tPA or EVT, post-stroke rehabilitation, recurrent AIS, secondary complications (infection occurs in 30% of AIS patients), and genetic variations. A recent study indicates that around 50% of patients with a good mRS (0-1) after AIS will have cognitive impairment or reintegration restrictions [31].

In conclusion, this study provides evidence of acute-phase inflammation at the AIS location, disclosing candidate players for disease biomarkers and modifying

pharmacological strategies beyond thrombectomy. The cellular origin and the temporal profile of specific inflammatory and vascular factors should be further examined.

Conflict of Interest.

None.

Data availability statement.

Data are available upon request.

Ethics Statement.

The studies involving human participants were reviewed and approved by Comité de Protection des Personnes «Sud-Méditerranée IV», Centre Hospitalier Universitaire de Montpellier, hôpital Saint-Eloi, 34295 Montpellier Cedex 5.

Authors' Contribution.

CD, VC, and NM: conception and design of the study, analysis of data, and drafting of the manuscript. MB, FdB, CH, PM: samples' technical analyses. MM: acquisition of patients' characteristics, registry. CD, VC, MM: emergency interventions, patients' recruitment and approval. CD, VC, ID, RR, GG, PHL, FR, JF, CA, ATS, RB: samples obtainment and circuit. All authors contributed to the article and approved the submitted version.

Table 1. Main baseline characteristics and outcomes of the study sample.

	Values
Age, yrs., mean (SD)	75 ± 11
Men	45 (60.0)
Medical history	
Hypertension	50 (66.7)
Diabetes	10 (13.3)
Hypercholesterolemia	19 (25.3)
Active smoking	10 (13.3)
Prior Stroke	11 (14.7)
Atrial fibrillation	19 (25.3)
Prior Antithrombotics	31 (41.3)
Aspirin	17 (22.7)
Plavix	2 (1.3)
Vitamin K antagonist	3 (4.0)
New oral anticoagulants	8 (10.7)
Ticagrelor	1 (1.3)
Examination findings at admission	
Pre-stroke mRS >1	72 (96.0)
NIHSS	17 (11 to 21)
ASPECTS *	8 (6 to 9)
Baseline infarct volume, ml†	14 (1 to 39)
Mismatch on perfusion imaging	51/55 (92.7%)
Systolic blood pressure, mmHg, mean (SD)	148 ± 27

Glucose, mmol/l	6.95 (5.9 to 8.4)
Cardioembolic stroke etiology	49 (65.3)
Site of occlusion	
MCA	43 (57.3)
ICA terminus	18 (24.0)
Basilar artery	1 (1.3)
Tandem	12 (16.0)
Cervical carotid artery	1 (1.3)
Acute stroke treatment	
Intravenous rtPA thrombolysis	29 (38.7)
General anesthesia	19 (25.3)
Symptom onset to groin puncture, minutes‡	287 (182 to 380)
Outcomes	
Reperfusion grade at end of endovascular thrombectomy	
mTICI 2b	13 (17.3)
mTICI 2c	17 (22.7)
mTICI 3	40 (53.3)
Symptom onset to successful reperfusion (mTICI ≥2b) time, minutes	337 (220.0 to 414.0)
mRS at 90-day	4 (2 to 6)
Favorable outcome	28 (37.3)
All-cause mortality at 90-days	
Any ICH	33 (45.2)
Symptomatic ICH	5 (6.9)
Infarct Volume at 24-hours, ml#	17 (6 to 69)
Infarct grow at 24-hours, ml§	5 (0 to 15)

Table 1. Values are number (%) or median (IQR) unless otherwise indicated. * 1 missing value; † 4 missing values; ‡ 3 missing values; # 5 missing values; § 9 missing values. Favorable outcome at 90-days was defined as mRS 0-2 or equal to pre-stroke mRS. Abbreviations: ASPECTS=Alberta stroke program early computed tomography score; ICA=internal carotid artery; ICH=intracranial cerebral hemorrhage; IQR=interquartile range; MCA=middle cerebral artery; mRS=modified rankin scale; mTICI= modified thrombolysis in cerebral infarction; NIHSS=national institutes of health stroke scale; rtPA=recombinant tissue plasminogen activator; SD=standard deviation.

