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Predicting reperfusion injury and functional status after stroke using blood biomarkers: the STROKELABED study

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Abstract

Background Ischemic stroke is a leading cause of disability and mortality, particularly among the elderly. Recanalization therapies, including thrombolysis and thrombectomy, are essential for restoring blood flow and saving ischemic tissue. However, these interventions may trigger reperfusion injury, worsening inflammation and tissue damage, leading to blood-brain-barrier (BBB) disruption, cerebral edema (CE) and adverse functional outcomes. Here we propose a model integrating circulating inflammatory biomarkers with metabolomic and lipoproteomic data able to help clinicians in predicting BBB disruption, CE at 24 h post stroke onset and poor post-stroke functional outcome (Modified Rankin Scale (mRS > 2).

Methods Peripheral blood from 87 patients was collected at admission and 24 h after stroke onset. The logistic LASSO regression algorithm was employed to identify the optimal combination of metabolites, lipoprotein-related parameters and circulating biomarkers to discriminate the groups of interest at the two time-points.

Results Multivariable logistic regression models included as covariates: age, sex, onset-to-treatment time, treatment with lipid-lowering medications before stroke, history of heart failure, history of atrial fibrillation and history of diabetes. The regression models showed that methionine, acetate, GlyA and MMP-2 were significant predictors of BBB disruption, methionine, acetate, TIMP-1 and CXCL-10 predicted 24-hours CE, whereas a poor functional outcome at three months was predicted by CXCL-10, IL-12 and LDL-5.

Conclusions As stroke has a heterogeneous pathophysiology, a personalized approach based on biomarkers, as presented in this study, shown to be effective in tackling patient individual risk and could help in developing novel diagnostic, prognostic, and therapeutic neuroprotective strategies for the management of stroke patients.

Keywords Stroke, Biomarkers, Inflammation, Blood brain barrier, Cerebral edema, Functional outcome

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Introduction

Ischemic stroke is a leading cause of disability and mortality worldwide. As individuals age, the risk of experiencing a stroke significantly increases, making stroke a major health concern among the elderly population [1]. Recanalization treatments (systemic thrombolysis, endovascular thrombectomy and their combination) can successfully re-open occluded vessels and save the ischemic brain tissue from death promptly. However, while reperfusion is essential for limiting neurological deficits, paradoxically, it can sometimes exacerbate tissue damage and inflammation through a phenomenon known as reperfusion injury (RI), thus leading to worse clinical outcomes and posing a great challenge to clinicians [2, 3].

The multifaceted nature of RI involves intricate molecular and cellular mechanisms, including oxidative stress, immune response, inflammation, platelet activation, activation of metalloproteinases (MMPs), which altogether lead to blood-brain-barrier (BBB) disruption and eventually to cerebral edema (CE), hemorrhagic transformation, and infarct grow [4, 5]. Advanced neuroimaging techniques can assess the extent of brain damage and BBB integrity, while the Modified Rankin Scale (mRS) is usually employed to measure the degree of disability and handicap three months after the ictal event [6].

Despite years of research efforts, the ability to accurately predict which patients are at higher risk for RI remains limited, underscoring the need for more precise and reliable stratification methods. Developing novel stratification tools can aid in optimizing treatment strategies, minimizing risks, and optimizing patient outcomes.

Experimental studies on cerebral ischemia have already demonstrated that inflammatory mediators (e.g., cytokines and chemokines), MMPs and their inhibitors, and endothelial function mediators may have a role in RI [5]. Metabolomics as well as lipoproteomics provide the molecular characterization of patients within the dynamic context of a disease process [7–10], and they have already provided insight into stroke scientific research [11–15]. Here we propose to integrate circulating biomarkers with metabolomic and lipoproteomic data to help predict BBB disruption, CE evaluated at 24 h post stroke onset and compromised post-stroke functional outcome defined as 3-month mRS > 2 in acute ischemic stroke (AIS) patients.

Methods

Ethical statements

All procedures were performed in compliance with relevant laws and institutional guidelines, and the study has been approved by the local Ethics Committee (ethics committee registration number: Regional Ethics Committee of Tuscany, Comitato Etico Area Vasta Centro [CEAVC] 16923_oss).

Signed informed consent was obtained from all the surviving patients included in the study.

