

ORIGINAL RESEARCH

Detection of GFAP and D-Dimer in a Point-of-Care Test for Large Vessel Occlusion Ischemic Stroke

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BACKGROUND: The blood biomarkers GFAP (glial fibrillary acidic protein) and D-dimer have been shown to accurately diagnose large vessel occlusion ischemic stroke. The Upfront DX LVOne is a novel lateral flow-based point-of-care test used for the detection of GFAP and D-dimer. This study sought to (1) assess the performance of LVOne against commercially available point-of-care test platforms, which independently detect GFAP and D-dimer (Abbott iSTAT TBI Plasma and LumiraDx D-dimer), and to (2) assess the diagnostic performance of LVOne in large vessel occlusion detection.

METHODS: Manufacturer-reported detection thresholds of the LVOne test for GFAP and D-dimer (213 pg/mL and 600 ng/mL, respectively) were compared against commercial hospital-based point-of-care test platforms using 20 randomly selected plasma samples for each biomarker. D-dimer correlation between LVOne and LumiraDx was assessed using Spearman's Rank correlation coefficient; GFAP correlation between LVOne and the Abbott iSTAT TBI results were assessed using point biserial correlation. Diagnostic performance of the LVOne point-of-care test in conjunction with clinical assessment for detection of large vessel occlusion in plasma samples from 210 patients with suspected stroke was validated.

RESULTS: There was a strong positive correlation ($Rho = 0.86$, $P < 0.001$) between the qualitative scoring of the lateral flow test and serum GFAP concentrations. Similarly, there was a significant positive correlation between the D-dimer quantification and LVOne semiquantitative measurements ($Rho = 0.90$; $P < 0.0001$). In our cohort of 210 patients with suspected stroke, the LVOne test in conjunction with clinical assessment had a sensitivity of 75% (95% CI, 51%–91%), a specificity of 92% (95% CI, 87%–95%), a positive predictive value of 48% (95% CI, 30%–67%), and a negative predictive value of 97% (95% CI, 94%–99%) for detection of large vessel occlusion.

CONCLUSION: Our results validate detection thresholds for the LVOne test in a representative cohort of plasma samples of patients with suspected stroke. Further studies are required to demonstrate the efficacy, safety, and diagnostic performance of LVOne in capillary blood during emergency care/clinical triage.

Key Words: biomarkers ■ D-dimer ■ glial fibrillary acidic protein ■ large vessel occlusion ■ point of care diagnostics ■ prehospital triage ■ stroke ■ thrombectomy

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Supplementary Material for this article is available at <https://www.ahajournals.org/doi/suppl/10.1161/SVIN.124.001559>

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Ischemic stroke is the second leading cause of death and the third leading cause of disability worldwide,¹ with global economic consequences estimated at over \$960 billion USD in 2019.² Large vessel occlusion (LVO), which makes up approximately one third of all ischemic stroke, is estimated to be responsible for 96% of poststroke disability, and as such, contributes disproportionately to the disease burden associated with ischemic stroke.³

Over the past decade, LVO management has drastically improved following the advent of endovascular thrombectomy (EVT).^{4–6} Effective treatment of LVO with EVT hinges on well-equipped/staffed comprehensive stroke centers⁷ and timely arrival at these centers.^{4,8} Accordingly, the stroke system should enable routing of patients with suspected LVO by emergency medical system providers directly to EVT capable centers when possible, as well as prompt transfer of patients with LVO from non-EVT-capable hospitals to EVT-capable facilities.⁹ To help facilitate LVO triage by emergency medical system, a number of clinical scales have been proposed, including face, arm, speech, time; field assessment stroke triage for emergency destination (FAST-ED),¹⁰ rapid arterial occlusion evaluation,¹¹ and emergency medical stroke assessment.¹² These scales lack the sensitivity and specificity required for reliable triage of LVO and commonly result in both false negatives (in patients with LVO) and false positives in patients with stroke mimics and/or hemorrhagic stroke.^{13–15} For instance, the rapid arterial occlusion evaluation (RACE) scale, one of the best performing prehospital scales,¹⁶ confers a sensitivity of only ~60% and specificity of ~81%, when prospectively validated in the field.¹⁷ Moreover, although scales such as FAST-ED have demonstrated adequate interrater reliability when used by paramedics,¹⁸ human factors associated with clinical scales inevitably introduce a degree of subjectivity.¹⁹ More accurate and objective diagnostic tests are thus urgently needed for the effective triage of LVO in the EVT era.

