

ORIGINAL RESEARCH

Prospective Validation of Glial Fibrillary Acidic Protein, D-Dimer, and Clinical Scales for Acute Large-Vessel Occlusion Ischemic Stroke Detection

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BACKGROUND: Large-vessel occlusion (LVO) ischemic stroke is responsible for significant morbidity and mortality. We have previously described a novel tool for acute LVO detection that combines blood-based biomarkers (glial fibrillary acidic protein and D-dimer) with stroke severity scales to achieve high accuracy. Accordingly, the present study sought to prospectively validate cutoff values that we had previously established for biomarkers and scales.

METHODS: The TIME (Testing for Identification Markers of Stroke) trial was designed as a prospective observational diagnostic accuracy study. All ambulance-identified stroke code activations <18 hours from symptom onset were recruited at Brandon Regional Hospital (Brandon, FL) between May 2021 and August 2022. Previously determined cutoff concentrations of plasma glial fibrillary acidic protein (213 pg/mL) and D-dimer (600 ng/mL) were used in combination with prehospital stroke scales to detect LVO. We compared rates of LVO detection against a reference standard using computed tomography/magnetic resonance angiography.

RESULTS: A total of 382 patients with suspected stroke were recruited. The final cohort was composed of 323 patients with suspected stroke with the following distribution: LVO ischemic stroke ($n = 29$, 9%), non-LVO ischemic stroke ($n = 48$, 15%), hemorrhagic stroke ($n = 13$, 4%), transient ischemic attack ($n = 12$, 3.9%), and stroke mimics ($n = 220$, 68.1%). Combining blood-based biomarkers (glial fibrillary acidic protein and D-dimer) with the scale field assessment stroke triage for emergency destination yielded the best performance for LVO detection, with specificity of 94% and sensitivity of 71%. Performance was found to be higher in a subanalysis focusing on patients presenting <6 hours from symptom onset, with 93% specificity and 81% sensitivity. Critically, application of the biomarker and stroke scale algorithms ruled out all patients with hemorrhage.

CONCLUSION: The present work prospectively validated the potential utility of previously defined glial fibrillary acidic protein and D-dimer cutoff levels (ie, 213 pg/mL and 600 ng/mL, respectively), demonstrating their value for discrimination of LVO stroke from differential diagnoses during code stroke workups. (ClinicalTrials.gov number, NCT04292600.)

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Key Words: biomarkers ■ glial fibrillary acidic protein (GFAP) ■ intracerebral hemorrhage (ICH) ■ large-vessel occlusion (LVO) ■ stroke ■ thrombectomy (EVT) ■ triage

The global disease burden of stroke is vast and continues to be a leading cause of human suffering.^{1–3} A considerable proportion of this burden is driven by ischemic strokes caused by large-vessel occlusions (LVOs). In fact, LVO accounts for 62% of poststroke disabilities and 96% of poststroke death.⁴

Acute ischemic stroke with LVO is one of the most readily treatable stroke types, attributable to a recent paradigm shift toward endovascular thrombectomy (EVT).⁵ Thrombectomy is effective but requires specialized teams and equipment, limiting its availability to comprehensive stroke centers and other EVT-capable centers, which represent a small subset of accredited stroke centers and an even smaller subset of emergency medical facilities.⁶ Transfers result in time delays, which in turn are associated with poor outcomes and significant increases in morbidity/death.⁷ For every 15-minute reduction from stroke onset to EVT treatment, there is a 3.9% decrease in long-term disability; 2.5 times more patients also achieve functional independence (modified Rankin scale 0–2).⁸ Delays in treatment often arise from interhospital transfer (ie, from primary stroke centers to EVT-capable centers are estimated to delay treatment >2 hours)⁸ or in the identification of candidate patients undergoing EVT with LVO strokes.⁹ Therefore, early identification of patients with LVO in the prehospital setting (eg, within an ambulance) is critical to facilitating appropriate triage and routing patients to EVT-capable centers in a timely manner.¹⁰ Furthermore, early identification can expedite time to EVT and may facilitate direct transfer to the angiography suite.¹¹

