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ORIGINAL ARTICLE

Coagulation markers and functional outcome in acute ischemic stroke: Impact of intensive versus standard hyperglycemia control

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Abstract

Objective: Alterations in coagulation could mediate functional outcome in patients with hyperglycemia after acute ischemic stroke (AIS). We prospectively studied the effects of intensive versus standard glucose control on coagulation markers and their relationships to functional outcomes in patients with AIS.

Approach: The Insights on Selected Procoagulation Markers and Outcomes in Stroke Trial measured the coagulation biomarkers whole blood tissue factor procoagulant activity (TFPCA); plasma factors VII (FVII), VIIa (FVIIa), and VIII (FVIII); thrombin-antithrombin (TAT) complex; D-dimer; tissue factor pathway inhibitor, and plasminogen activator inhibitor-1 (PAI-1) antigen in patients enrolled in the Stroke Hyperglycemia Insulin Network Effort trial of intensive versus standard glucose control on functional outcome at 3 months after AIS. Changes in biomarkers over time (from baseline ≈12 hours after stroke onset) to 48 hours, and changes in biomarkers between treatment groups, functional outcomes, and their interaction were analyzed by two-way analysis of variance.

Results: A total of 125 patients were included (57 in the intensive treatment group and 68 in the standard treatment group). The overall mean age was 66 years; 42% were women. Changes from baseline to 48 hours in coagulation markers were significantly different between treatment groups for TFPCA (P = 0.02) and PAI-1 (P = .04) and FVIIa (P = .04). Increases in FVIIa and decreases in FVIII were associated with favorable functional outcomes (P = .04 and .04, respectively). In the intensive treatment group, reductions in TFPCA and FVIII and increases in FVIIa were greater in patients with favorable than unfavorable outcomes (P = .02, 0.002, 0.03, respectively). In the

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standard treatment group, changes in FVII were different by functional outcome (P = .006).

Conclusions: Intensive glucose control induced greater alterations in coagulation biomarkers than standard treatment, and these were associated with a favorable functional outcome at 3 months after AIS.

KEYWORDS
acute ischemic stroke, coagulation markers, diabetes mellitus, functional outcome, hyperglycemia control, tissue factor

Essentials

- Hyperglycemia stimulates blood coagulation after acute ischemic stroke (AIS).
- Alterations in coagulation with glucose control may mediate functional outcome in patients with AIS.
- We compared coagulation markers between intensive and standard insulin treatment groups.
- Changes in coagulation markers with hyperglycemia control are linked to improved outcome in AIS.

1 | INTRODUCTION

Hyperglycemia is a prothrombotic[1-3] and proinflammatory state[2,4] and is associated with worse functional outcomes after acute ischemic stroke (AIS).[5,6] These deleterious effects may be mediated by the activation of the tissue factor pathway of blood coagulation.[3,7,8] Tissue factor (TF) is a membrane-bound protein and a cofactor in the proteolytic conversion of coagulation factor VII to its activated form (FVIIa) to initiate blood coagulation. The resulting TF-FVIIa complex activates factors IX and X leading to the conversion of prothrombin to thrombin.[9] TF is highly expressed in atherosclerotic plaques and initiates thrombus formation when the vessel wall is injured or plaques are fissured.[10] There is also a pool of circulating TF in blood associated with monocytes and other cells and microparticles; elevated levels of circulating TF are associated with a prothrombotic state.[10,11]

Patients with hyperglycemia and type 2 diabetes mellitus (T2DM) have marked alterations in blood coagulation[7] and fibrinolytic mechanisms.[12] We and others have shown that patients with hyperglycemia during AIS have marked increases in circulating TF procoagulant activity (TFPCA) and plasma coagulation factors VII (FVII), VIIa (FVIIa), and VIII (FVIII)[8,13]; markers of thrombin generation (prothrombin fragment factor 1.2, and thrombin-antithrombin [TAT] complexes)[8,13]; and markers of fibrinolysis (D-dimer and plasminogen activator inhibitor [PAI-1]).[12,14] After AIS, elevated TF may contribute to enhanced coagulation by promoting thrombin generation, fibrin deposition, and thrombus formation.[15] In addition, FVIIa and TAT levels are related to stroke severity,[16,17] and high FVIII and F1.2 levels are associated with recurrent stroke following transient cerebral ischemia.[13,18] While hyperglycemia is known to have prothrombotic effects, the effects of hyperglycemia control on blood coagulation or fibrinolysis in patients with AIS are unknown. The Insights on Selected Procoagulation Markers and Outcomes in Stroke Trial (iSPOT) (ClinTrials.gov NCT01811550) was designed to compare the effects of intensive versus standard treatment of hyperglycemia on selected markers of blood coagulation and fibrinolysis and their relationship to functional outcomes in patients with AIS enrolled in the Stroke Hyperglycemia Insulin Network Effort (SHINE)[19] trial (ClinTrials.gov NCT01369069).

2 | METHODS

2.1 | Patient population

iSPOT was prospectively designed as an ancillary study to the SHINE clinical trial. A full description of the SHINE trial methodology and rationale has been reported.[19,20] The key SHINE inclusion criteria were ischemic stroke onset within 12 hours, baseline blood glucose >110 mg/dL with history of T2DM or baseline blood glucose ≥150 mg/dL without history of T2DM, and baseline National Institute of Health Stroke Score (NIHSS) of 3 to 22. Patients in the intensive treatment group received continuous intravenous insulin infusion guided by an electronic decision support tool (target blood glucose 80-130 mg/dL).

Patients in the standard treatment group received only subcutaneous insulin (target blood glucose 80-179 mg/dL). The primary outcome in the SHINE trial was the modified Rankin Scale (mRS) at 90 days adjusted to baseline stroke severity. Favorable clinical outcome was defined as mRS of 0 for patients with baseline NIHSS 3 to 7 (mild stroke severity), mRS 0 to 1 for patients with baseline NIHSS 8 to 14 (moderate stroke severity), or mRS 0 to 2 for patients with baseline NIHSS 15 to 22 (severe stroke). Of 63 enrolling SHINE sites, 58 also participated in iSPOT, and 34 of these sites enrolled at least one patient in the study. In addition to the SHINE exclusions, the iSPOT exclusion criteria included treatment with recombinant tissue-type plasminogen activator (rt-PA), anticoagulation therapy (other than standard deep vein thrombosis prophylaxis) or endovascular thrombectomy, known coagulopathy or hypercoagulable

disorder, or significant liver impairment assessed by evidence of jaundice or hepatic encephalopathy.

Enrollment in iSPOT began in October 2012. Of 63 enrolling SHINE sites, 58 also participated in iSPOT, and 34 of these sites enrolled at least one patient in the study.