Table 2. Association of selected biomarkers with favorable outcome at 90-day

Biomarkers	Unfavorable outcome (n=47)	Favorable outcome (n=28)	Unadjusted OR (95%CI)	Adjusted OR (95%CI)
IL-6				
Intracranial values	280 (207 to 419)	241 (203 to 440)	0.46 (0.25 to 0.82)	0.51 (0.27 to 0.95)
Peripheral values	261 (211 to 336)	223 (181 to 267)	0.63 (0.38 to 1.05)	0.80 (0.46 to 1.38)
TARC				
Intracranial values	65 (48 to 134)	98 (74 to 120)	1.66 (1.00 to 2.75)	2.07 (1.14 to 3.76)
Peripheral values	108 (69 to 158)	106 (78 to 284)	1.36 (0.83 to 2.21)	1.48 (0.85 to 2.57)
SAA				
Intracranial values	3028720 (1247552 to 17834003)	1364720 (613130 to 3522763)	0.53 (0.29 to 0.94)	0.59 (0.31 to 1.11)
Peripheral values	5682883 (2263737 to 20459506)	2614920 (1017315 to 6332840)	0.52 (0.29 to 0.92)	0.60 (0.31 to 1.14)

Values are the median (IQR) of biomarkers unless otherwise indicated.

OR indicates the odds ratio of favorable outcome (defined as 90-day mRs 0-2 or equal to pre-stroke mRs) per one standard deviation increase in log-transformed biomarkers values, estimated from logistic regression models before and adjusted for age, intravenous thrombolysis, pre-treatment NIHSS and ASPECT scores. Abbreviations: ASPECTS = Alberta Stroke Program Early Compute Score; CI = confidence interval; NIHSS = National Institute of Health Stroke Score

Supplemental Table 1. Median (interquartile range) of intracranial and peripheral blood biomarker levels and their correlations.

Biomarkers	Intracranial levels	Peripheral levels*	Intra-patients variations		
			Difference in intracranial vs. peripheral values)	P-Value	r (95%CI)
IFN- γ , pg/ml	0.005 (0.001 to 0.020)	3.93 (2.46 to 6.93)	-3.89 (-6.78 to -2.14)	<0.0001	0.85 (0.77 to 0.91)
IL-10, pg/ml	0.65 (0.41 to 2.27)	0.58 (0.38 to 1.56)	0.02 (-0.07 to 0.11)	0.25	0.92 (0.87 to 0.95)
IL-13, pg/ml	2.91 (1.91 to 3.68)	1.58 (0.62 to 2.57)	1.15 (0.34 to 2.28)	<0.0001	0.43 (0.22 to 0.60)
IL-1 β , pg/ml	0.27 (0.14 to 0.38)	0.13 (0.04 to 0.27)	0.09 (0.00 to 0.19)	<0.0001	0.61 (0.44 to 0.74)
IL-2, pg/ml	0.33 (0.16 to 0.56)	0.09 (0.04 to 0.27)	0.19 (0.04 to 0.29)	<0.0001	0.71 (0.56 to 0.81)
IL-4, pg/ml	0.07 (0.04 to 0.10)	0.05 (0.02 to 0.07)	0.03 (-0.009 to 0.06)	<0.0001	0.32 (0.09 to 0.52)
IL-6, pg/ml	3.11 (1.90 to 7.46)	2.44 (1.47 to 5.55)	0.34 (-0.09 to 0.92)	<0.0001	0.83 (0.74 to 0.89)
IL-8, pg/ml	16.7 (11.5 to 24.6)	6.47 (4.21 to 11.14)	9.16 (6.28 to 15.40)	<0.0001	0.65 (0.48 to 0.77)
TNF- α , pg/ml	1.67 (1.31 to 2.39)	1.51 (1.29 to 2.09)	0.18 (0.00 to 0.43)	<0.0001	0.83 (0.74 to 0.89)
IL-12/IL-23p49, pg/ml	115 (68 to 205)	132 (86 to 217)	-11.45 (-36.20 to -1.40)	<0.0001	0.88 (0.80 to 0.93)
IL-15, pg/ml	2.89 (2.5 to 3.37)	3.44 (2.91 to 4.16)	-0.55 (-0.96 to -0.09)	<0.0001	0.57 (0.38 to 0.71)
IL-16, pg/ml	277 (207 to 423)	248 (198 to 328)	28 (-39 to 174)	0.006	0.45 (0.24 to 0.62)
IL-17A, pg/ml	1.91 (1.38 to 3.49)	4.90 (2.86 to 8.04)	-2.92 (-5.50 to -1.00)	<0.0001	0.70 (0.56 to 0.81)
IL-1- α , pg/ml	16.6 (11.1 to 34.3)	13.9 (10.3 to 18.2)	4.08 (0.01 to 15.67)	<0.0001	0.84 (0.75 to 0.90)