A growing body of work has, therefore, evaluated prehospital stroke assessment scales in an effort to identify LVO strokes in the field. However, such severity scales lack the sensitivity and specificity required for triaging LVO patients with confidence, resulting in false negatives in patients with LVO as well as false positives in patients with stroke mimics or hemorrhagic stroke.^{12–14} More accurate diagnostic tests complementing such clinical assessment scores are desperately needed. We have previously described a retrospective observational cohort study, demonstrating that combination of D-dimer and glial fibrillary acidic protein (GFAP) with established stroke scales can accurately identify patients with LVO.¹⁵

Herein, we describe the TIME (Testing of Identification Markers for Stroke) trial, which aims to prospectively assess the specificity and sensitivity of our predetermined biomarker/scale cutoff values for the identification of LVO versus non-LVO stroke (ie, ischemic

non-LVO, hemorrhagic, stroke mimics, and transient ischemic attacks [TIAs]).

METHODS

Study Design and Sample Collection

Data are available on reasonable request. We conducted an observational prospective cohort clinical trial wherein consecutive patients admitted by ambulance or the emergency department at the Brandon Regional Hospital in Florida between May 2021 and August 2022 were included if (1) they were referred for a suspected stroke and (2) the time from symptom onset was known to be <18 hours. The decision to include patients within 0 to 18 hours of symptom onset was based on the premise that the ability to detect LVO should lead to an actionable intervention, that is, leaving a window of time for advanced imaging, triage, and treatment (0–24 hours). Patients were excluded if they received thrombolytic therapy before collection of blood, if there was an anticipated inability to provide a blood sample, if the time from stroke onset was >18 hours, or if they were participating in a clinical trial related to an investigational medicinal product at the time of consent.

Collected venous whole blood was centrifuged at 4°C for 15 minutes at 10 000g and the obtained plasma was then frozen at –80°C until analysis. Frozen samples were transferred to Pockit Diagnostics Ltd for the assaying of biomarkers. WCG Western Institutional Review Board reviewed and approved this study (Institutional Review Board tracking number: 20193270; study number: 1294597).

Clinical Data Collection

The following routine clinical data were collected for eligible patients: demographics (age, sex), clinical characteristics (blood pressure, pulse, atrial fibrillation, hypertension), clinical laboratory results (hematology, biochemistry, blood lipids), admission National Institutes of Health Stroke Scale (NIHSS) score by neurology-trained clinicians, time since last known well, imaging findings within 1 hour, and final clinical diagnosis.

Assigning a Diagnostic Category

The clinical data were used to assign patients to 1 of the following diagnostic categories: (1) LVO, (2) non-LVO

ischemic stroke, (3) hemorrhagic stroke, and (4) TIA/stroke mimic condition.

1. LVO required computed tomography or magnetic resonance (MR) angiography confirmation of filling defect (ie, via a neuroradiology report) of: cervical or intracranial internal carotid artery, bifurcation of the internal carotid artery, cervical or intracranial vertebral artery, basilar artery, or first segment of middle cerebral artery (M1) before the major bifurcation/trifurcation or the insular turn, whichever occurred later, or second middle cerebral carotid artery segment (before the lateral insular turn) (M2), or the first or second segments of the anterior cerebral artery or posterior cerebral artery.
2. Non-LVO ischemic stroke required computed tomography or MR angiography exclusion via a neuroradiology report; that is, confirming the absence of LVO. In addition, diffusion restriction MR imaging was used to confirm ischemic stroke.
3. Hemorrhagic stroke was assigned on the basis of the presence of hemorrhage on computed tomography (hyperdensity) or MR angiography (gradient echo or susceptibility weighted sequences or evident blood product on T1 or T2 sequences).
4. TIA and stroke mimic(s) were assigned on the basis of expert clinical opinions and lack of pertinent findings on imaging.

The non-LVO category used for determination of diagnostic performance was composed of TIAs, stroke mimics, hemorrhagic stroke, and non-LVO ischemic stroke. The nonhemorrhagic suspected stroke subgroup used to compare GFAP levels versus hemorrhagic patients was composed of TIAs, stroke mimics, LVO ischemic stroke, and non-LVO ischemic stroke.