An increasingly promising strategy for LVO identification is the combination of blood biomarkers with clinical scales^{20–22}; as evidenced in our recently published TIME (Testing for Identification Markers of Stroke) trial, clinical scales, combined with GFAP (glial fibrillary acidic protein) (cutoff = 213 pg/mL) and D-dimer (cutoff = 600 ng/mL), prospectively yielded a specificity for LVO of 93% and sensitivity of 80%.²¹ The TIME trial measured plasma D-dimer and GFAP using commercially available enzyme-linked immunosorbent assays that were read using microplate readers, allowing for exact quantification.²¹ To allow for portable, more readily available testing for LVO, the lateral flow test LVOne (Upfront Diagnostics Ltd) has been developed to be used with fingerprick capillary blood. LVOne,

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
POCT	point-of-care test
RACE	rapid arterial occlusion evaluation
TIME	Testing for Identification Markers of Stroke
RTW	return to work

CLINICAL PERSPECTIVE

- These results validate previously established glial fibrillary acidic protein and D-dimer thresholds for the detection of large vessel occlusion using the LVOne device in combination with stroke scales.
- Implementation of LVOne in the pre-hospital setting can aid in rapid and accurate triage of stroke patients.
- Further work is necessary to better delineate the diagnostic performance of LVOne in capillary blood in emergency and prehospital settings.

specifically designed for LVO detection, allows for rapid results in <15 minutes (average of ~10 min) of fingerprick via the semiquantification of D-dimer levels (using a scorecard of 1–10; ≥ 4 being elevated) and qualitative binary readout (positive/not positive) of GFAP levels. Although promising, the ability for LVOne to detect LVO has not yet been validated in a representative population of patients with suspected stroke. The consistency of LVOne with other point-of-care test (POCT) platforms for the detection of elevated GFAP/D-dimer (Abbott iSTAT TBI Plasma and LumiraDx D-dimer), which are already commercially available in certain hospitals, also remains to be determined.

Accordingly, the aim of the present study was to (1) assess the manufacturer-reported detection threshold(s) of the LVOne test for GFAP and D-dimer (213 pg/mL and 600 ng/mL, respectively) against commercial/hospital-based POCT platforms; and (2) validate the diagnostic performance of the LVOne POCT for detection of LVO in plasma from patients with suspected stroke.

METHODS

Data are available upon reasonable request via the corresponding authors. This study was conducted according to the Standards for Reporting Diagnostic Accuracy guidelines.²³

Patient Samples

In this study, we used venous plasma samples collected as part of the previously published TIME study. Briefly, stroke alert patients admitted to the emergency department at Brandon Regional Hospital (Florida) were prospectively recruited if the time from symptom onset was <18 hours before administration of thrombolytic therapy and/or any brain imaging. Collected venous whole blood was centrifuged at 4 °C for 15 minutes at 10 000 × *g* and the obtained plasma was then frozen at −80 °C until analysis.

To compare the LVOne test against commercially available platforms, 20 plasma samples, for each biomarker, were randomly selected from the TIME cohort by an operator blinded to patient diagnosis. Samples were selected spanning the analytical range for both biomarkers, yielding a total sample size of 40 in accordance with the Clinical Laboratory Standard Institute Guideline EP03, third edition, “Measurement Procedure Comparison and Bias Estimation Using Patient Samples.”^{21,24} For this reason, different patient samples were used for the D-dimer and GFAP test comparison.