IL-5, pg/ml	0.57 (0.27 to 0.93)	0.56 (0.30 to 0.87)	-0.01 (-0.30 to 0.22)	0.35	0.49 (0.29 to 0.65)
IL-7, pg/ml	1.91 (1.22 to 2.70)	4.72 (2.81 to 6.58)	-2.48 (-3.80 to -1.06)	<0.0001	0.50 (0.29 to 0.65)
TNF- β , pg/ml	0.05 (0.00 to 0.15)	0.31 (0.19 to 0.42)	-0.21 (-0.32 to -0.12)	<0.0001	0.60 (0.42 to 0.73)
VEGF-A, pg/ml	57 (42 to 80)	61 (38 to 112)	-3 (-41 to 12)	0.13	0.51 (0.31 to 0.67)
Eotaxin, pg/ml	908 (577 to 1297)	278 (215 to 479)	584 (317 to 852)	<0.0001	0.40 (0.18 to 0.58)
Eotaxin-3, pg/ml	70 (44 to 119)	21 (15 to 33)	49 (27 to 90)	<0.0001	0.51 (0.31 to 0.66)
IP-10, pg/ml	448 (299 to 588)	399 (312 to 551)	5 (-26 to 74)	0.26	0.88 (0.81 to 0.93)
MCP-1, pg/ml	200 (129 to 263)	181 (140 to 242)	2.8 (-34 to 66)	0.48	0.64 (0.47 to 0.76)
MCP-4, pg/ml	232 (164 to 356)	101 (72 to 156)	136 (60 to 199)	<0.0001	0.36 (0.14 to 0.55)
MDC, pg/ml	626 (515 to 821)	691 (555 to 840)	-62 (-161 to 56)	0.002	0.74 (0.62 to 0.84)
MIP-1 α , pg/ml	18.6 (13.7 to 24.6)	19.1 (15.8 to 24.4)	-0.4 (-3.2 to 2.4)	0.31	0.75 (0.62 to 0.84)
MIP-1, β , pg/ml	72 (52 to 84)	78 (61 to 103)	-8 (-17 to 3)	0.0008	0.78 (0.67 to 0.86)
TARC, pg/ml	82 (52 to 84)	107 (71 to 206)	-22 (-74 to -5)	<0.0001	0.61 (0.44 to 0.74)
bFGF, pg/ml	24.7 (12.5 to 42.3)	9.4 (5.2 to 20.9)	10.3 (0.8 to 29.1)	<0.0001	0.23 (-0.01 to 0.44)
FLT-1, pg/ml	137 (84 to 213)	127 (97 to 168)	6 (-23 to 43)	0.14	0.64 (0.50 to 0.78)
PlGF, pg/ml	4.5 (3.5 to 5.9)	7.2 (6.1 to 9.1)	-2.5 (-3.7 to -1.5)	<0.0001	0.62 (0.45 to 0.75)
Tie-2, pg/ml	3282 (2532 to 2735)	4863 (4282 to 5618)	-1562 (-2197 to -1056)	<0.0001	0.55 (0.35 to 0.69)
VEFG-C, pg/ml	694 (461 to 1132)	1594 (966 to 2514)	-775 (-1503 to -317)	<0.0001	0.65 (0.48 to 0.77)
VEGF-D, pg/ml	1163 (867 to 1552)	753 (530 to 1070)	395 (247 to 574)	<0.0001	0.78 (0.67 to 0.86)