Derivation of Stroke Scales Fatrrom NIHSS Score

The same stroke scales used in our retrospective study were selected for consistency.¹⁵ The face, arm, speech, time score was calculated by assigning 1 point for the presence of facial paresis (NIHSS item 4), 1 point for any arm weakness (NIHSS item 5a/b), and 1 point for any speech impairment (NIHSS item 9 or NIHSS item 10). Field assessment stroke triage for emergency destination (FAST-ED) was calculated as described by Lima et al,¹⁶ rapid arterial occlusion evaluation (RACE) score was calculated as described by Pérez de la Ossa,¹⁷ Cincinnati stroke triage assessment tool was calculated as described by Katz et al,¹⁸ and emergency medical stroke assessment was calculated as described by Groppen et al.¹⁹

Nonstandard Abbreviations and Acronyms

EVT	endovascular thrombectomy
FAST-ED	field assessment stroke triage for emergency destination
GFAP	glial fibrillary acidic protein
ICH	intracerebral hemorrhage
LVO	large-vessel occlusion
NIHSS	National Institutes of Health Stroke Scale
RACE	rapid arterial occlusion evaluation

CLINICAL PERSPECTIVE

- The present study validates the effectiveness of using plasma biomarkers (GFAP [glial fibrillary acidic protein] and D-dimer) in combination with stroke severity scales to detect large-vessel occlusion ischemic strokes. High specificity (93/94%) and sensitivity (81/71%) was demonstrated at <6 and 18 hours.
- Implementation of these biomarkers in the pre-hospital setting can aid in rapid and accurate triage of stroke patients.
- The biomarker algorithm effectively rules out hemorrhagic strokes, minimizing the risk of inappropriate treatment and facilitating targeted care.

Measurement of Blood Biomarkers

Plasma GFAP and D-dimer were measured using commercially available enzyme-linked immunosorbent assays following the manufacturer's instructions. D-dimer enzyme-linked immunosorbent assays kits (ab260076) and GFAP matched antibody pairs (ab222279) were purchased from Abcam (Cambridge, UK). All samples were analyzed in duplicate, and the mean value was used for quantification. For all patients, the coefficient of variation between replicates had to be <20%. All readings were performed with a CLARIO PLUS 430-2070 ACU and Clariostar Plus Reader Control and MARS Software version 3.42 R5 (BMG Labtech, Cary, NC). Biomarker quantification was achieved via regression analysis of an 8-point standard curve with known concentrations of analyte.

Statistical Analysis

The intended use of the blood biomarkers was to rule in patients with LVO allowing for redirection of patients to EVT-capable centers; accordingly, the study was powered for specificity. With a prevalence of 10% and a minimum specificity of 90%, a 2-tailed 5% type I error rate (α) and 90% power (β), a sample size of 292 suspected patients with 29 LVOs would yield a specificity of 95% (95% CI, 90%–100%) for LVO. Ultimately, the final sample size was increased by $\approx 30\%$ in an effort to account for possible data loss–related exclusion(s) criteria and the like.

To compare the levels of blood biomarkers and clinical variables in patients with and without LVO, the Wilcoxon–Mann–Whitney U test, median, and interquartile range (IQR) were used. Categorical variables were dichotomized and assessed via a Pearson's χ^2 test. Multiple hypothesis correction was performed using the Benjamini–Hochberg method.

Clinical decision rules combining previously identified cutoff values for D-dimer, GFAP, and clinical scales were used to assign a “test-positive” or “test-negative” value.^{15,20} In short, the cutoff used for plasma D-dimer and GFAP were 600 ng/mL and 213 pg/mL, respectively. The cutoffs used for each prehospital clinical scale were as follows: Cincinnati stroke triage assessment tool >1 , emergency medical stroke assessment >2 , face, arm, speech, time >1 , FAST-ED >2 , RACE >2 . A “test-positive” value was assigned on the basis of the following decision rule: clinical scale $>$ cutoff + D-dimer > 600 ng/mL + GFAP < 213 pg/mL.

To determine the diagnostic performance of each combination of biomarkers and clinical scale, confusion matrices were constructed using the LVO or non-LVO diagnosis (see Assigning a Diagnostic Category section) and the assigned test-positive or test-negative values.

A subanalysis was conducted using only patients who presented within the first 6 hours since the time of last known well.

All analyses were performed with RStudio version 2023.03.0+386 (RStudio, PBC, Boston, MA). The data collected during the study are available from the corresponding authors upon reasonable request.