To validate the diagnostic performance of the LVOne test for LVO, samples from TIME study patients presenting within 6 hours from stroke symptom onset were used. Only samples from patients with a recorded FAST-ED scale ≥2 were measured with the LVOne test. This was to simulate a real-world clinical application where the LVOne test is performed only on patients scoring ≥2 points on the FAST-ED scale, as previously described.²¹ The selected plasma samples were thawed for 30 minutes at room temperature and then analyzed on the commercial POCT platforms or the LVOne test.

Based on imaging, patients were assigned to 1 of 4 diagnostic categories: (1) LVO, (2) non-LVO ischemic stroke, (3) hemorrhagic stroke, and (4) transient ischemic attack/mimic condition (defined in Table S1).²¹ Ethics approval was obtained from WCG Western Institutional Review Board (Institutional Review Board tracking number: 20193270; study number: 1294597).

Biomarker Quantification With Commercial POCT Platforms

The concentration of GFAP in selected patient samples was analyzed using the Abbott iSTAT TBI Plasma

cartridge and the Abbott iSTAT Alinity instrument (04×6425), following the manufacturer's instructions. Briefly, 20 µL of venous plasma were added to the test cartridge and analyzed. The concentration of D-dimer was measured using the LumiraDx D-dimer test strips and the LumiraDx instrument (L005000101012), following the manufacturer's instructions. Briefly, the test strip was inserted in the instrument and once heated and prompted, 20 µL of venous plasma was added to the test strip and analyzed. GFAP and D-dimer quantifications appeared on the Abbott/LumiraDx instrument display screens and were manually transferred to a separate spreadsheet for subsequent analyses.

LVOne Test

LVOne test measurements of GFAP and D-dimer were derived following the manufacturer's instructions.²⁵ Briefly, 10 µL of plasma sample were added to the test's sample port, followed by addition of 100 µL chase buffer. Tests were incubated for 15 minutes before the LVOne test line intensities were visually inspected. For the LVOne GFAP assay, the presence/absence of a test line was recorded as a positive/negative LVOne GFAP result. For D-dimer, the test line color intensity was compared against a colorimetric reference scorecard by 2 blinded independent operators, and concordant measurements were recorded. Where there was discordance between the 2 operators, the test result was reexamined jointly, and a consensus reached on the value to be recorded. An LVOne D-dimer test was assigned as “positive” if a test line intensity ≥4 was present (test lines of 4–10); otherwise the LVOne D-dimer result was “negative” (test lines 0–3). An overall LVOne test result was assigned as positive in the presence of a negative GFAP assay result and a positive D-dimer result. All other combinations were assigned as a LVOne test negative result.

Statistical Analysis

The demographics and clinical findings were summarized using descriptive statistics with medians and interquartile range for continuous variables and count (%) for categorical variables. Differences between patients with LVO and without LVO were analyzed using the Wilcoxon Mann–Whitney *U* test for continuous variables and the Fisher's exact test for categorical variables; *P* values were adjusted using the Benjamini–Hochberg procedure, an α -level of 0.05 was selected. Sensitivity and specificity for the LVOne test were calculated based on contingency matrix built on the presence/absence of LVO (defined in Table S1) and the LVOne test results (positive/negative), 95% CIs of performance characteristics were obtained using Collett's exact method.²⁶

Table 1. Clinical Characteristics of Suspected Stroke Patients Used to Compare the LVOne Test Results Against Commercial Platforms

Parameter	N = 40
Age, y, median (IQR)	73 (20)
Sex, % female	67.5
Body mass index, kg/m ² , median (IQR)	25.4 (6.8)
Systolic BP, median (IQR)	149 (43)
Diastolic BP, median (IQR)	81 (15)
Dyslipidemia (%)	50
History of diabetes (%)	37.5
Peripheral arterial disease (%)	2.5
Hypertension (%)	72.5
Heart failure (%)	10
Myocardial infarction (%)	2.5
Coronary artery disease (%)	25
Atrial fibrillation (%)	15
National Institutes of Health Stroke Scale score, median (IQR)	5 (13)
Onset time (min), median (IQR)	260 (592)
Race	
Black (%)	15
White (%)	77.5
Other (%)	7.5
Final diagnosis	
Ischemic stroke LVO (%)	5
Ischemic stroke non-LVO (%)	10
Hemorrhagic stroke (%)	25
Transient ischemic attack/mimic condition (%)	60

Median (IQR) of continuous and number (%) of categorical variables.