Study population

This observational, prospective, single-center, hospital-based study included 87 patients with anterior circulation ischemic stroke, all within 12 h of last being seen well, treated either with intravenous thrombolysis and/or endovascular thrombectomy. These patients are a subset of those enrolled, by the outpatient clinic of the Careggi University Hospital in Florence, in the framework of the Acute ischemic STROKE– from laboratory to the Patient's BED (STROKELABED) study. The study protocol is registered at https://clinicaltrials.gov/study/NCT05725694?term=NCT05725694%26;rank=1 (ClinicalTrials.gov ID: NCT05725694, 23/01/2023 retrospectively registered) and details on its study design, inclusion/exclusion criteria, and methodology can be retrieved from the protocol previously described [16, 17]. A complete neuroimaging assessment, including non-contrast computed tomography (NCCT), CT-angiography (CTA), and CT-perfusion (CTP) was performed at hospital arrival before acute interventions, according to guidelines in place at the time of treatment [18, 19]. All patients underwent a 24-h follow-up NCCT.

Outcome measures

Functional outcome at 3 months after stroke onset was assessed for all enrolled patients using the mRS, either in person during an outpatient visit or via telephone interview if the patient was unable to attend.

BBB disruption is defined as K trans value > 0.63 on CTP at admission. K trans values were expressed in $ml \cdot min^{-1} \cdot (100\ g)^{-1}$. The imaging protocol has been already detailed [20].

Two trained neurologists independently evaluated each patient's 24-h NCCT and assessed the presence of CE according to CE classification used by the Helsinki Stroke Thrombolysis Registry Group [21], where:

- CED1 = Focal brain swelling up to one-third of the hemisphere;
- CED2 = Focal brain swelling greater than one-third of the hemisphere;
- CED3 = Brain swelling with midline shift;
- NONE = absence of cerebral edema.

Sample collection

Peripheral blood was collected twice, at admission (t0) and 24 h after stroke onset (t1). Not all patients provided both serum samples as expected, thus the total number of samples collected was 133: 60 t0 samples and 73 t1 samples, 87 patients provided at least one sample (t0 or t1). Whole venous blood was collected in tubes without

anticoagulant and with sodium citrate (3.2%, 0.109 M). Tubes without and with anticoagulants were centrifuged at room temperature at 1500 g for 15 min, and the supernatants were stored in aliquots at − 80 °C until the measurement of blood biomarkers and NMR analyses. Biomarker measurements were blind to clinical data.

Circulating biomarkers protocol

Biomarkers were selected according to a previous review by our research group [22]. Samples were analyzed in a unique central laboratory. A broad panel of cytokines/chemokines levels [interleukin (IL)-4, IL-6, IL-8, IL-10, Tumor Necrosis Factor alpha (TNF-alpha), chemokine (C-C motif) ligand 2 (CCL2) also referred to as monocyte chemoattractant protein 1, C-X-C motif chemokine ligand 10 (CXCL-10) also known as Interferon gamma-induced protein 10, Intercellular Adhesion Molecule-1 (ICAM-1), Vascular cell adhesion protein 1 (VCAM-1) and Vascular-Endothelial Growth Factor (VEGF)] and metalloproteinases (MMP-2, MMP-7, MMP-8, MMP-9, MMP-12), extracellular matrix metalloproteinase inducer (EMMPRIN) and tissue inhibitors of metalloproteinases (TIMP-1, TIMP-2, TIMP-3 and TIMP-4) were determined on serum samples using a Bio-Plex suspension array system and R&D Kits (R&D System, Milan, Italy) according to the manufacturer's instructions. The coefficients of variation of inflammatory markers, MMPs and TIMPs assays were < 6%.

Von Willebrand Factor (VWF) Antigen circulating levels were determined on citrated plasma samples by a latex particle-enhanced immunoturbidimetric assay (Werfen, Milan, Italy). Plasminogen Activator Inhibitor type 1 (PAI-1) Antigen levels were assessed on citrated samples by an immunoenzymatic assay (Hyphen Biomed, Neuville-sur-Oise, France).

NMR analysis

Serum samples were thawed at room temperature and shaken before use. A total of 350 µL of sodium phosphate buffer (75 mM $Na_2HPO_4 \times 7H_2O$; 20% (v/v) ^2H_2O, 4.6 mM 3-(Trimethylsilyl) propionate-2,2,3,3-d4; 6.1 mM NaN_3; pH 7.4) was added to 350 µL of each sample. After homogenization by vortexing for 30 s, 600 µL of each mixture was transferred into a 5 mm NMR tube.

The ^{1}H-NMR spectra of all samples have been acquired using a Bruker 600 MHz spectrometer (Bruker BioSpin) operating at 600.13 MHz proton Larmor frequency and equipped with a 5 mm PATXI ^{1}H-^{13}-^{15} N and ^{2}H-decoupling probe including a z-axis gradient coil, an automatic tuning-matching (ATM) unit and an automatic refrigerated (6 °C) sample changer (SampleJet, Bruker BioSpin). A BTO 2000 thermocouple has been utilized for temperature stabilization at the level of approximately 0.1 K at the sample. Before starting the NMR acquisition, each sample was maintained inside the NMR probe for at least 300 s to equilibrate temperature at 310 K. Spectrometer calibration was performed daily following standard operating procedures which ensure high spectral quality and reproducibility.