RESULTS

Study Enrollment

Of 382 patients recruited, 59 patients were excluded from further analyses (as outlined in Figure 1). Reasons for exclusion were absence of computed tomography/MR angiography data ($n = 7$), no NIHSS score documented at admission ($n = 20$), unknown time since last known well ($n = 10$), or absence of a blood sam-

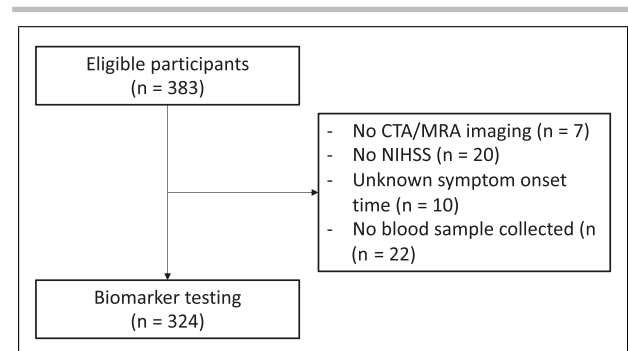


Figure 1. Standards for Reporting Diagnostic Accuracy study flow diagram. CTA indicates computed tomography angiogram; MRA indicates magnetic resonance angiogram; and NIHSS, National Institute of Health Stroke Scale.

ple ($n = 22$). The final cohort of 323 patients with suspected stroke was categorized as follows: LVO ischemic stroke ($n = 29$ [9%]), non-LVO ischemic stroke ($n = 48$ [15%]), hemorrhagic stroke ($n = 13$ [4%]), TIA ($n = 12$ [3.7%]), and stroke mimics ($n = 220$ [68.1%]). Among the stroke mimic population, the diagnoses were as follows: encephalopathy ($n = 26$ [11.8%]), hyperglycemia ($n = 2$ [0.9%]), hypertensive emergency ($n = 7$ [3.2%]), migraine ($n = 10$ [4.5%]), posterior reversible encephalopathy syndrome ($n = 1$ [0.5%]), seizure ($n = 17$ [7.7%]), and undetermined ($n = 157$ [71.4%]). Among patients with LVO, 19 (66%) received EVT treatment. The following occlusions were observed in patients with LVO: A2 ($n = 1$ [3.4%]), internal carotid artery ($n = 9$ [31%]), middle cerebral artery M1 ($n = 13$ [44.9%]), middle cerebral carotid artery M2 ($n = 5$ [17.3%]), and posterior cerebral artery ($n = 1$ [3.4%]).

Clinical Characteristics

Clinical characteristics for patients with LVO and all patients without LVO presenting within 18 hours are reported in Table 1; clinical characteristics of patients presenting within 6 hours are reported in Table S1. Compared with all other patients with suspected stroke, patients with LVO scored significantly higher on the admission NIHSS (LVO, 14 ± 11 versus non-LVO, 2 ± 5 ; $P < 0.001$) and were more likely to present with atrial fibrillation (LVO, 38% versus 1 non-LVO, 2%; $P < 0.05$). Of note, age, sex, and time from symptom onset were not significantly different between patients with LVO and patients without LVO. An overview of stroke subtypes is presented in Table S2.

Blood Biomarkers

The concentration of plasma D-dimer was significantly higher in patients with LVO as compared with patients with non-LVO suspected stroke (LVO suspected stroke, 1213 ng/mL [IQR, 733–1609] versus non-LVO

Table 1. Clinical Characteristics of Patients With LVO and Non-LVO Suspected Stroke at 18 Hours

Parameter	All	LVO	Non-LVO	P value
Age, y	65 (24.5)	73 (22)	65 (25)	>0.05
Sex, female, %	59.2	50.0	60.1	>0.5
BMI	28 (9.1)	26.7 (7.6)	28.3 (9.8)	>0.1
Systolic BP	154 (39)	163 (40)	154 (39)	>0.5
Diastolic BP	83 (20)	83 (9)	83 (21)	>0.5
Dyslipidemia, %	49.7	62.1	48.5	>0.5
History of diabetes, %	33.9	31	34.1	>0.5
Peripheral artery disease, %	0.9	3.4	0.7	>0.5
Hypertension, %	74.2	79.3	73.7	>0.5
Heart failure, %	7.1	10.3	6.8	>0.5
Myocardial infarction, %	4.3	3.4	6.7	>0.5
Coronary artery disease, %	20.2	37.9	17.4	>0.05
Atrial fibrillation, %	14.6	37.9	12.3	≤0.01*
Race or ethnicity				
Black, %	17.1	17.2	17.1	>0.5
Asian, %	1.6	3.4	1.4	>0.5
White, %	63	21.1	64.5	>0.1
Other, %	16.1	27.6	15	>0.1
NIHSS score	2 (6)	14 (11)	2 (5)	≤0.001***
Patient arrival, ambulance vs walk-in, %	68.4%	89.7%	66.2%	>0.05
Onset time, min	211 (451)	106 (475)	220 (436)	>0.05