BP blood pressure; IQR, interquartile range; and LVO, large vessel occlusion.

The median, interquartile range, and range of LVOne D-dimer quantitative score were calculated. Correlation between the quantitative LVOne D-dimer score and the LumiraDx D-dimer measurement was obtained using Spearman's Rank correlation coefficient. Correlation between the LVOne GFAP results and the Abbott iSTAT TBI results was obtained using Point Biserial correlation. Measurement reliability for LVOne D-dimer and GFAP was assessed by calculating the Cohen's Kappa coefficient and *P* value. All statistical analyses were conducted with R Software version 4.4.1.

RESULTS

To compare the LVOne test results with commercial platforms, we tested 40 plasma samples from patients with suspected stroke; 20 were tested with the LVOne GFAP assay and the Abbott iSTAT TBI Plasma and 20 were tested with the LVOne D-dimer assay and the LumiraDx D-dimer test. The demographics and clinical presentation of these patients are presented in Table 1.

Median age was 73 (20), 27 (67%) patients were female, and the median National Institutes of Health Stroke Scale score at admission was 5 (13). The final diagnoses were ischemic stroke due to LVO in 2 patients (5%), ischemic stroke not due to LVO in 4 patients (10%), hemorrhagic stroke in 10 patients (25%), and stroke mimic/transient ischemic attack in 24 patients (60%).

Plasma GFAP concentrations measured by the Abbott iSTAT TBI Plasma platform ranged from 30 to 10 000 pg/mL, with 5 samples below and 2 samples above the manufacturer's quantification limits (30–10 000 pg/mL). Of all samples, 12/20 had a positive LVOne GFAP result, with 1 false positive (Figure). The sample resulting in false positive had a GFAP concentration of 194 pg/mL, as measured by the Abbott platform. Although this concentration level is below the declared cutoff level of 213 pg/mL for the LVOne GFAP assay, it is within the error range reported by Abbott for the iSTAT TBI measurement precision ($194 \text{ pg/mL} \pm 10\% = 173.6\text{--}213.4 \text{ pg/mL}$). There was a significant positive correlation between the Abbott iSTAT TBI measurements and the LVOne GFAP results ($r = 0.86$; $P < 0.00001$).

Plasma D-dimer concentrations measured by the LumiraDx D-dimer test ranged from 200 to 4000 ng/mL, with 1 sample below, and 1 sample above, the manufacturer's quantification limits (200–4000 ng/mL). Of all samples, 13/20 had a positive LVOne D-dimer result (Figure). All samples quantified as having D-dimer concentrations $>600 \text{ ng/mL}$ resulted in positive LVOne D-dimer results and vice versa. No false positives or negatives were observed. There was a significant positive correlation between the LumiraDx quantification and LVOne semiquantitative measurements (spearman $\rho = 0.90$; $P < 0.0001$). The median D-dimer and range for each LVOne D-dimer semiquantitative score is provided in Table 2. Samples resulting in LVOne scores of 5 or 6 had overlapping D-dimer concentration ranges. All LVOne tests ran correctly with no void results. Six of the 210 tests (3%) were interpreted differently by the 2 independent operators and had to be reexamined. However, the measurement reliability for both LVOne D-dimer or GFAP was highly significant (Cohen's Kappa for LVOne D-dimer or GFAP = 0.643 or 0.696, respectively; $P < 0.001$). None of the 6 differently interpreted results would have led to a different test interpretation.