2.2 | Sample size calculations

The study was designed to test the effect of treatment on the change from baseline to 48-hour biomarker levels. Assuming the magnitude of a clinically relevant difference in FVIIa between treatment and control to be 25%,[3,8] at an overall significance level of 5% (with a Bonferroni adjustment for 5 biomarkers leading to 1% for each biomarker), 80% power, and a standard deviation of 25 units, a total of 315 patients (148 in each treatment group plus 6% additional subjects for attrition) would be needed.

There was a lower-than-expected accrual of patients in the iSPOT study. Accrual in iSPOT was contingent on identifying eligible patients among those enrolled in the SHINE trial. Over the course of iSPOT enrollment, many SHINE-enrolled patients were ineligible for iSPOT primarily because they were treated with intravenous rt-PA (63% of SHINE-enrolled patients) with or without endovascular thrombectomy (13%).[19] However, the primary analysis of iSPOT data was not compromised with the lower sample size. Simulations were used to estimate the sample size required, under appropriate assumptions of uncertainty, to detect both interaction and main effects. Sample size calculations were repeated based on unblinded interim information when protocol modifications with respect to inclusion of tissue-type plasminogen activator were considered. The variability observed in biomarkers was less than estimated initially from prior studies; we estimated there was sufficient power to detect even a fairly conservative effect size with 96 subjects per SHINE treatment group for a total of 192 subjects. All post hoc comparisons from the General Linear Models approach used to analyze the data were adjusted using Tukey. Sequential recruitment to iSPOT continued until the completion of SHINE enrollment. At the end of the study, we accrued a total of 125 patients for the per-protocol analysis, which is smaller than the 192 expected. However, the effect sizes for several biomarkers (see Results section) were adequate enough to reach statistical significance.

2.3 | Measurement of biomarkers

Venous blood samples were collected into one-tenth volume of 3.2% sodium citrate at the time of SHINE randomization (baseline) and 46 to 54 hours later (48-hour sample). Whole blood and plasma aliquots were stored at −70 to −80°C. All assays were performed in a blinded manner with respect to treatment group assignment and to functional outcome. TFPCA was measured in whole blood cell lysates as previously described[3,11] with a two-stage clotting assay using recombinant human FVIIa (Sekisui Diagnostics LLC, Framingham, MA, USA), human factor X (Haematologic Technologies Inc, Essex Junction, VT, USA), and pooled normal human plasma (George King Bio-Medical, Inc, Overland Park, KS, USA) containing phospholipid vesicles. This assay measures cell membrane–bound and microparticle-associated TF activity in lysed whole blood. HemosIL RecombiPlastin 2G (Instrumentation Laboratory, Bedford, MA, USA) was used as a standard. FVII and FVIII activities were measured by standard clotting assays. FVIIa activity was measured by a commercially available assay (STACLOT VIIa-rTF; Diagnostica Stago Inc, Parsippany, NJ, USA). Plasma thrombin-antithrombin complexes (Enzygnost TAT micro, Siemens Healthcare Diagnostics, Malvern, PA, USA), D-dimer (IMUCLONE D-Dimer, Biomedica Diagnostics, Windsor, NS, Canada), TFPI (IMUBIND total TFPI, Louisville APL Diagnostics, Inc, Atlanta, GA, USA) and PAI-1 antigen (Biomedica Diagnostics) were measured using ELISAs. Reference ranges for the biomarkers are provided in the Supporting Information.

2.4 | Statistical analysis

The main dependent variable for analysis was the change in the biomarker levels from baseline to 48 hours when both time points were available. Data were analyzed using a single two-way analysis of variance with the independent factors being (1) treatment group (intensive and standard), (2) functional outcome at 90 days (favorable and unfavorable), and (3) the interaction between treatment group and functional outcome. For each marker, interaction between treatment group and functional outcome was tested, and linear contrasts were constructed to test the difference between functional outcomes for each treatment group. The assumptions were tested for the residuals and found adequate. Variables that were significantly different between treatment groups (see Table 1) were, along with other covariates that were considered clinically relevant, included in the model, and the analyses described above were repeated. Covariates include demographic variables such as age, race, and gender; medical history variables including hypertension, prior stroke, congestive heart failure, coronary artery disease, and hyperlipidemia; baseline measurements such as hemoglobin A_{1c}, NIHSS at randomization, and blood glucose level; and other potential factors such as total insulin dose given before a 48-hour blood draw, use of VTE prophylaxis during 48 hours, and time of stroke onset to baseline blood draw. A backward selection approach was employed to identify useful covariates. Conclusions regarding group differences are based on an overall significance level of .05. Results of both unadjusted and adjusted analyses are provided.

3 | RESULTS

Between October 2012 and August 2018, 149 patients were enrolled (Figure 1); 24 patients were excluded from the analysis who received prohibited medications including systemic thrombolytic

TABLE 1 Characteristics of the patients

Characteristic	Intensive treatment (N = 57)	Standard treatment (N = 68)
Age, y, median (interquartile range)	69 (55-75)	67 (59.5-73)
Female sex,n (%)[a]	18 (31.6)	34 (50)
Race, n (%)		
Black	19 (33.3)	22 (32.4)
White	37 (64.9)	37 (54.4)
Other	1 (1.8)	9 (13.2)
Ethnicity, n (%)		
Hispanic	8 (14)	10 (14.7)
Not Hispanic or Unknown	49 (86.0)	58 (85.3)
Baseline BG level, (mg/dL), median (interquartile range)	160 (115-246)	199 (155.5-237.5)
48-h BG level, mg/dL, median (interquartile range)[a]	107 (96-134)	190.5 (152.5-232)
Medical history, n (%)		
Previous ischemic stroke	11 (19.3)	11 (16.2)
Diabetes mellitus	49 (86.0)	55 (80.9)
Hyperlipidemia[a]	35 (61.4)	29 (42.6)
Hypertension	52 (91.2)	58 (85.3)
Eligibility POC blood glucose, mg/dL, median (interquartile range)	201 (155-267.5)	220 (178.5-265.5)
NIHSS at randomization, median (interquartile range)	7 (5-12)	7 (4.5-11)
Stroke onset to BL blood draw, h, median (interquartile range)[a]	9.6 (7.7-12.3)	10.6 (8.8-12.3)

Abbreviations: BG, blood glucose; BL, baseline; NIHSS, National Institute of Health Stroke Score; POC, point of care.