Median (and interquartile range) are reported. BMI indicates body mass index; BP, blood pressure; LVO, large-vessel occlusion; and NIHSS, National Institutes of Health Stroke Scale.

* and *** indicate *P* values <0.05 and 0.001, respectively.

Table 2. Diagnostic Performance of D-Dimer and GFAP Combined With Different Clinical Scales

Scale+biomarkers	18 h		6 h	
	Sensitivity	Specificity	Sensitivity	Specificity
C-STAT+biomarkers	59	96	70	95
EMSA+biomarkers	72	91	80	91
FAST+biomarkers	70	92	80	92
FAST-ED+biomarkers	69	94	80	93
RACE+biomarkers	72	94	75	94

Performance in the time windows 0–18 and 0–6 h from symptom onset is shown. C-STAT indicates Cincinnati stroke triage assessment tool; EMSA, emergency medical stroke assessment; FAST, face, arm, speech, time; FAST-ED, field assessment stroke triage for emergency destination; GFAP, glial fibrillary acidic protein; and RACE, rapid arterial occlusion evaluation.

suspected stroke, 617 ng/mL [IQR, 377–1345]; $P<0.001$). GFAP was significantly increased in the plasma of patients with hemorrhagic stroke as compared with all other patients with suspected stroke (hemorrhagic stroke, 1464 pg/mL [IQR, 292–2580] versus nonhemorrhagic suspected stroke, 48 pg/mL [IQR, 12–98]; $P<0.005$).

Diagnostic Performance for LVO Detection

Table 2 shows the diagnostic performance of the combination of plasma D-dimer and GFAP with a number of different prehospital clinical symptom scales. Combinations of the blood biomarkers with the scales FAST-ED or RACE showed the best performance for LVO detection, with a specificity of 94% (for either scale combination) and a sensitivity of 71% for both scales.

A subanalysis considering only patients presenting within the first 6 hours from symptom onset showed improved sensitivity, with the combination of biomarkers and FAST-ED resulting in a specificity of 93% and sensitivity of 81% (Table 2). Figure 2 shows the proportion of stroke subtypes selected as test positive by the combination of D-dimer, GFAP, and FAST-ED. Importantly, we found that no patients with hemorrhagic stroke were selected as test positive. Biomarkers combined with stroke scales had a significantly higher ($P\leq 0.05$) positive predictive value than stroke scales alone with respect to all stroke scales evaluated, with the exception of RACE and emergency medical stroke assessment at <18 hours, which only approached significance ($P = 0.06$ and 0.10 , respectively) (Table S3).