The clinical characteristics of the cohort used in the diagnostic performance cohort are presented in Table S2. When combined with the FAST-ED scale, the LVOne test had an overall specificity of 92% (95% CI, 87%–95%), a sensitivity of 75% (95% CI, 51%–91%), a positive predictive value of 48% (95% CI, 30%–67%), and a negative predictive value of 97% (95%

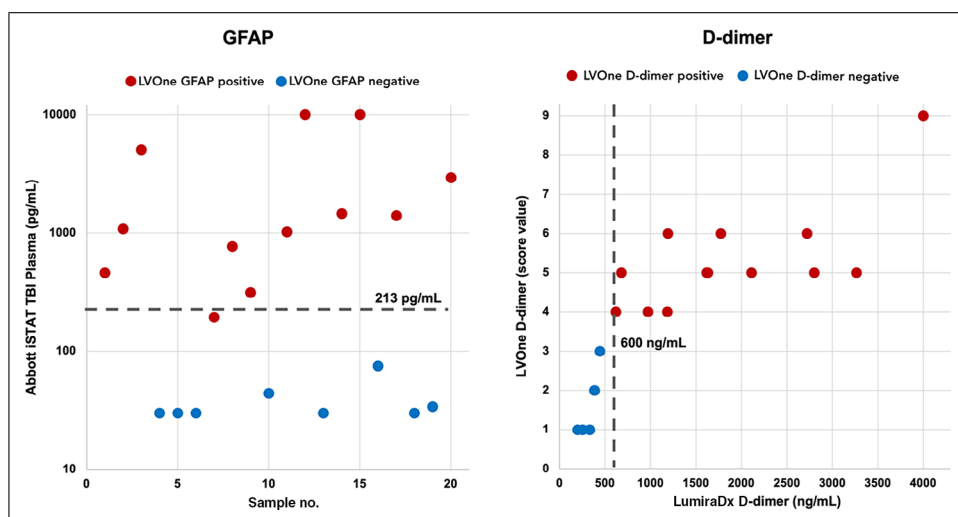


Figure. Comparison of GFAP and D-dimer levels between Upfront DX LVOne and commercial POCT platforms in samples taken from patients with suspected stroke. Left: Abbott iSTAT TBI Plasma results of combined with results from the LVOne GFAP assay. The values obtained with the Abbott platform are shown in logarithmic scale. Right: LumiraDx D-dimer results combined with LVOne D-dimer assay. Red/blue dots represent positive/negative LVOne GFAP or D-dimer results. The grey dashed lines indicate the nominal cutoff values for the LVOne GFAP or D-dimer assay (213 pg/mL or 600 ng/mL, respectively). GFAP indicates glial fibrillary acidic protein; and POCT, point-of-care test.

Table 2. Median D-Dimer Per Semiquantitative LVOne D-Dimer Score

LVOne score	Number of samples	LumiraDx D-dimer range (ng/mL)	LumiraDx D-dimer (ng/mL) (median [(interquartile range)])
1	4	200–332	253 [239–274]
2	2	384–385	385 [384–385]
3	1	N/A	445 [N/A]
4	3	621–1187	973 [797–1080]
5	6	680–3266	1871 [1622–2630]
6	3	1193–2722	1773 [1483–2248]
7	0	N/A	N/A
8	0	N/A	N/A
9	1	N/A	4000 [N/A]

Table 3. 2×2 Table of LVOne Results

	LVO present	LVO absent	Total
LVOne + (%)	15 (48.4)	16 (51.6)	31 (100)
LVOne – (%)	5 (2.8)	174 (97.2)	179 (100)
Total	20 (9.5)	190 (90.5)	210 (100)

LVO indicates large vessel occlusion.