For each serum sample, a standard nuclear Overhauser effect spectroscopy (NOESY) sequence [23] was applied using 32 scans, 98,304 data points, a spectral width of 18,028 Hz, an acquisition time of 2.7 s, a relaxation delay of 4 s and a mixing time of 0.01 s to detect the NMR signals of both high and low molecular weight molecules in concentrations above the NMR detection limit. Before applying Fourier transform, free induction decays were multiplied by an exponential function equivalent to a 0.3 Hz line-broadening factor. Transformed spectra were automatically corrected for phase and baseline distortions and calibrated to the anomeric glucose doubled at δ 5.24 ppm.

A panel of 41 metabolites were unambiguously identified and quantified using the Plasma/Serum B.I.Quant-PS platform™ (version 2.0.0). Metabolites with more than 20% of observation under the limit of quantification were excluded from the present analysis, thus the system was reduced to 26 metabolites. In addition, the signals of glycoproteins GlycA at δ 2.04 and GlycB at δ 2.08 ppm were quantified by integration using an in-house developed R script. The identification and quantification of 112 lipoprotein-related parameters was performed utilizing the B.I. LISA platform™ (version 1.0.0). This platform provides information about the concentrations of triglycerides, cholesterol, free cholesterol, phospholipids, Apo-A1, Apo-A2 and Apo-B100 of the main fractions and subfractions of VLDL, IDL, LDL and HDL classes [24].

Statistical analysis

All data analyses were performed in the "R" statistical environment. The logistic LASSO regression algorithm was employed to identify the optimal combination of metabolites, lipoprotein-related parameters and circulating biomarkers to discriminate the groups of interest. Missing samples were excluded from the analysis. We selected the LASSO regression algorithm because our aim was to select a limited number of predictors from the entire set of variables. LASSO was chosen based on its well-established ability to perform variable selection and regularization simultaneously, which makes it particularly suitable for high-dimensional data where the number of predictors is large compared to the number of observations or when potential multicollinearity among predictors is present. LASSO is known to promote sparsity in the model, effectively identifying the most relevant predictors and reducing overfitting, which is crucial when working with complex datasets [25, 26]. LASSO regression models were calculated using the "glmnet"

function of the eponymous R package. The tuning parameter lambda for each model was chosen by 10-fold cross-validation (R function "cv.glmnet"). To avoid overfitting, the model that maximized accuracy while using the fewest variables was selected for each outcome of interest. Multivariable logistic regression models included as covariates: age, sex, onset-to-treatment time, treatment with lipid-lowering medications before stroke, history of heart failure, history of atrial fibrillation and history of diabetes. Each model was internally validated by means of a leave-one-out cross-validation (LOO-CV) scheme, each area under the receiver operating characteristic curve (AUROC) and each 95% confidence interval were calculated using the functions included in the R package "pROC". AUROC of each model were assessed for significance against the null hypothesis of no prediction by means of a 100 randomized class-permutations test.

To point out differences among patients included in the groups of interest, metabolites, lipoprotein-related parameters and circulating biomarkers were examined using the Wilcoxon rank-sum test. *P*-values were adjusted for multiple testing using the false discovery rate (FDR) procedure with the Benjamini-Hochberg correction at $\alpha = 0.1$.

Table 1 Demographic and clinical characteristics of the baseline study cohort ($n = 87$)

Demographic and Clinical Characteristics	**Total Cohort *n* = 87**
Age (yrs), median (IQR)	79.3 (70.7–84.8)
Sex M, n (%)	45 (51.7)
Current Smokers, n (%)	14 (16.1)
Hyperlipidemia, n (%)	48 (55.2)
Heart Failure, n (%)	18 (20.7)
Atrial Fibrillation, n (%)	28 (32.2)
Diabetes, n (%)	11 (12.6)
Hypertension, n (%)	21 (24.1)
Blood Brain Barrier Disruption, n (%)	37 (42.5)
Cerebral Edema, n (%)	34 (39.1)
Relevant HT*, n (%)	15 (17.2)
3-month Modified Rankin Scale > 2, n (%)	41 (47.1)
Acute Ischemic Stroke Treatments	
Systemic Thrombolysis	36 (41.4)
Endovascular Thrombectomy	26 (29.9)
Endovascular Thrombectomy with Systemic Thrombolysis	25 (28.7)
Stroke Treatment Latency (min), median (IQR)	155 (120–293)
NIH Stroke Scale, median (IQR)	17 (11–22)
Lipid-Lowering Medications, n (%)	31 (35.6)
Corticosteroids, n (%)	2 (2.3)

* Relevant HT was defined as hemorrhagic infarction type two or any type of parenchymal hemorrhage (PH1 and PH2) on the 24 h NCCT according to Larrue et al. [27]

Results

Overall population characteristics

The analyses were conducted on 87 patients diagnosed with ischemic stroke at the moment of hospital admission. Demographic, clinical and pharmacological information of the study population are reported in Table 1 for the entire population. Information on ischemic stroke treatments is reported in the table as well as the time latency between the stroke event and the treatment.