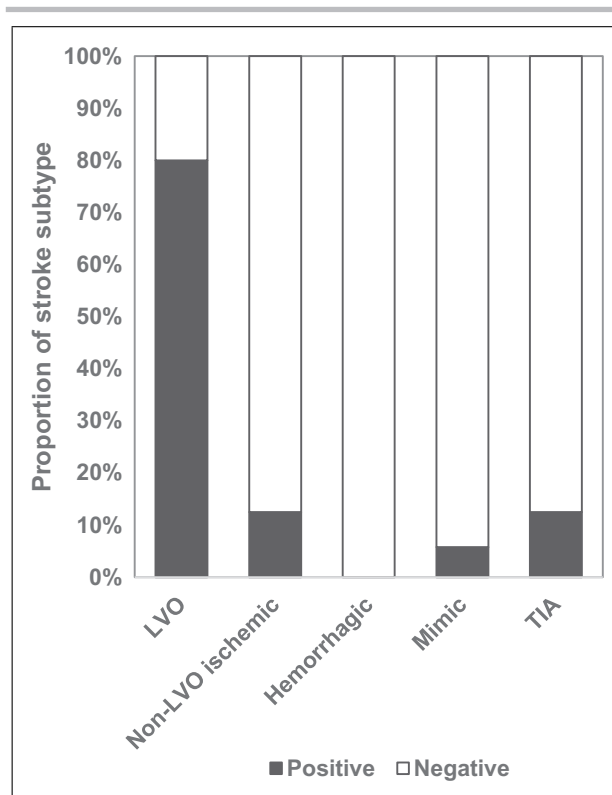


Figure 2. Test-positive patients as proportion of stroke subtypes. Bar graph showing the percentages of each stroke subtype resulting as “test positive” and “test negative” (see Methods). The combination of D-dimer, GFAP, and FAST-ED was used to determine “test-positive/negative”. FAST-ED indicates field assessment stroke triage for emergency destination; GFAP, glial fibrillary acidic protein; LVO, large-vessel occlusion; and TIA, transient ischemic attack.

Discussion

The TIME trial is the first study to validate blood biomarker cutoff values for LVO detection using a prospective cohort design. The TIME trial results demonstrate that a combination of D-dimer and GFAP in conjunction with established clinical stroke scales, including FAST-ED and RACE, is effective in the identification of patients with LVO stroke. Furthermore, these results validate our previously defined cutoff values for D-dimer and GFAP of 600 ng/mL and 213 pg/mL, respectively.¹⁵ These results may aid in the development of a point-of-care diagnostic test capable of guiding prehospital LVO stroke triage.

In a previous prospective observational study, Ramos-Pachón et al in 2021 reported that D-dimer was associated with LVO²¹; using a D-dimer cutoff point of 1664.15 ng/mL with an NIHSS score ≥ 10 , LVOs were detected with a specificity of 93% and a sensitivity of 35%.²¹ In our subsequent retrospective observational study, combining D-dimer/GFAP with FAST-ED resulted in a sensitivity of 82% and a specificity of 98%.¹⁵ The

results of the present study demonstrated a lower, albeit high, sensitivity (71%), and a high specificity (94%) for identification of LVO stroke using D-dimer/GFAP combined with FAST-ED. In addition to the use of different stroke severity scales, the improvement in sensitivity compared with the earlier work by Ramos-Pachón is likely due to the use of a lower D-dimer cutoff value (600 versus 1664 ng/mL). The addition of GFAP to D-dimer and clinical scales likely contributed to maintaining high levels of specificity, by excluding patients with hemorrhagic stroke. This is in line with prior work demonstrating that GFAP levels are elevated in patients with hemorrhagic stroke as compared with ischemic stroke, stroke mimics and TIAs.^{22,23} Therefore, the use of a lower D-dimer cutoff with the addition of GFAP may provide a significant improvement over previous studies as demonstrated here. Of note, diagnostic specificity was prioritized over sensitivity in our earlier retrospective work to minimize the selection of patients without LVO who are unlikely to benefit from admission to an EVT-capable hospital (ie, non-LVO ischemic and stroke mimic/TIAs), or patients who should be stabilized as soon as possible (ie, hemorrhagic stroke).

In both the present prospective work and our previous retrospective study, patients with hemorrhagic stroke had significantly elevated levels of GFAP.¹⁵ Given that clinical stroke scales in patients with hemorrhagic stroke frequently suggest LVO and that these patients are not candidates for EVT, a tool capable of ruling out hemorrhage and identifying only nonhemorrhagic ischemic LVO is essential. This dilemma was further highlighted in the RACECAT (Transfer to the Closest Local Stroke Center Versus Direct Transfer to Endovascular Stroke Center of Acute Stroke Patients With Suspected Large Vessel Occlusion in the Catalan Territory) trial,²⁴ and in a recent secondary analysis of its data (ie, the inappropriate triage of patients with intracerebral hemorrhage [ICH] led to worse outcomes).²⁵ Patients were randomized to either direct transport to an EVT or the closest local stroke center. No clinical benefit was demonstrated when using the RACE stroke scale to triage alone,²⁴ highlighting the need for effective blood biomarkers to identify nonhemorrhagic LVO stroke.²⁶ Accordingly, the RACECAT secondary analysis highlights the importance of accurately ruling out ICH from nonhemorrhagic LVO in the field.²⁵