CRP, ng/ml	2097154 (1803307 to 2711367)	2167460 (1900109 to 2646626)	-86593 (-469589 to 286755)	0.41	0.64 (0.48 to 0.76)
SAA, ng/ml	2099721 (1071460 to 8722393)	3638280 (1948803 to 15486136)	-1408179 (-5573681 to -536136)	<0.0001	0.93 (0.89 to 0.96)
ICAM-1, ng/ml	515804 (399829 to 613604)	508850 (388147 to 611477)	-3519 (-77813 to 113421)	0.55	0.63 (0.47 to 0.76)
VACM-1, ng/ml	890106 (330532 (3030935)	1052269 (313885 to 3319126)	-24136 (-339998 to 93993)	0.040	0.38 (0.17 to 0.57)

Values are median (interquartile range values) * unavailable for 1 patient; † P-values computed using Wilcoxon-signed rank tests.
r indicates Spearman's rank correlation coefficients. P-values and widths of the confidence intervals for r values were not adjusted for multiple comparisons and may not be used for hypothesis testing.

REFERENCES

- 1 Dargazanli C, Zub E, Deverdun J, *et al.* Machine Learning Analysis of the Cerebrovascular Thrombi Proteome in Human Ischemic Stroke: An Exploratory Study. *Front Neurol.* 2020;11:575376.
- 2 Kollikowski AM, Schuhmann MK, Nieswandt B, *et al.* Local leukocyte invasion during hyperacute human ischemic stroke. *Ann Neurol.* 2020;ana.25665.
- 3 Aliena-Valero A, Baixauli-Martín J, Torregrosa G, *et al.* Clot Composition Analysis as a Diagnostic Tool to Gain Insight into Ischemic Stroke Etiology: A Systematic Review. *J Stroke.* 2021;23:327–42.
- 4 Maglinger B, Sands M, Frank JA, *et al.* Intracranial VCAM1 at time of mechanical thrombectomy predicts ischemic stroke severity. *J Neuroinflammation.* 2021;18:109.
- 5 Zhu H, Hu S, Li Y, *et al.* Interleukins and Ischemic Stroke. *Front Immunol.* 2022;13:828447.
- 6 Alhadidi QM, Bahader GA, Arvola O, *et al.* Astrocytes in functional recovery following central nervous system injuries. *J Physiol.* Published Online First: 13 September 2023. doi: 10.1113/JP284197
- 7 Giannoni P, Badaut J, Dargazanli C, *et al.* The pericyte-glia interface at the blood-brain barrier. *Clin Sci Lond Engl 1979.* 2018;132:361–74.
- 8 Lyden PD. Cerebroprotection for Acute Ischemic Stroke: Looking Ahead. *Stroke.* 2021;52:3033–44.
- 9 Jickling GC, Liu D, Ander BP, *et al.* Targeting neutrophils in ischemic stroke: translational insights from experimental studies. *J Cereb Blood Flow Metab.* 2015;35:888–901.
- 10 Kollikowski AM, Pham M, März AG, *et al.* Platelet Activation and Chemokine Release Are Related to Local Neutrophil-Dominant Inflammation During Hyperacute Human Stroke. *Transl Stroke Res.* 2022;13:364–9.
- 11 Warner JJ, Harrington RA, Sacco RL, *et al.* Guidelines for the Early Management of Patients With Acute Ischemic Stroke: 2019 Update to the 2018 Guidelines for the Early Management of Acute Ischemic Stroke. *Stroke.* 2019;50:3331–2.
- 12 Furie KL, Jayaraman MV. 2018 Guidelines for the Early Management of Patients With Acute Ischemic Stroke. *Stroke.* 2018;49:509–10.
- 13 Maegerlein C, Berndt MT, Mönch S, *et al.* Further Development of Combined Techniques Using Stent Retrievers, Aspiration