Univariate analysis of metabolites, lipoproteins and circulating biomarkers

We investigated the association of the three outcomes of interest with metabolites, lipoprotein-related parameters and circulating biomarkers. Increased methionine and reduced GlycA and GlycB levels at t0 showed to be significantly associated with altered BBB permeability, whereas higher glucose levels were associated with cerebral edema at t1 (Fig. 1).

Five parameters related to the LDL-5 subfraction showed to be significantly reduced at t0 in patients with compromised post-stroke functional outcome (mRS > 2) (Fig. 2 and Supplementary Fig. S1).

Tissue inhibitor of metalloproteinases 1 (TIMP-1) levels at t0 were significantly decreased in patients with cerebral edema. C-X-C motif chemokine ligand 10 (CXCL-10) levels at t0 were increased in patients with compromised post-stroke functional outcome, whereas CXCL-10 levels at t1 showed an inverse association with cerebral edema (Fig. 3).

Multivariable models to predict reperfusion injury and functional outcome

Reperfusion injury is a pathophysiological term that describes complex biochemical mechanisms that may boost the damage of the ischemic tissue. We investigated the possibility of predicting BBB disruption and cerebral edema, as two of the major conditions associated with RI. Furthermore, we included in our analysis also the prediction of patient poor functional outcome (defined as mRS > 2 at three months). The predictive models were calculated separately for t0 and t1 samples. For each model, cross-validated AUROC, *p*-value and the variables included are reported in Table 2. Results of the models calculated using clinical variables only were also provided in Table 2 and showed less significant predictivity.

Discussion

In clinical practice, RI is still unpredictable, and it is associated with the worst clinical outcomes in stroke patients [28]. CE is reported to occur in 10–78% of patients with any type of ischemic stroke, moreover, it can result in massive cerebral swelling that, in turn, carries 80% of the case-fatality rate [29]. The literature assessing the

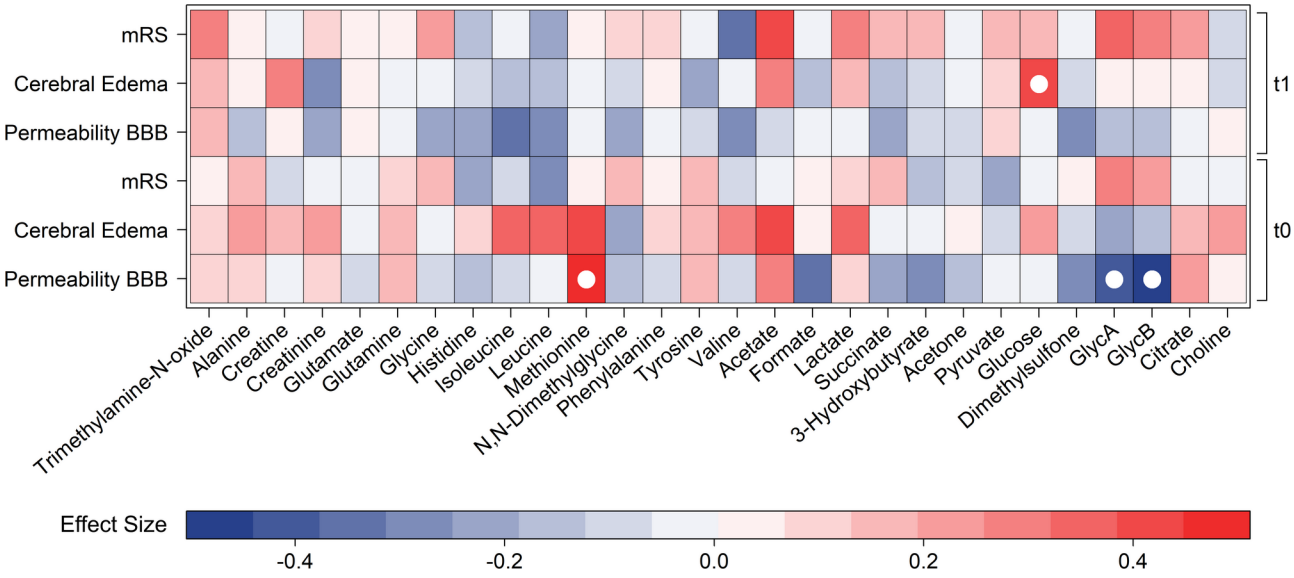

Fig. 1 Heatmap of metabolite concentrations in the groups determined by the three outcomes of interest (altered BBB permeability, cerebral edema, mRS > 2). Metabolite levels were measured at t0 and t1. The Cliff's Delta effect size is represented with red (blue) for higher (lower) levels in patients with worse prognosis as illustrated in the color key. White dots encode for metabolites with *p*-values FDR adjusted < 0.1