[a]Denotes difference between treatment groups at .05 significance level.

and anticoagulant therapy, were treated with mechanical thrombectomy, had a history of hypercoagulable condition, or were ultimately determined to have had a stroke mimic. Thus, 125 patients (57 in the intensive treatment group and 68 in the standard treatment group) were included in the analyses. The key baseline characteristics were compared between the intensive and standard treatment groups (Table 1). By design, the 48-hour blood glucose was significantly lower in the intensive as compared to the standard treatment group. There were more women in the standard as compared to the intensive treatment group (Table 1). There were more patients with a history of hyperlipidemia in the intensive than in the standard treatment group.

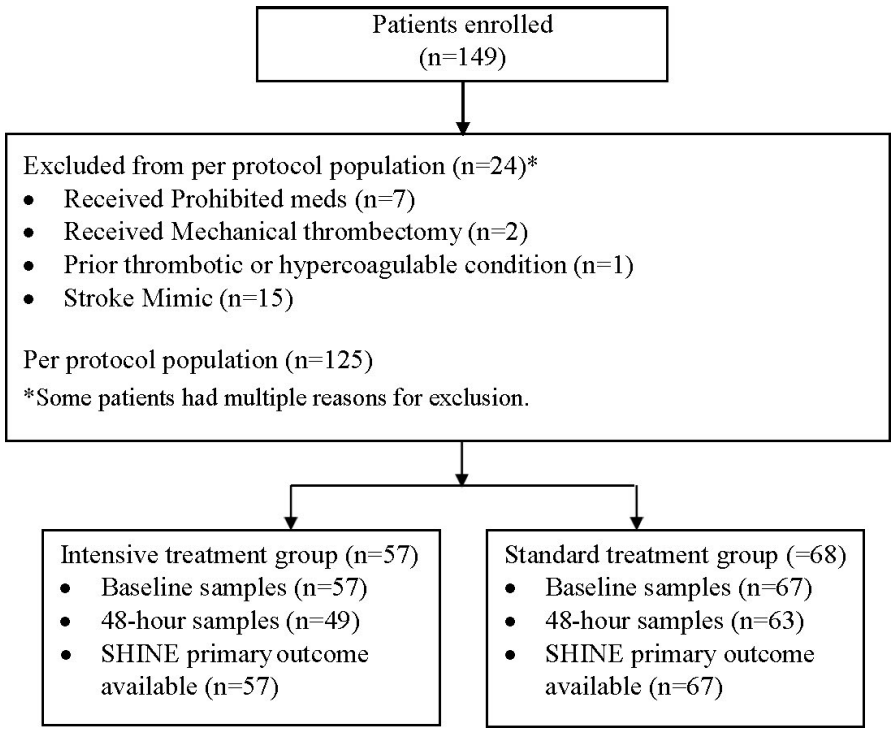
3.1 | Changes in biomarkers from baseline to 48 hours

Figure 2 shows biomarker levels at baseline and at ≈48 hours separated by insulin treatment groups. In the standard treatment group, levels of FVII and FVIIa were lower at 48 hours compared to baseline. In the intensive treatment group, 48-hour TFPCA, FVII, and PAI-1 levels were lower than at baseline. The 48-hour levels of other biomarkers were not different from baseline in the two treatment groups.

3.2 | Changes in biomarker levels (TFPCA, FVIIa, and PAI-1) by insulin treatment group

The main dependent variable for analysis for this study was the change in the biomarker levels from baseline to 48 hours. Changes from baseline to 48 hours in TFPCA, FVIIa, and PAI-1 were significantly different between intensive and standard treatment groups (Figure 3). TFPCA fell by −32.10 ± 10.04 U/mL (mean ±SEM) from 102.96 ± 13.05 U/mL at baseline (BL) to 70.86 ± 13.05 U/mL at 48 hours in the intensive treatment group compared with a small increase of 2.62 ± 11.21 U/mL from 68.49 U/mL ± 14.56 U/mL to 71.11 ± 14.56 U/mL in the standard treatment group (difference, −34.72 U/mL; 95% confidence interval [CI], −64.58 to −4.87; P = .02). FVIIa increased by 7.30 ± 6.42 mU/mL from 55.29 ± 7.54 mU/mL to 62.59 ± 7.54 mU/mL in the intensive treatment and fell −12.06 ± 6.66 mU/mL from 63.28 ± 7.83 to 51.23 ± 7.83 mU/mL with standard treatment (difference, 19.36 mU/mL; 95% CI, 1.01-37.70; P = 0.04). PAI-1 levels decreased by −6.31 ± 3.39 ng/mL from 35.74 ± 5.15 ng/mL to 29.43 ± 5.15 ng/mL from BL to 48 hours with intensive treatment compared to a rise of 3.74 ± 3.51 ng/mL from 42.43 ± 5.34 ng/mL to 46.17 ng/mL ± 5.34 ng/mL with standard treatment (difference, −10.06 ng/mL; 95% CI, −19.73 to −0.39;P = .04). Changes from baseline to 48 hours in the other biomarkers were not different between the standard and

FIGURE 1 Enrollment diagram



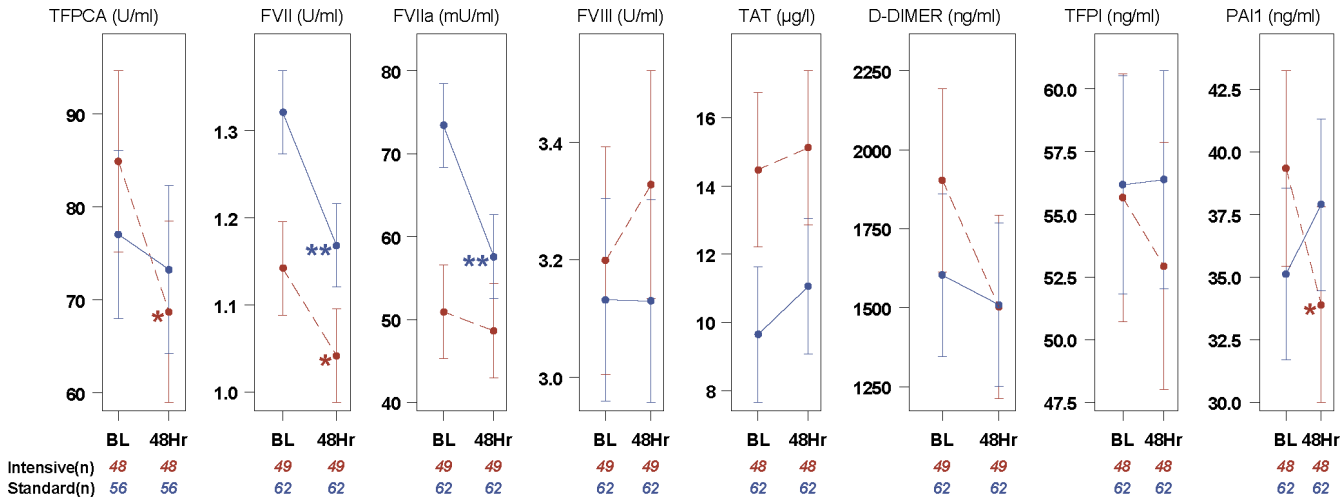


FIGURE 2 Temporal changes in markers of blood coagulation after acute ischemic stroke by insulin treatment groups: intensive (red, hatched line) and standard (blue, solid line) treatment. Average levels of biomarkers measured at baseline (BL) and 48 h are shown. Comparisons were made between baseline and 48-h levels for each marker within each treatment group. *$P < .05$, **$P < .01$

intensive treatment groups. Mean changes and mean BL and 48-hour biomarker levels in patients treated with intensive and standard insulin treatment are included as Table S1a.