- Catheters and BGC : The PROTECTPLUS Technique. *Clin Neuroradiol.* 2020;30:59–65.
- 14 Hacke W, Kaste M, Bluhmki E, *et al.* Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med.* 2008;359:1317–29.
 - 15 Albers GW, Thijs VN, Wechsler L, *et al.* Magnetic resonance imaging profiles predict clinical response to early reperfusion: the diffusion and perfusion imaging evaluation for understanding stroke evolution (DEFUSE) study. *Ann Neurol.* 2006;60:508–17.
 - 16 Mechtouff L, Bochaton T, Paccalet A, *et al.* Association of Interleukin-6 Levels and Futile Reperfusion After Mechanical Thrombectomy. *Neurology.* 2021;96:e752–7.
 - 17 Mechtouff L, Bochaton T, Paccalet A, *et al.* A lower admission level of interleukin-6 is associated with first-pass effect in ischemic stroke patients. *J Neurointerventional Surg.* 2022;14:248–51.
 - 18 Hotter B, Hoffmann S, Ulm L, *et al.* IL-6 Plasma Levels Correlate With Cerebral Perfusion Deficits and Infarct Sizes in Stroke Patients Without Associated Infections. *Front Neurol.* 2019;10:83.
 - 19 Armstead WM, Hekierski H, Pastor P, *et al.* Release of IL-6 After Stroke Contributes to Impaired Cerebral Autoregulation and Hippocampal Neuronal Necrosis Through NMDA Receptor Activation and Upregulation of ET-1 and JNK. *Transl Stroke Res.* 2019;10:104–11.
 - 20 Waje-Andreassen U, Kråkenes J, Ulvestad E, *et al.* IL-6: an early marker for outcome in acute ischemic stroke. *Acta Neurol Scand.* 2005;111:360–5.
 - 21 Suzuki S, Tanaka K, Suzuki N. Ambivalent aspects of interleukin-6 in cerebral ischemia: inflammatory versus neurotrophic aspects. *J Cereb Blood Flow Metab Off J Int Soc Cereb Blood Flow Metab.* 2009;29:464–79.
 - 22 Monsour M, Croci DM, Agazzi S, *et al.* Contemplating IL -6, a double-edged sword cytokine: Which side to use for stroke pathology? *CNS Neurosci Ther.* 2023;29:493–7.
 - 23 Hagihara K, Nishikawa T, Isobe T, *et al.* IL-6 plays a critical role in the synergistic induction of human serum amyloid A (SAA) gene when stimulated with proinflammatory cytokines as analyzed with an SAA isoform real-time quantitative RT-PCR assay system. *Biochem Biophys Res Commun.* 2004;314:363–9.
 - 24 Ye RD, Sun L. Emerging functions of serum amyloid A in inflammation. *J Leukoc Biol.* 2015;98:923–9.
 - 25 Jenny NS, Callas PW, Judd SE, *et al.* Inflammatory cytokines and ischemic stroke risk: The REGARDS cohort. *Neurology.* 2019;92:e2375–84.
 - 26 Smith CJ, Emsley HCA, Gavin CM, *et al.* Peak plasma interleukin-6 and other peripheral markers of inflammation in the first week of ischaemic stroke correlate with brain infarct volume, stroke severity and long-term outcome. *BMC Neurol.* 2004;4:2.
 - 27 Gertz K, Kronenberg G, Kälin RE, *et al.* Essential role of interleukin-6 in post-stroke angiogenesis. *Brain.* 2012;135:1964–80.
 - 28 Simats A, García-Berrocoso T, Montaner J. Neuroinflammatory biomarkers: From stroke diagnosis and prognosis to therapy. *Biochim Biophys Acta.* 2016;1862:411–24.
 - 29 Arumugam TV, Granger DN, Mattson MP. Stroke and T-cells. *Neuromolecular Med.* 2005;7:229–42.