CI, 94%–99%) for the detection of LVO (Table 3). The pretest probability of LVO in our study cohort was 9.5%. Among patients with a positive LVOne score, 15 (48%) had LVO, 9 (29%) had stroke mimicking conditions (4 encephalopathy, 4 unknown, 1 seizure), 6 (20%) had non-LVO ischemic stroke, and 1 (3%) had a transient ischemic attack (Table 4). LVOne was able to rule out all patients with hemorrhagic stroke. Of note, varying the LVOne D-dimer cut-off (1–9) affected the diagnostic performance for LVO prediction (Table S3).

DISCUSSION

This study explores the use of a POCT for the detection of patients with LVO ischemic stroke from a representative population of suspected strokes. In patient samples, the POCT discriminated clinically relevant GFAP (213 pg/mL) and D-dimer (600 ng/mL) levels with performance matching that of commercially available platforms. We further demonstrated significant positive correlation between LumiraDx quantified D-dimer concentrations and the semiquantitative score provided by the LVOne D-dimer assay, as well as between Abbott iSTAT TBI GFAP quantifications and the LVOne GFAP qualitative results. In our cohort, the LVOne test demonstrated a specificity of 92%, with an excellent negative predictive value of 97% and a moderate positive predictive value of 48% in detecting LVO stroke.

Table 4. LVOne Positive and Negative Results Distributed Among Subtypes of Suspected Stroke

	LVO ischemic	Non-LVO ischemic	Mimic	TIA	ICH	Total
LVOne + (%)	15 (48.4)	6 (19.4)	9 (29.0)	1 (3.2)	0 (0.0)	31 (100)
LVOne – (%)	5 (2.8)	26 (14.5)	131 (73.2)	7 (3.9)	10 (5.6)	179 (100)

ICH indicates intracerebral hemorrhage; LVO, large vessel occlusion.

The utility of using GFAP and D-dimer in the diagnosis of LVO has become increasingly evident over the last few years.^{20,21,27} Ramos-Pachón et al. in 2021 established the benefit of using D-dimer (cutoff = 1664.15 ng/mL) combined with National Institutes of Health Stroke Scale (≥ 10) for LVO diagnosis, yielding high specificity (93%) albeit with low sensitivity (35%).²⁷ By decreasing the D-dimer cutoff from 1664 to 600 ng/mL, a higher sensitivity could be achieved in our retrospective (82%)²⁰ and prospective (80%)²¹ studies when used in combination with the FAST-ED scale. Moreover, the high specificity as demonstrated by Ramos-Pachón et al.²⁷ could be maintained in both the retrospective (96%)²⁰ and prospective (93%) studies,²¹ via the addition of GFAP to D-dimer and clinical scales, which excludes patients with hemorrhage; GFAP levels are known to be elevated in hemorrhagic stroke compared with ischemic stroke, stroke mimics, and/or transient ischemic attacks.^{28,29} This is believed to relate to the immediate necrosis of astrocytes in intracerebral hemorrhage (ICH), leading to the immediate release of GFAP into the bloodstream, compared with slower release of GFAP following ischemic stroke.³⁰ Of note, specificity was intentionally optimized to limit the selection of patients without LVO who are unlikely to benefit from admission to an EVT-capable hospital. Crucially, in a 2023 reanalysis of 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, it was demonstrated that patients with ICH who bypassed the closest stroke center for an EVT capable center had worse functional outcomes at 90 days, highlighting the importance of ruling out ICH in the prehospital setting using a platform such as LVOne.³¹ Importantly, ICH was correctly ruled out in all instances in this work, highlighting the safety of triaging patients with positive tests to EVT capable centers.