3.3 | Changes in biomarker levels (FVIII and FVIIa) by functional outcome

Figure 4 shows that, with both intensive and standard insulin treatment groups combined, FVIII decreased −0.46 ± 0.28 U/mL from 3.19 ± 0.35 U/mL at BL to 2.73 ± 0.35 U/mL at 48 hours in patients with a favorable outcome but increased 0.17 ± 0.11 U/mL from 3.17 ± 0.14 U/mL at BL to 3.33 ± 0.14 U/mL at 48 hours with unfavorable outcome (difference, −0.63 U/mL; 95% CI, −1.23 to −0.03;, $P = .04$). FVIIa increased 7.17 ± 8.60 mU/mL from 55.98 mU/mL ± 10.10 mU/mL at BL to 63.14 ± 10.10 mU/mL at 48 hours in patients with favorable outcome but decreased −11.92 ± 3.43 mU/mL from 62.60 ± 4.03 mU/mL at BL to 50.68 ± 4.03 mU/mL at 48 hours in patients with unfavorable outcomes (difference, 19.09 mU/mL; 95% CI, 0.74-37.43; $P = .04$). Changes in the other biomarkers were not significantly different by functional outcome. Mean changes and BL and 48-hour biomarker levels separated by functional outcome groups are included as Table S1b.

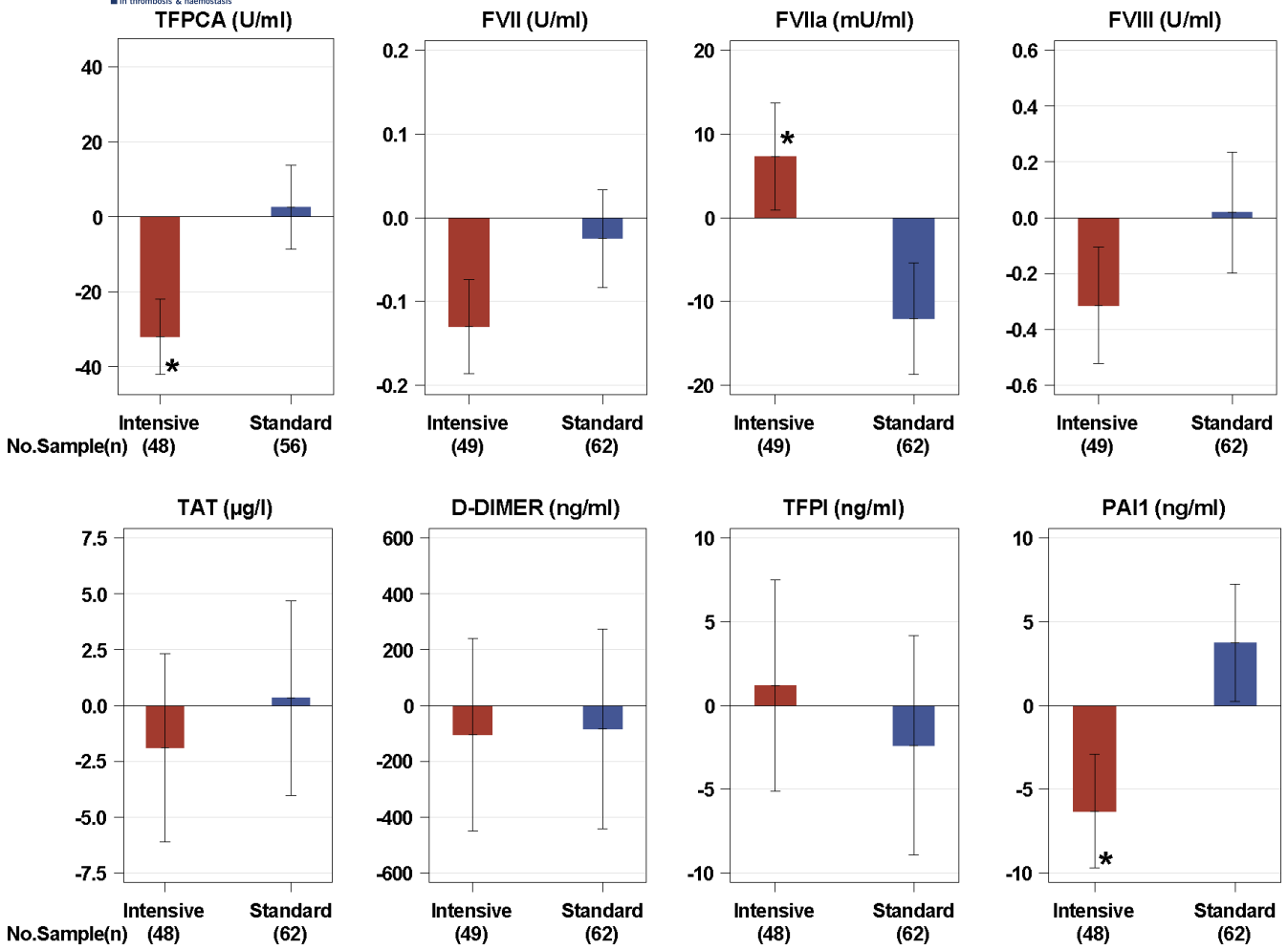


FIGURE 3 Average changes from baseline to 48-h in levels of biomarkers measured in patients treated with intensive (shown in red) and standard treatment (blue) after acute ischemic stroke. Comparisons were made between intensive and standard treatment for each marker. *$P < .05$. FVII, factor VII; FVIIa, factor VIIa; FVIII, factor VIII; PAI-1, plasminogen activator inhibitor-1; TAT, thrombin-antithrombin; TFPI, tissue factor pathway inhibitor; TFPCA, tissue factor procoagulant activity