- 30 Deng S, Jin P, Sherchan P, *et al.* Recombinant CCL17-dependent CCR4 activation alleviates neuroinflammation and neuronal apoptosis through the PI3K/AKT/Foxo1 signaling pathway after ICH in mice. *J Neuroinflammation*. 2021;18:62.
- 31 Kapoor A, Lanctôt KL, Bayley M, *et al.* “Good Outcome” Isn’t Good Enough. *Stroke*. 2017;48:1688–90.

Figure captions

Figure 1: Flowchart of the study

Figure 2: A-B) Example of blood sampling in a patient with right M1 occlusion (arrow, left image), with a microcatheter in the M2 segment (arrow, right image). C-C1) Example of protein check by SDS gel electrophoresis and depletion to unmask all proteins in the peri-clot and peripheral samples.

Figure 3: Association of the level of disability assessed by the modified Rankin score (mRS) at 90-day with biological markers assessed before thrombectomy from intracranial (A) and peripheral (B) blood samples. r indicates Spearman's rank correlation coefficients, calculated before and after adjustment for age, intravenous thrombolysis, pre-treatment NIHSS and ASPECT scores. The widths of the confidence intervals were not adjusted for multiple comparisons and may not be used for hypothesis testing; * significant at 0.05 level after adjustment for false discovery rate. Abbreviations: ASPECT = Alberta Stroke Program Early Compute Score; CI = confidence interval; NIHSS = National Institute of Health Stroke Score

Figure 4: Association of the Ischemic Stroke Volume at 24-hours with biological markers assessed before thrombectomy from intracranial (A) and peripheral (B) blood samples. r indicates Spearman's rank correlation coefficients, calculated before and after adjustment for age, intravenous thrombolysis, pre-treatment NIHSS and ASPECT scores. The widths of the confidence intervals were not adjusted for multiple comparisons and may not be used for hypothesis testing. None of the adjusted correlation coefficients reached the significance level after adjustment for false discovery rate. Abbreviations: ASPECT= Alberta Stroke Program Early Compute score; CI = confidence interval; NIHSS = National Institute of Health Stroke Score

Supplemental Figure 1: Association of the Ischemic Stroke Volume at baseline with biological markers assessed before thrombectomy from intracranial (A) and peripheral (B) blood samples. r indicates Spearman's rank correlation coefficients, calculated before and after adjustment for age, intravenous thrombolysis, pre-treatment NIHSS and ASPECT scores. The widths of the confidence intervals were not adjusted for multiple comparisons and may not be used for hypothesis testing. None of the adjusted correlation coefficients reached the significance level after adjustment for false discovery rate. Abbreviations: ASPECT= Alberta Stroke Program Early Compute score; CI = confidence interval; NIHSS = National Institute of Health Stroke Score

Supplemental Figure 2: Association of the Ischemic Stroke Volume at baseline with biological markers assessed before thrombectomy from intracranial (A) and peripheral (B) blood samples. r indicates Spearman's rank correlation coefficients, calculated before and after adjustment for age, intravenous thrombolysis, pre-treatment NIHSS and ASPECT scores. The widths of the confidence intervals were not adjusted for multiple comparisons and may not be used for hypothesis testing. None of the adjusted correlation coefficients reached the significance level after adjustment for false discovery rate. Abbreviations: ASPECT= Alberta Stroke Program Early Compute score; CI = confidence interval; NIHSS = National Institute of Health Stroke Score