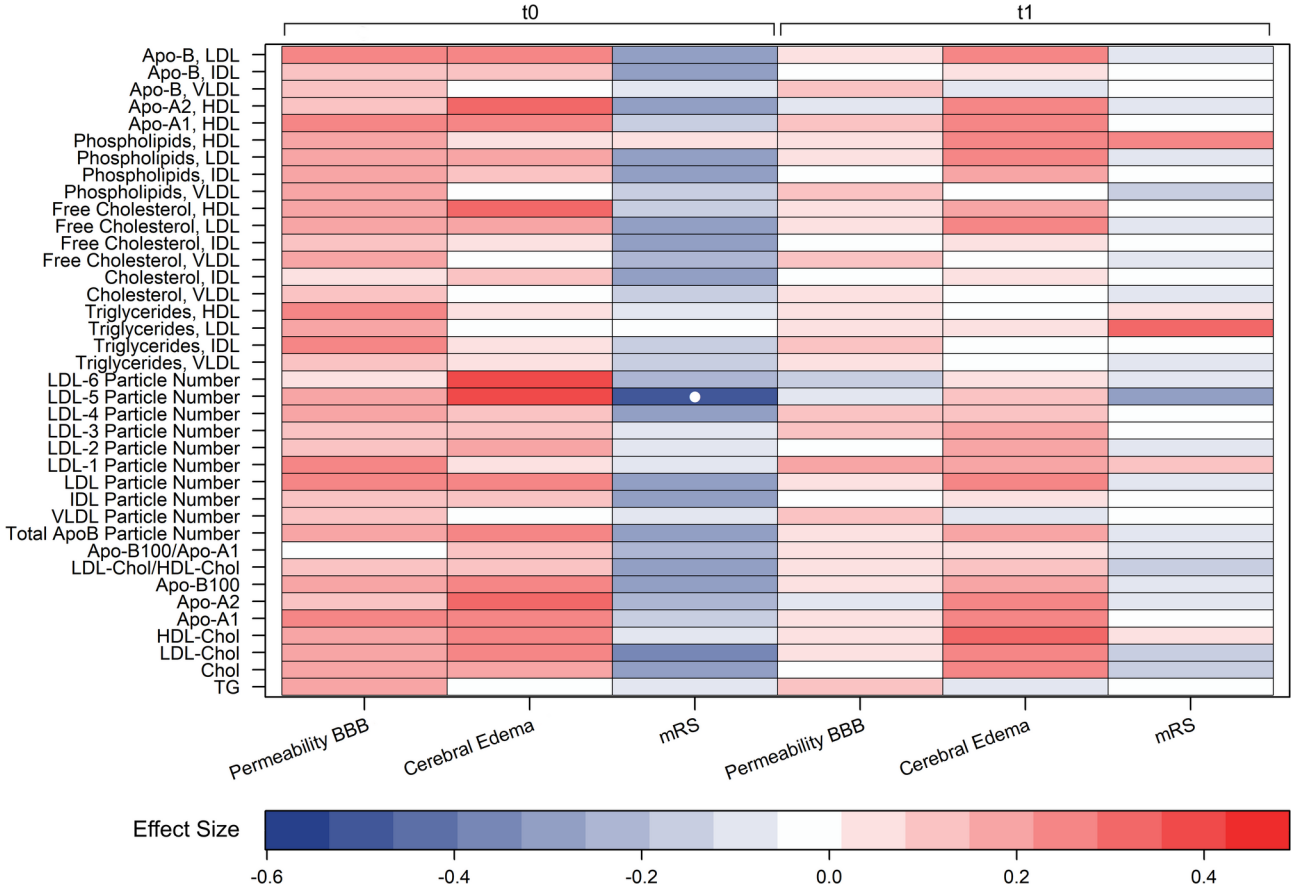

Fig. 2 Heatmap of concentrations of the lipoprotein-related main fractions in the groups determined by the three outcomes of interest (altered BBB permeability, cerebral edema, mRS > 2). Lipoprotein levels were measured at t0 and t1. The Cliff's Delta effect size is represented with red (blue) for higher (lower) levels in patients with worse prognosis as illustrated in the color key. White dots encode for metabolites with *p*-values FDR adjusted < 0.1.