3.4 | Changes in biomarker levels by both treatment and functional outcome

Patients in the intensive treatment group with favorable outcome had a greater reduction in TFPCA (−55.98 ± 18.34 U/mL) than patients with unfavorable outcome (−8.22 ± 8.20 U/mL; $P = .02$; Figure 5). Patients in the intensive treatment group with favorable outcome had reductions in FVIII levels (−0.97 ± 0.38 U/mL) as compared with increases (0.34 ± 0.17 U/mL) in patients with unfavorable outcome ($P = 0.002$). FVIIa levels rose (21.55 ± 11.74 mU/mL) in patients in the intensive treatment group with favorable outcome but decreased in patients with unfavorable outcome (−6.95 ± 5.19 mU/mL; $P = 0.03$). Patients in the standard treatment group with favorable outcome had a small increase in FVII (0.14 ± 0.11 U/mL) as compared with reduction (−0.19 ± 0.04 U/mL; $P = 0.006$) with unfavorable outcome.

3.5 | Analysis of covariates

In the adjusted models, changes from baseline to 48-hour level in FVIII was significantly different by history of hypertension, FVII by baseline blood glucose and history of hyperlipidemia, TFPI by baseline blood glucose, and PAI-1 by history of hypertension. After adjusting for potential confounding variables, FVIII was not significantly different between functional outcome groups (−0.61 ± 0.29 U/mL in favorable versus −0.10 ± 0.17 U/mL in patients with unfavorable outcome; $P = 0.09$). However, as in the unadjusted analyses, there were significant differences in PAI-1 between intensive (−10.38 ± 3.79 ng/mL) and standard treatment groups (0.33 ± 3.77 ng/mL; $P = .03$), in FVIII in the intensive treatment group with favorable (−1.13 ± 0.38 U/mL) as compared to unfavorable outcome (0.05 ± 0.22 U/mL; $P = .005$), and in FVII in patients in the standard treatment group with favorable (0.03 ± 0.14 U/mL) as compared to unfavorable outcome (−0.24 ± 0.11 U/mL; $P = .02$).

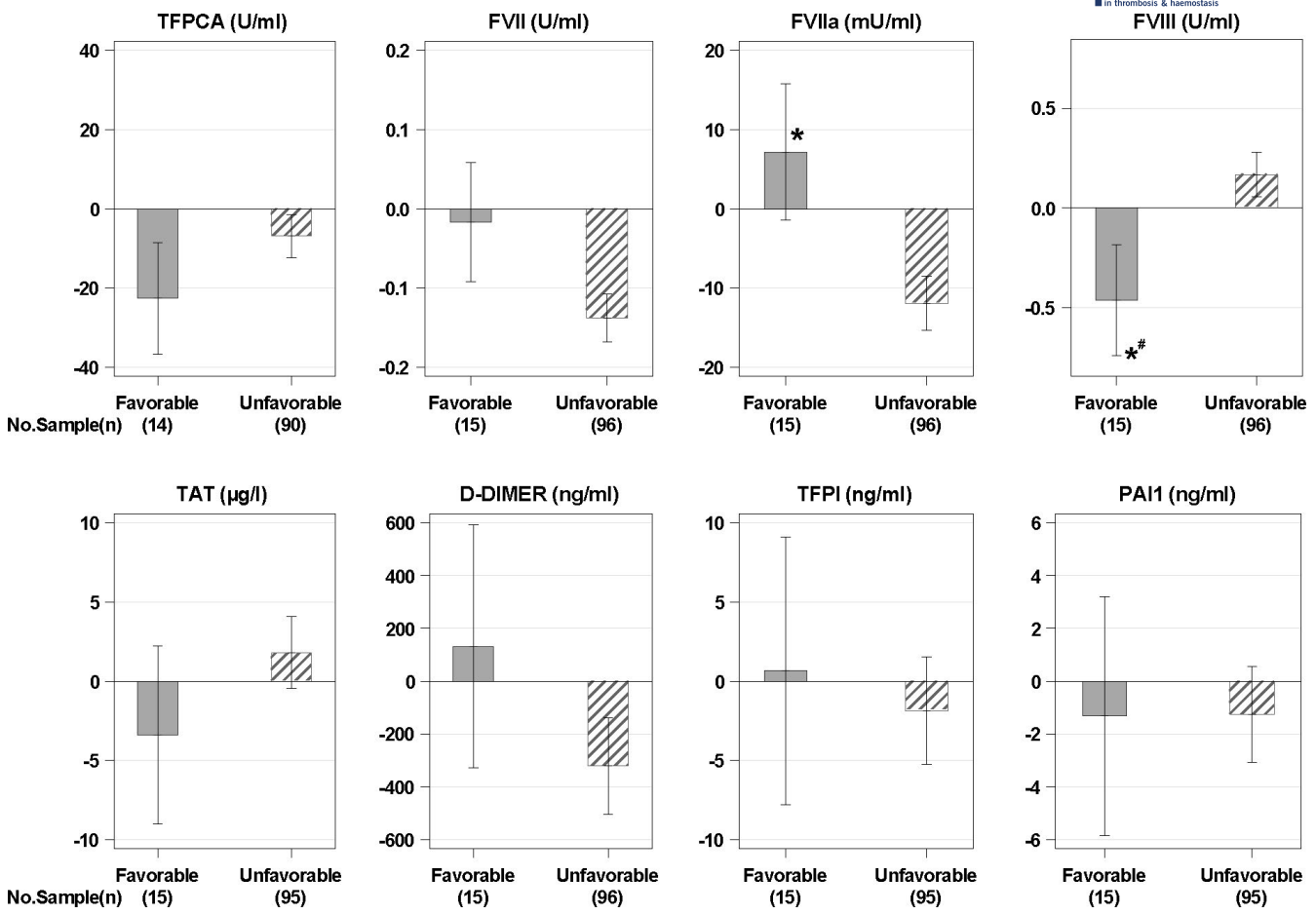


FIGURE 4 Average changes from baseline to 48-h in levels of biomarkers measured in patients with favorable (solid bar) and unfavorable SHINE outcome (hatched) at 3 months after acute ischemic stroke. Comparisons were made between favorable and unfavorable functional outcomes. All significance (* denoting 5% levels; $P < .05$) hold for both adjusted and unadjusted analyses, except for FVIII where a # symbol is added to denote significance at 5% level only for unadjusted analysis. FVII, factor VII; FVIIa, factor VIIa; FVIII, factor VIII; PAI-1, plasminogen activator inhibitor-1; TAT, thrombin-antithrombin; TFPI, tissue factor pathway inhibitor; TFPCA, tissue factor procoagulant activity