LVOne, being a lateral flow based POCT, allows for the rapid diagnosis of LVO using a simple portable assay, making it suitable for triage in ambulances/emergency departments. The test takes approximately 1 minute to perform, and results are delivered in ~10 minutes. This allows for other emergency assessments and procedures to take place while test results are pending. Given that scene times are on average 15 minutes in the United States,³² per-

forming LVOne is unlikely to meaningfully alter emergency triage times. Additionally, an ambulance feasibility study is currently evaluating this, and other aspects of using the LVOne in the prehospital environment (ISRCTN12414986). Moreover, the portable nature of the test clearly allows for triaging decisions to be made *en route* to a stroke center. Further work is underway assessing how LVOne can be used to rationalize clinical workflows to allow LVO patients to be treated more rapidly, for instance via using a positive test to triage patients immediately to EVT, bypassing computed tomography and/or magnetic resonance angiography. The direct-to-angiography suite approach has been demonstrated to improve long-term functional outcomes in a recent randomized controlled trial.³³

Although not optimized for detection of ICH, low D-dimer/high GFAP may also have potential for ruling in ICH; it is for instance noteworthy that there were no examples of ICH being categorized as LVO in the TIME trial.²¹ The importance of accurate and rapid diagnosis of ICH was highlighted in the recent ENRICH (Early Minimally Invasive Removal of Intracerebral Hemorrhage) trial wherein patients receiving early minimally invasive hematoma evacuation had superior functional outcomes at 180 days.³⁴ Moreover, in a recent multicenter trial from Germany, surgical evacuation was shown to benefit patients with large cerebellar ICH.³⁵ In line with these results, further work is underway assessing the extent to which the clinical decision rule offered by LVOne could be adapted to rule in patients with ICH for early therapeutic intervention.

There are several limitations to the present work. First, the D-dimer scorecard was subjectively assessed, in contrast to the direct quantification provided by commercially available POCT platforms. However, only ~3% of tests were interpreted differently by the raters and required a consensus ruling. Second, stroke scores in the TIME trial were calculated from National Institutes of Health Stroke Scale scores, which were calculated by emergency department clinicians rather than directly by emergency medical system staff. This may introduce bias as emergency department clinicians may have had access to additional data and the FAST-ED and rapid arterial occlusion evaluation scales are known to be more complex to conduct than face, arm, speech, time/emergency medical stroke assessment. Third, although the high negative predictive value con-

firms that a negative result can effectively rule out LVO, the positive predictive value of 48% indicates that further testing may be necessary once triaged to the EVT capable center. Importantly, however, ICH was correctly ruled out in all instances of a positive LVOne test, highlighting the relative safety of foregoing the closest stroke center in favor of an EVT capable center. However, the extent to which bypassing the local hospital could affect the patient's eligibility for thrombolytic therapy should be carefully considered, as has been highlighted by American Heart Association guidelines.³⁶ Finally, plasma from whole venous blood could give different results compared with capillary blood from a fingerprick, for which further validation is underway.

CONCLUSIONS

In conclusion, these results further validate our previously established thresholds for detection of LVO using the blood biomarkers GFAP and D-dimer in combination with stroke scales. Further work is necessary to better delineate the diagnostic performance of LVOne in capillary blood in the emergency and prehospital settings.

ARTICLE INFORMATION

Received August 4, 2024; Accepted November 26, 2024

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Acknowledgments

The authors acknowledge the radiographers, paramedics, and nurses, without whom this work would be impossible.

Sources of Funding

This study was supported by Innovate UK grant 104640, SBRI Healthcare grant SBRIH18P2003, and by private funding.

Disclosures

Dr Gaude declares grant funding from the UK Research and Innovation small business research initiative. Dr Gaude is the author of patent number WO2021209732A1. Dr Gaude and Dr Bernstock have positions and equity in Pockit Diagnostics Ltd. Dr Bernstock also has an equity position in Treovir Inc.

and is on the boards of Centile Bio and NeuroX1. All other authors have no pertinent disclosures to make.

Supplemental Materials

Supplemental Table 1. Assignment of diagnostic category.

Supplemental Table 2. Clinical characteristics of LVO and non-LVO suspected stroke patients presenting within 6h.

Supplemental Table 3. Diagnostic performance at varying LVOne D-dimer cut-off levels.

Supplementary Figure 1. Receiver operating characteristic (ROC) curve of using the FAST-ED scale alone or in combination with the LVOne results for LVO detection.

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