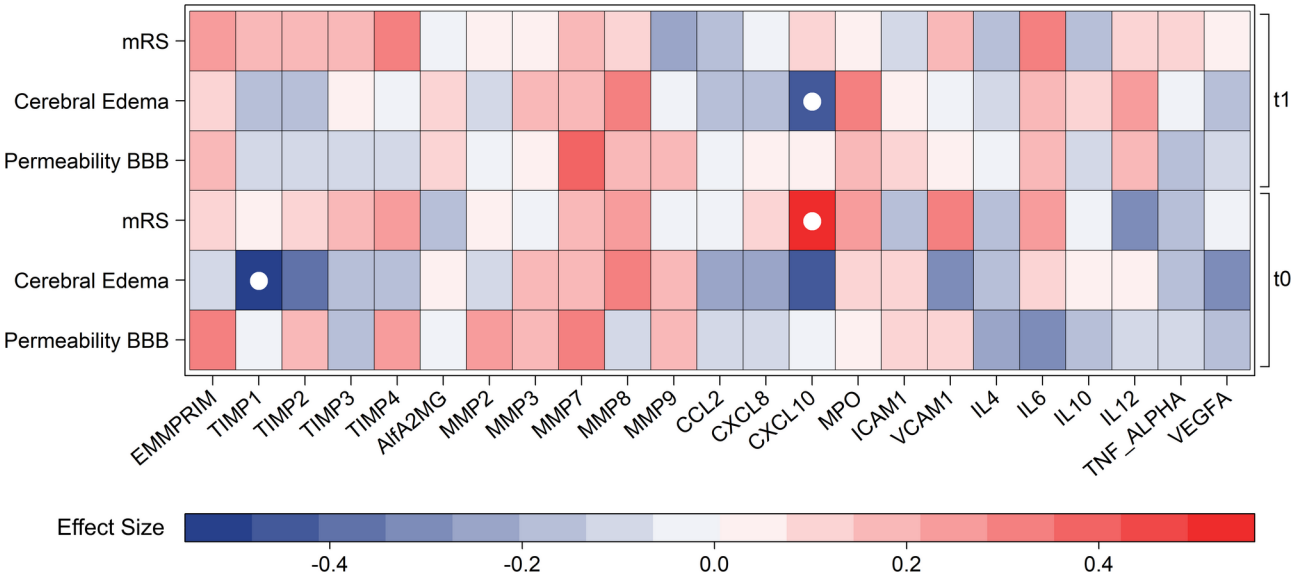

Fig. 3 Heatmap of concentrations of circulating biomarkers in the groups determined by the three outcomes of interest (altered BBB permeability, cerebral edema, mRS > 2). Circulating biomarker levels were measured at t0 and t1. The Cliff's Delta effect size is represented with red (blue) for higher (lower) levels in patients with worse prognosis as illustrated in the color key. White dots encode for metabolites with *p*-values FDR adjusted < 0.1

Table 2 Results of the multivariable regression models calculated to predict the three outcomes of interest: BBB disruption, cerebral edema and mRS > 2 at three months after stroke. Models were adjusted for potential confounding factors: age, sex, onset-to-treatment delay, being on lipid-lowering medications before stroke, history of heart failure, history of atrial fibrillation and history of diabetes. Results obtained using clinical variables only are reported as well.

Outcome	Clinical Variables		Predictors adjusted for clinical variables		
	AUROC (95% CI)	*p*-value	AUROC (95% CI)	*p*-value	Predictors
BBB disruption	0.52 (0.36–0.58)	0.86	0.77 (0.63–0.90)	0.04	t0: Methionine, Acetate, GlycA, MMP2
	0.63 (0.50–0.77)	0.22	0.82 (0.72–0.92)	0.02	t1: Isoleucine, MMP7, MMP9
Cerebral edema	0.72 (0.58–0.86)	0.09	0.73 (0.59–0.88)	0.04	t0: Methionine, Acetate, TIMP1, CXCL-10
	0.53 (0.39–0.67)	0.77	0.83 (0.75–0.93)	0.01	t1: Creatine, Acetate, Free Cholesterol HDL-4, CXCL-10, IL12
mRS > 2 at three months after stroke	0.71 (0.58–0.86)	0.07	0.81 (0.69–0.94)	0.01	t0: Particle Number LDL-5, CXCL-10, IL12
	0.52 (0.39–0.67)	0.86	0.82 (0.72–0.92)	0.01	t1: Glycine, Valine, Acetate, GlycA, Triglycerides LDL-2, Triglycerides LDL-4, Cholesterol LDL-5, IL10"

post-recanalization period of acute stroke is still scanty but growing, and the pathophysiology and its implications in clinical outcomes are still a matter of debate [30]. Both preclinical and clinical research are still looking for drugs that can antagonize reperfusion injury (including CE) with good efficacy and safety profile, similar to what is recently turning out as for the glenzocimab related to the no-reflow phenomenon [31, 32].