4 | DISCUSSION

We found that intensive treatment of hyperglycemia induced greater alterations in markers of blood coagulation compared to standard treatment (Figure 3), and these were associated with a favorable functional outcome at 3 months (Figures 4 and 5). There were significantly greater reductions in TFPCA and PAI-1 and increases in FVIIa with intensive as compared with standard insulin treatment. Further, in the intensive treatment group, favorable outcome was associated with greater reductions in TFPCA and FVIII and greater increases in FVIIa (Figure 5). Together, these results demonstrate that intensive insulin treatment reduces biomarkers reflecting the hyperglycemia-induced procoagulant state; and this effect is associated with improved functional outcome after AIS. In a prior study, circulating TFPCA was markedly elevated in AIS patients with hyperglycemia; and the levels correlated with stroke severity.[8] We now show that reductions in TFPCA are associated with improved functional outcome after AIS. This is important because TF is known to enhance coagulation and thrombosis,[15] and elevated TFPCA has been associated with increased risk of arterial events.[21]

The increase in plasma FVIIa levels with intensive insulin treatment observed (Figure 3) likely reflects the decrease in TF, the principal ligand for FVIIa. A reduction in TF leads to a decrease in FVIIa binding and concomitant increase in plasma FVIIa. This inverse relationship has been reported in patients with hyperglycemia[3,7] and in patients with AIS.[8] High plasma FVIIa is generally considered prothrombotic; however, in the present study, an increase in FVIIa was associated with improved outcome. Plasma FVIIa has been shown to reduce thrombin-mediated endothelial barrier disruption[22] and to have cytoprotective and anti-inflammatory effects in vivo.[23] Pretreatment with FVIIa has been shown to downregulate proinflammatory cytokines in response to lipopolysaccharide (LPS) administration, decrease microvascular endothelial cell apoptosis, and may have a protective role following experimental brain contusion.[24] In the present study, hyperglycemia control could have, by decreasing TF activation, reduced thrombus generation and improved regional blood flow limiting infarct size. Higher FVIIa levels may also have provided protection against penumbral microvascular leakage[24] and hyperpermeability,[23] ameliorating brain damage and edema. In the present study, elevations in FVIIa and reductions in

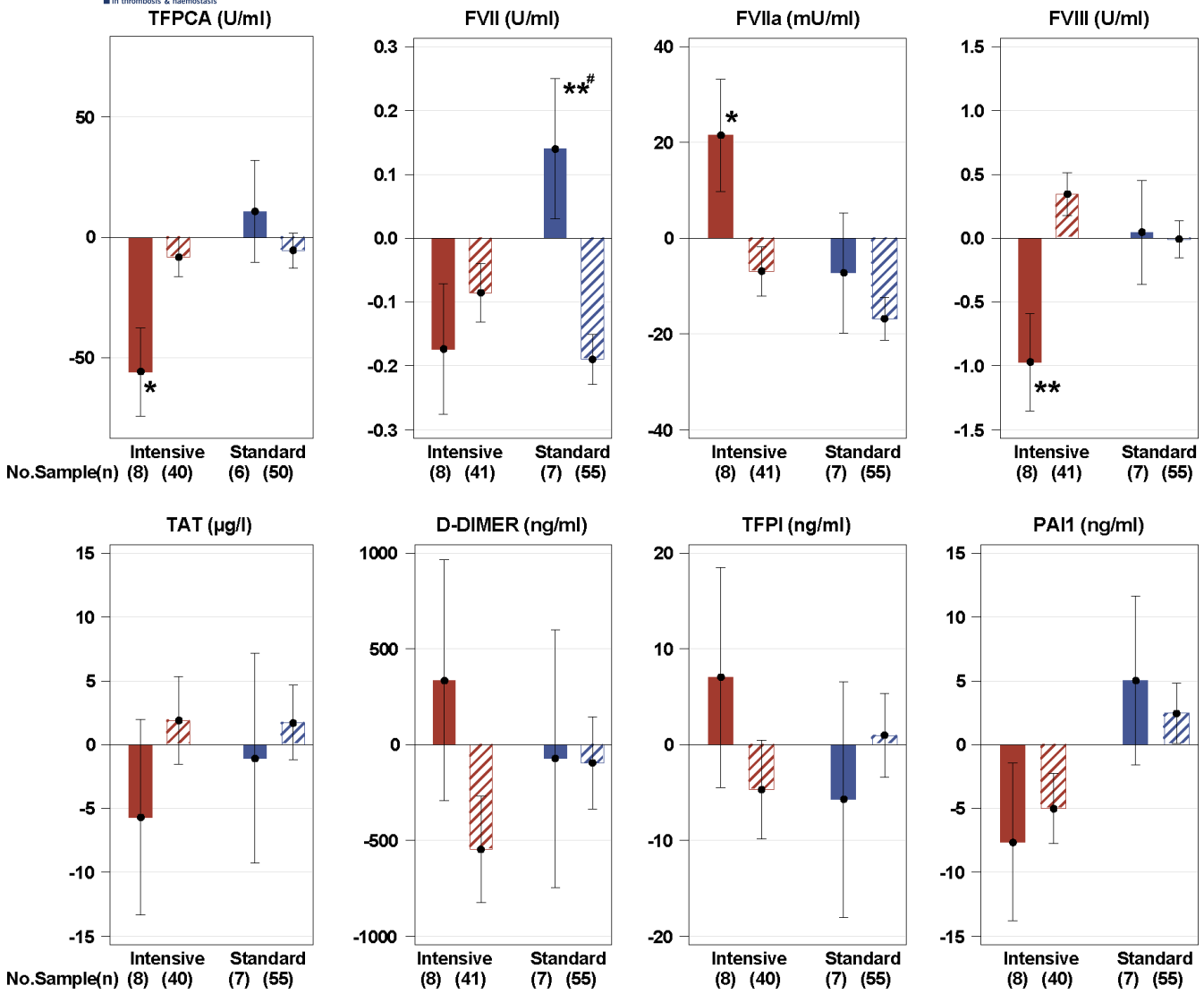


FIGURE 5 Average changes from baseline to 48-h biomarker levels in patients treated with intensive (left panel, shown in red) or standard treatment (right panels, blue) with favorable (solid bar) and unfavorable outcome (hatched) after acute ischemic stroke (AIS). Comparisons were made between favorable and unfavorable functional outcomes measured 3-months after AIS. All significance (*, ** denoting 5% and 1% levels, respectively) hold for both adjusted and unadjusted analyses, except for FVII where $^{**}P < .01$ for unadjusted and # denotes $P < .05$ for the adjusted analysis. FVII, factor VII; FVIIa, factor VIIa; FVIII, factor VIII; PAI-1, plasminogen activator inhibitor-1; TAT, thrombin-antithrombin; TFPI, tissue factor pathway inhibitor; TFPCA, tissue factor procoagulant activity

FVIII, a cofactor that amplifies thrombin generation, were associated with improved 3-month functional outcome with intensive and standard glucose treatment groups combined (Figure 4). These findings suggest that potential strategies that selectively reduce TF or reduce FVIII including glycemic control could improve outcome in AIS patients with hyperglycemia.