Although not the focus of our present study, early identification of ICH may also be beneficial. The early positive results of the recent ENRICH (Early Minimally Invasive Removal of Intracerebral Hemorrhage) trial, emphasizes the importance of accurately identifying ICH.²⁷ Although prior randomized controlled trials have demonstrated limited clinical benefit of ICH clot evacuation,^{28,29} the early ENRICH results

suggest improved 180-day modified Rankin scale scores and death in patients undergoing minimally invasive transsulcal parafascicular surgical ICH clot evacuation.²⁷ In addition, a recent comprehensive retrospective analysis of 5 centers in Germany between 2008 and 2021 demonstrated the benefit of surgical decompression in patients with larger stroke volumes.³⁰ Such evidence highlights the potential of our biomarker profile to rule in ICH in the setting of low D-dimer/high GFAP, and prompt neurosurgical referral may also have therapeutic utility.

Compared with our previous study, a decreased sensitivity was observed.¹⁵ This could be explained, at least in part, by inclusion of patients up to 18 hours from symptom onset. In a subgroup analysis of the current study, including only patients presenting within the first 6 hours of symptom onset increased the sensitivity considerably (71% versus 81% for the FAST-ED combination), with no apparent change in specificity (94% versus 93%). This difference may also be due to blood biomarker kinetics or to resolving symptoms at later presentations. However, the performance observed in both time windows (0–18 and 0–6 hours) is well within the CIs defined in our previous study for both specificity (92%–100%) and sensitivity (57%–96%).¹⁵

The clinical scales FAST-ED and RACE have recently been prospectively validated.^{31,32} FAST-ED showed a specificity of 48% and sensitivity of 80%, while RACE demonstrated a specificity of 65% and a sensitivity of 71% for LVO. The present work definitively shows that a combination of GFAP and D-dimer with either FAST-ED or RACE yields improved performance compared with the clinical scales alone.

A high number (≈67%) of suspected strokes were categorized as stroke mimics, higher than the mimic rate reported in larger cohorts (≈30%). Merino et al³³ assessed predictors of acute stroke mimics in 8187 consecutive patients seen at the National Institutes of Health program over a 10-year period; of note, female sex and arrival in a personal vehicle were predictors of stroke mimics. In our cohort, the relatively high percentage of female patients (≈60%) and patients presenting by private vehicle (≈31%) may in part explain our high mimic rate.

Given the likely reasonable cost versus imaging of the combined GFAP/D-dimer biomarker profile, stroke triage systems could be rationalized in settings beyond high-income countries. This is of particular importance as the majority of the current and predicted stroke burden is within low- and middle-income countries.^{1,34} The importance of cost-effective stroke care resource allocation is, therefore, of considerable interest. Importantly, the recent 2023 Lancet Commission on “Pragmatic Solutions to Reduce the Global Burden of Stroke” specifically highlighted diagnostics/biomarkers

as an important component in combating global stroke disease burden.³⁴

Several limitations should be noted in the present work. First, stroke scores were derived from NIHSS scores performed by emergency department clinicians and not directly collected by emergency medical services staff. This may be important for FAST-ED and RACE, both known to be more complex compared with FAST or emergency medical stroke assessment. Additionally, NIHSS reporting bias may have arisen as clinicians conducting NIHSS scoring may have had access to additional data as compared with stroke scales that would be collected by emergency medical services staff. Second, we used frozen blood samples for biomarker measurements. Only sparse data are available on the stability of frozen D-dimer and GFAP^{35,36}; we cannot exclude the possibility that some of our negative results could be due to protein degradation. Third, diagnostic adjudication by an independent vascular neurologist was not performed in this study. Although official medical records were used, we cannot exclude that an independent adjudicator would have interpreted imaging and clinical data differently. Fourth, by choosing biomarker cutoffs designed to yield highly specific results, a certain number of LVOs are missed. However, as such patients default into “standard-of-care” triaging pathways, such a decision is unlikely to represent much clinical risk. Finally, biomarker measurements were performed with laboratory immunoassays; as such, prospective validation with point-of-care assays in pertinent clinical settings will ultimately be required.

In conclusion, the prospective evidence presented herein further validates that the D-dimer and GFAP cutoff levels of 600 ng/mL and 213 pg/mL, respectively, combined with stroke scales is capable of accurately identifying patients with nonhemorrhagic LVO stroke.

ARTICLE INFORMATION

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Supplemental Materials

Table S1-S3

Figure S1

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