We used circulating biomarkers, along with metabolomic and lipoproteomic data, to develop regression models that significantly predicted BBB disruption, CE at 24 h post stroke onset, and poor post-stroke functional outcome three months after stroke. The models were calculated using both the samples collected before treatment (baseline-t0), and 24 h after stroke onset (t1); however, the results obtained using the samples collected at the time of hospital admission are the most relevant, being CE widely reported to occur within 24 h. Furthermore, baseline measurements meet the temporal criteria necessary to be considered true predictors of early complications such as BBB disruption and CE.

The regression model based on t0 samples for predicting BBB disruption included methionine, acetate, GlycA and MMP2. Our metabolomic analysis revealed a significant association between methionine levels and BBB disruption. It is well known that homocysteine is a key intermediate in the metabolism of methionine and cysteine, and several clinical studies have demonstrated homocysteine involvement in the pathogenesis of ischemic stroke [33–35]. Acute severe hypermethioninemia has be associated with adverse neurological outcomes, including death [36]. Furthermore, there is evidence that hypermethioninemia may contribute to brain edema [37], although the underlying mechanisms remain poorly understood. Recent studies have reported that acute administration of methionine and/or oxidized methionine in rats induces oxidative stress and disrupts cholinergic signaling in the hippocampus, striatum, and

cerebellum of young rats, also leading to cerebral edema [38].

Surprisingly, increasing of acetate and reduction of GlycA were found in patients that will develop BBB disruption. These trends contrast with those described in the limited literature currently available and will require further investigations.

Accumulating evidence suggests that MMPs, particularly MMP-2, are involved in neuropathological processes by disrupting the extracellular matrix and the tight junctions that maintain the integrity of the BBB [39]. Metalloproteinases such as MMP-9, MMP-2, and MMP-3 are central to the regulation of BBB permeability. MMP-2, derived from astrocytes, endothelial cells, and potential leukocytes, has been shown to increase in the brain within 1 to 3 h after stroke onset in rodent and primate models, remaining elevated for several days [40]. The increase of MMP-2 is associated with early BBB opening and degradation of tight junction proteins such as claudin-5 and occludin in rodent models of middle cerebral artery occlusion. In addition, direct injection of MMP-2 into rodent brain disrupts the BBB and results in hemorrhage, further supporting the role of MMP-2 in early BBB disruption following stroke [41].

The regression model based on t0 samples for predicting cerebral edema included methionine, acetate, TIMP1, and CXCL-10. Interestingly, in this study we observed lower levels of TIMP-1, an inhibitor of several MMPs that is involved in maintaining and remodeling the extracellular matrix. In fact, TIMPs, along with cytokines, growth factors, hormones, and α2-macroglobulin can inhibit the activity of MMPs. Thus, a favorable balance between MMPs activity and inhibition by TIMPs is essential for preventing various pathological conditions, including neurodegenerative disorders, atherosclerosis, and fibrosis.

TIMP-1 is up-regulated by several factors such as interleukin (IL)-1β, transforming growth factor (TGF)-β1, epithelial growth factor (EGF), IL-6 and other inflammatory molecules, highlighting its close association with inflammatory processes.

Animal studies have shown that TIMP-1 exerts neuroprotective effects in the central nervous system by supporting BBB integrity: TIMP-1 interacts with extra cellular matrix components by inhibiting MMPs and reduces calcium influx following excitotoxic stress [42]. Therefore, in our patient population, the lower levels of TIMP-1 may have contributed, at least in part, to the development of cerebral edema observed within 24 h from stroke.

Unexpectedly, in our study, we observed lower levels of CXCL-10 at baseline (t0) in patients who developed cerebral edema. This finding is not readily explained by current understanding of the pathophysiological mechanisms underlying ischemic stroke. Neuroinflammation following stroke is typically characterized by the infiltration of leukocytes into the central nervous system through the BBB, a process primarily controlled by chemokines such as CXCL-10 [43]. Although CXCL-10 has been suggested to play both detrimental and protective roles in disease progression and resolution, recent in vivo and ex vivo studies indicate that its net effect in the context of ischemic injury is more likely to be damaging than protective [43]. However, CXCL-10 is not solely an attractor of leukocytes to inflamed tissues, but it is also has non-chemotactic functions within the CNS. Therefore, the impact of modulating the function of CXCL-10 is likely to be both highly disease- and context-specific [44]. Moreover, chemokines have time-dependent roles in regulating cell recruitment, BBB permeability, angiogenesis, and neurogenesis. In early after phases following ischemic stroke, they tend to exacerbate cerebral injury by promoting BBB breakdown and leukocyte infiltration. At later stages, however, chemokines may contribute to recovery by attracting neural progenitor cells to damaged regions of brain [45]. In our study, cerebral edema was associated with lower levels of CXCL-10 at t0, while poor functional outcome (mRS > 2) was associated with higher CXCL-10 levels at t0. These findings suggest that CXCL-10 may play distinct roles during the acute and recovery phases of stroke, potentially contributing to both injury and repair depending on the timing and context.