Intensive insulin treatment was associated with reductions in PAI-1, an inhibitor of fibrinolysis (Figure 3). However, changes in PAI-1 were not related to functional outcome in this study (Figures 4 and 5). Insulin administration has been previously shown to reduce PAI-1 levels, and reductions in PAI-1 levels have been shown to enhance fibrin clot lysis[25] and augment blood flow in the stroke penumbral area.[26] However, these effects depend on the stroke subtype. The impact of PAI-1 expression is different in thrombotic as compared with atherosclerotic stroke etiologies.[12] Further analyses are needed to understand the relationship between changes in PAI-1 and functional outcome in our study.

Potential explanations for the observed changes in TF and other factors in the intensive treatment arm can be offered by the distinct effects of hyperglycemia and insulin.[2,4] Hyperglycemia induces a procoagulant state and insulin has antithrombotic and anti- inflammatory effects.[2] Hyperglycemia reduces local microvascular perfusion[27] compromising regional blood flow in the penumbral areas of an ischemic stroke.[28] Decreasing TF activation, intensive insulin treatment could restore regional blood flow limiting stroke size and reducing brain damage.[29] Insulin has anti-inflammatory effects and inhibits expression of transcription factors (nuclear factor kappa B, epidermal growth factor receptor 1, activator protein 1, toll-like

receptor 4(TLR4), and generation of reactive oxygen species.[2,4] Monocytes and macrophages, a major source of tissue factor, have insulin receptors and insulin inhibits LPS-induced increases in TF and TLR4.[30] In our study, intensive insulin treatment could, by suppressing prothrombotic and proinflammatory mediators, decrease the disruption of the blood-brain barrier, preventing cerebral edema, leakage of plasma proteins, and inflammatory cells,[29] and thereby attenuate the detrimental inflammatory cascade.

In iSPOT, we found that intensive insulin treatment is associated with changes in coagulation markers that are associated with improved functional outcome after stroke. However, the parent SHINE trial showed no beneficial effect of intensive versus standard insulin treatment on functional outcome.[19] One possible explanation is that the SHINE trial included rt-PA and non–rt-PA treated patients, whereas the iSPOT primary analyses included only non–rt-PA–treated patients. Since over 63% of SHINE patients received rt-PA, it is possible that the positive effects of intensive glucose treatment on blood coagulation observed in non–rt-PA–treated in the present study were obscured. The present findings are nonetheless relevant to patients with AIS since, unlike the SHINE trial cohort, the majority of patients with AIS are not treated with rt-PA.[31,32] Further studies are needed to understand the relative effects of insulin treatment alone and thrombolytic therapy on functional outcome in AIS.

The present study was limited by the relatively small number of patients. While the effect sizes for several comparisons were substantial and reached statistical significance, we might have failed to achieve significance for some biomarker effects and some important differences may have been missed due to lack of power. We believe is it is unlikely that an increased sample size would substantially alter the findings in this study. On review of the analyses, there were no comparisons in which the differences were "close" to significance, that is, none with *P* values between .05 and 0.10 that we felt could have achieved statistical significance if the study were more adequately powered. The study may also be limited by the inclusion of patients with and without diabetes and patients with different stroke subtypes and severities. In addition, there was a wide range of blood glucose values within treatment arms including a higher rate of hypoglycemia in the intensive arm in the SHINE trial.[19] These factors may have impacted stroke outcome[33-36]; further analyses are warranted to address their influence on the relationships between blood coagulation, hyperglycemia control, and stroke outcomes.

In summary, the iSPOT trial showed that intensive insulin treatment of hyperglycemia during the first 48 hours of acute stroke induced alterations in blood coagulation that were associated with improved functional outcome. Leveraging the effects of glycemic control on the coagulation pathway for therapeutic purposes, however, will require further insight into these complex relationships.

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RELATIONSHIP DISCLOSURE

None.

AUTHOR CONTRIBUTIONS

NTG and AKR are co-principal investigators primarily responsible for the design, execution, and data analysis and interpretation of the project. HR and FDC-C are responsible for the data collection and measurements. VR and QP are responsible for the data analysis plan and data management. WGB and AB contributed to the interpretation of the data and review of the manuscript. They serve as liaison to the Neurological Emergencies Treatment Trials Network (NETT) Investigators and with the SHINE investigators, respectively. All authors have reviewed and accepted the manuscript and have attested to any conflict of interest.

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REFERENCES

1. Grant PJ. Diabetes mellitus as a prothrombotic condition. *J Intern Med*. 2007;262:157-172.
2. Dandona P, Chaudhuri A, Ghanim H, Mohanty P. Insulin as an anti-inflammatory and antiatherogenic modulator. *J Amer Coll Cardiol*. 2009;53(5):S14-S20.
3. Vaidyula VR, Rao AK, Mozzoli M, Homko C, Cheung P, Boden G. Effects of hyperglycemia and hyperinsulinemia on circulating tissue factor procoagulant activity and platelet CD40 ligand. *Diabetes*. 2006;55:202-208.
4. Sun Q, Li J, Gao F. New insights into insulin: the anti-inflammatory effect and its clinical relevance. *World J Diabetes*. 2014;5(2):89-96.
5. Bruno A, Levine SR, Frankel MR, et al. Admission glucose level and clinical outcomes in the NINDS rtPA stroke trial. *Neurology*. 2002;59:669-674.
6. Desilles JP, Meseguer E, Labreuche J, et al. Diabetes mellitus, admission glucose, and outcomes after stroke thrombolysis: a registry and systematic review. *Stroke*. 2013;44:1915-1923.
7. Boden G, Vaidyula VR, Homko C, Cheung P, Rao AK. Circulating tissue factor procoagulant activity and thrombin generation in patients with type 2 diabetes: effects of insulin and glucose. *J Clinical Endocrin Metab*. 2007;92:4352-4358.
8. Gentile NT, Vaidyula VR, Kanamalla U, DeAngelis M, Gaughan J, Rao AK. Factor VIIa and tissue factor procoagulant activity in diabetes mellitus after acute ischemic stroke: impact of hyperglycemia. *Thromb Haemost*. 2007;98:1007-1013.
9. Rauch U, Nemerson Y. Tissue factor, the blood, and the arterial wall. *Trends Cardiovasc Med*. 2000;10:139-143.
10. Mackman N. Role of tissue factor in hemostasis, thrombosis, and vascular development. *Arterioscler Thromb Vasc Biol*. 2004;24:1015-1022.

11. Key NS, Slungaard A, Dandelet L, et al. Whole blood tissue factor procoagulant activity is elevated in patients with sickle cell disease. *Blood*. 1998;91:4216-4223.
12. Tjärnlund-Wolf A, Brogren H, Lo EH, Wang X. Plasminogen activator inhibitor-1 and thrombotic cerebrovascular diseases. *Stroke*. 2012;43(10):2833-2839.
13. Chang TR, Albright KC, Boehme AK, et al. Factor VIII in the setting of acute ischemic stroke among patients with suspected hypercoagulable state. *Clin Appl Thromb Hemost*. 2014;20(2):124-128.
14. Barber M, Langhorne P, Rumley A, Lowe GD, Stott DJ. Hemostatic function and progressing ischemic stroke: D-dimer predicts early clinical progression. *Stroke*. 2004;35(6):1421-1425.
15. Grover SP, Mackman N. Tissue factor: an essential mediator of hemostasis and trigger of thrombosis. *Arterioscler Thromb Vasc Biol*. 2018;38(4):709-725.
16. He M, Wen Z, He X, et al. Observation on tissue factor pathway and some other coagulation parameters during the onset of acute cerebrocardiac thrombotic diseases. *Thromb Res*. 2002;107:223-228.
17. Berge E, Friis P, Sandset PM. Hemostatic activation in acute ischemic stroke. *Thromb Res*. 2001;101:13-21.
18. Cote R, Wolfson C, Solymoss S, et al. Hemostatic markers in patients at risk of cerebral ischemia. *Stroke*. 2000;8:1856-1862.
19. Johnston KC, Bruno A, Pauls Q, Hall CE, Barrett KM, Barsan W, et al.; for the Neurological Emergencies Treatment Trials Network and the SHINE Trial Investigators. Intensive vs standard treatment of hyperglycemia and functional outcome in patients with acute ischemic stroke. *JAMA*. 2019;322(4):326-335.
20. Bruno A, Durkalski VL, Hall CE, et al; for the SHINE investigators. The Stroke Hyperglycemia Insulin Network Effort (SHINE) trial protocol: a randomized, blinded, efficacy trial of standard vs. intensive hyperglycemia management in acute stroke. *Int J Stroke*. 2014;9(2):246-251.
21. Tutar E, Ozcan M, Kilickap M, et al. Elevated whole-blood tissue factor procoagulant activity as a marker of restenosis after percutaneous transluminal coronary angioplasty and stent implantation. *Circulation*. 2003;108(13):1581-1584.
22. Kondreddy V, Wang J, Keshava S, Esmon CT, Rao LVM, Pendurthi UR. Factor VIIa induces anti-inflammatory signaling via EPCR and PAR1. *Blood*. 2018;131(21):2379-2392.
23. Sen P, Gopalakrishnan R, Kothari H, et al. Factor VIIa bound to endothelial cell protein C receptor activates protease activated receptor-1 and mediates cell signaling and barrier protection. *Blood*. 2011;117(11):3199-3208.
24. Yuan Q, Zhang D, Wu S, et al. FVIIa prevents the progressive hemorrhaging of a brain contusion by protecting microvessels via formation of the TF-FVIIa- FXa complex. *Neuroscience*. 2017;348:114-125.
25. Nagai N, Suzuki Y, Van Hoef B, Lijnen HR, Collen D. Effects of plasminogen activator inhibitor-1 on ischemic brain injury in permanent and thrombotic middle cerebral artery occlusion models in mice. *J Thromb Haemost*. 2005;3:1379-1384.
26. Griemert EV, Recarte Pelz K, Engelhard K, Schäfer MK, Thal SC. PAI-1 but not PAI-2 gene deficiency attenuates ischemic brain injury after experimental stroke. *Transl Stroke Res*. 2019;10(4):372-380.
27. Duckrow RB, Beard DC, Brennan RW. Regional cerebral blood flow decreases during chronic and acute hyperglycemia. *Stroke*. 1987;18:52-58.
28. Kawai N, Keep RF, Betz AL. Nagao S Hyperglycemia induces progressive changes in the cerebral microvasculature and blood-brain barrier transport during focal cerebral ischemia. *Acta Neurochir Suppl*. 1998;71:219-221.
29. Garg R, Chaudhuri A, Munschauer F, Dandona P. Hyperglycemia, insulin, and acute ischemic stroke: a mechanistic justification for a trial of insulin infusion therapy. *Stroke*. 2006;37(1):267-273.
30. Singh A, Boden G, Rao AK. Tissue factor and toll-like receptor (TLR)4 in hyperglycaemia-hyperinsulinaemia: effects in healthy subjects, and type 1 and type 2 diabetes mellitus. *Thromb Haemost*. 2015;113(4):750-758.
31. Fang MC, Cutler DM, Rosen AB. Trends in thrombolytic use for ischemic stroke in the United States. *J Hosp Med*. 2010;5(7):406-409.
32. Scherf S, Limburg M, Wimmers R, et al. Increase in national intravenous thrombolysis rates for ischaemic stroke between 2005 and 2012: is bigger better? *BMC Neurol*. 2016;16:53.
33. Bagoly Z, Szegedi I, Kálmándi R, Tóth NK, Csiba L. Markers of coagulation and fibrinolysis predicting the outcome of acute ischemic stroke thrombolysis treatment: a review of the literature. *Front Neurol*. 2019;21(10):513.
34. Masrur S, Cox M, Bhatt DL, et al. Association of acute and chronic hyperglycemia with acute ischemic stroke outcomes post-thrombolysis: Findings from Get with the Guidelines–Stroke. *J Am Heart Assoc*. 2015;4(10):e002193.
35. Arboix A, Rivas A, García-Eroles L, de Marcos L, Massons J, Oliveres M. Cerebral infarction in diabetes: clinical pattern, stroke subtypes, and predictors of in-hospital mortality. *BMC Neurol*. 2005;5(1):9.
36. Miedema I, Luijckx GJ, Brouns R, De Keyser J, Uyttenboogaart M. Admission hyperglycemia and outcome after intravenous thrombolysis: is there a difference among the stroke-subtypes? *BMC Neurol*. 2016;16:104.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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