The regression model based on t0 samples for predicting patient functional outcome at three months from stroke included particle number LDL-5, CXCL-10 and IL12. We found a general decrease in LDL cholesterol fractions and subfractions, particularly LDL-5 subfractions.

While small dense LDL particles are known to be more atherogenic, their association with stroke remains controversial. These particles penetrate the arterial wall more easily, are more prone to oxidation, and bind readily to glycosaminoglycans, suggesting they may be independent risk factors for coronary and peripheral artery disease as well as carotid atherosclerosis, compared to LDL cholesterol. Regarding functional outcomes, a hospital-based follow-up study in Chinese AIS patients found a significant association between poor functional outcome and small density LDL levels [46]. Conversely, reduced LDL cholesterol level has also been associated with severe neurological deficits in hemorrhagic stroke, increased risk of unfavorable outcomes at discharge (mRS > 2), and increased mortality after intracerebral hemorrhage [47–49]. This may be due to cholesterol's essential role in maintaining membrane integrity and fluidity of vascular vessels, which is vital for normal vascular function [49].

Although our study is innovative, some limitations should be acknowledged. The first is the small number

of patients, due to strict inclusion criteria designed to ensure the greatest possible homogeneity among enrolled participants. However, this lack of diversity in demographics (e.g., ethnicity, comorbidities) limits the generalizability of this single-center study, and a larger sample size would improve the robustness of our models. Therefore, our findings need to be reproduced and validated in larger, multi-center cohorts in future studies. We measured BBB disruption by the means of pretreatment CTP. However, the short image acquisition time might underestimate BBB disruption in ischemic areas with severely reduced blood flow (i.e., ischemic core), as insufficient contrast extravasation may occur under these conditions [20]. Additionally, our panel of circulating biomarkers did not include markers specific to tight junctions limiting our ability to investigate neurovascular unit dysfunction. This is significant, as the neurovascular unit plays a critical role in the biphasic increase in BBB permeability, which is driven by disintegration and redistribution of tight junctions [50].

Conclusions

Neuroinflammation, along with various metabolic pathways, can have both beneficial and detrimental effects. The dual role of the inflammatory response presents a significant challenge for clinical trials targeting neuroinflammation. In conclusion, our results suggest that early changes in specific biomarkers and metabolites may enhance the ability to predict BBB disruption, CE, and functional status three months after stroke in AIS patients.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-025-06498-z.

Supplementary Figure S1. Heatmap of concentrations of the lipoprotein-related subfractions in the groups determined by the three outcomes of interest (altered BBB permeability, cerebral edema, mRS > 2). Lipoprotein levels were measured at t0 and t1. The Cliff's Delta effect size is represented with red (blue) for higher (lower) levels in patients with worse prognosis as illustrated in the color key. White dots encode for metabolites with ***p***-values FDR adjusted < 0.1.

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Author contributions
Conceptualization: MB, BP, AMG, BG; Data curation: AV, BP, AS, VP; Formal analysis: AV, ES, AMG, AK, LT; Funding acquisition: LT, MB; Investigation: AV, VP, EF, BP, AS, FA, ES, AMG, BG, AK; Methodology: AV, ES, MB, BP, FA, AMG, LT; Project administration: MB; Resources: EF, CS; Supervision: CS; Visualization: AV; Writing– original draft: AV, AMG, LT, MB; Writing– review and editing: AV, AMG, LT, MB.

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Data availability
The data that support the findings of this study are available on reasonable requests from the corresponding authors. The data are not publicly available due to privacy and/or ethical restrictions.

Declarations

Ethics approval and consent to participate
This study is compliant with all the relevant national regulations, institutional policies and in accordance with the tenets of the Helsinki Declaration and has been approved by the local Ethics Committee (ethics committee registration number: Comitato Etico Area Vasta Centro [CEAVC] 16923_oss).

Consent for publication
Not applicable.

Competing interests
The authors declare that the research was conducted without any commercial or financial relationships that could be construed as potential conflicts of interest.

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