Endothelial Permeability and Hemostasis in Septic Shock

Results From the ProCESS Trial

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BACKGROUND: We studied patients from the Protocolized Care in Early Septic Shock (ProCESS) trial to determine the effects of alternative resuscitation strategies on circulating markers of endothelial cell permeability and hemostasis and the association between biomarkers and mortality.

METHODS: This was a prospective study of biomarkers of endothelial cell permeability (vascular endothelial growth factor [VEGF], soluble fms-like tyrosine kinase 1 [sFLT-1], angiopoietin 2 [Ang-2]) and biomarkers of hemostasis (von Willebrand factor [vWF], thrombomodulin [TM], tissue plasminogen activator [tPA]) in 605 of the 1,341 ProCESS participants in a derivation cohort and 305 participants in a validation cohort. Analyses assessed (1) the impact of varying resuscitation strategies on biomarker profiles and (2) the association of endothelial biomarkers with 60-day in-hospital mortality. The study was conducted in 31 US EDs in adult patients with septic shock. Patients were randomly assigned to one of three resuscitation strategies. Blood samples were collected at enrollment, at 6 h, and at 24 h.

RESULTS: There were 116 (19.2%) and 52 (17.0%) deaths in the derivation and validation cohorts, respectively. There was no significant association between treatment strategy and any biomarker levels. Permeability (Ang-2 and sFLT-1) and hemostasis (vWF, TM, tPA) biomarkers were higher and VEGF levels were lower in nonsurvivors ($P < .05$ for all). At baseline, sFLT-1 had the highest point estimate for mortality discrimination (derivation area under the curve [AUC], 0.74; validation, 0.70), similar to lactate (AUC, 0.74) and Sequential Organ Failure Assessment score (AUC, 0.73). In an analysis including all time points and adjusted for age, presence of cancer, and Charlson comorbidity score, the adjusted AUC for sFLT-1 was 0.80.

CONCLUSIONS: We found no relationship between different resuscitation strategies and biomarker profiles in sepsis, but we did find that elevated levels of endothelial cell biomarkers of permeability and hemostasis were associated with increased mortality.

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ABBREVIATIONS: Ang-1 = angiopoietin 1; AUC = area under the curve; EGDT = early goal-directed therapy; ELISA = enzyme-linked immunoassay; ProCESS = Protocolized Care in Early Septic Shock; sFLT-1 = soluble fms-like tyrosine kinase 1; TM = thrombomodulin; tPA = tissue plasminogen activator; VEGF = vascular endothelial growth factor; vWF = von Willebrand factor

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Sepsis triggers high morbidity, mortality, and health care costs.¹ The vascular endothelial lining is in continuous contact with circulating components of the blood, including cells, molecules, proteins, and hormones.² The endothelium helps regulate vascular permeability, hemostasis, inflammation, and microcirculatory flow, all of which are important determinants of the pathophysiology of sepsis. This makes the endothelium an attractive diagnostic and therapeutic target in patients with sepsis.^{3,4}

Vascular endothelial growth factor (VEGF), soluble fms-like tyrosine kinase 1 (sFLT-1; a soluble VEGF receptor), and angiopoietin-2 (Ang-2) are angiogenic factors that help mediate neovascularization and regulate endothelial barrier function.⁵ Each of these angiogenic markers is associated with vascular permeability and adverse outcomes in both experimental and human sepsis.^{3,5-9} There is also a disruption of the hemostatic balance during sepsis that leads to a largely procoagulant phenotype and results in

microcirculatory disturbances, leading to impaired perfusion and tissue hypoxia. Accordingly, thrombomodulin (TM), tissue plasminogen activator (tPA), and von Willebrand factor (vWF) are key proteins expressed by endothelial cells that regulate local hemostatic balance.² Few sepsis studies have simultaneously assessed multiple endothelial biomarkers across these two key domains. Moreover, little data exist to detail whether the endothelial and coagulation disturbances respond differently to varying resuscitation approaches.

We sought to (1) determine the effects of alternative resuscitation strategies on circulating markers of endothelial cell permeability and hemostasis, (2) study the association between endothelial cell biomarkers of permeability/hemostasis and 60-day in-hospital mortality in sepsis, and (3) investigate the added prognostic value of combining multiple biomarkers into a panel to identify patients with septic shock who have an increased risk of death.

Methods

Study Design

This was an investigation in patients who participated in the Protocolized Care for Early Septic Shock (ProCESS) trial, a patient-level randomized multicenter interventional trial of alternative resuscitation strategies in ED sepsis ([clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00793442) NCT00793442).¹⁰ In the ProCESS trial, patients with sepsis and evidence of hypoperfusion (see enrollment criteria further on) randomly received one of three resuscitation strategies: early goal-directed therapy (EGDT), as originally described by Rivers et al,¹¹ delivered by a study team; a noninvasive protocolized care strategy delivered by a study team; or usual care without any protocol or prompts and delivered by the clinical team. In the ProCESS clinical trial, 31 centers recruited 1,341 subjects between March 2008 and May 2013. For the current study, we collected blood for analysis of

circulating endothelial-related biomarkers from a subset of these patients. The primary outcome was in-hospital mortality or survival to 60 days, whichever came first. This study was registered with [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00793442) under the identifier NCT00793442, and ethics committee approval was received from participating sites. The University of Pittsburgh was the coordinating center for the clinical trial, and Beth Israel Deaconess Medical Center Committee for Clinical Investigations (protocol No. 2008P-000089) was the coordinating center for the substudy. Thus, all study procedures have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from each study subject or their legal authorized representative in compliance with ethics committee regulations.

Participants

Our study participants came from the ProCESS trial participants.¹⁰ In brief, ProCESS enrollment criteria were the following: They were patients being seen in the ED in whom sepsis was suspected according to the treating physician. They were at least 18 years of age, met two or more criteria for systemic inflammatory response syndrome, and had refractory hypotension or a serum lactate level of 4 mmol/L or higher. Refractory hypotension was defined as systolic BP that was either < 90 mm Hg or required vasopressor therapy to maintain 90 mm Hg even after an IV fluid challenge. The exclusion criteria are listed in the Methods section of the Supplementary Appendix of the ProCESS clinical trial publication.¹⁰ From the ProCESS subjects, we included those who were (1) recruited by participating centers who participated in other components of this ancillary study or (2) who were sequentially enrolled from periods derived from the beginning, middle, and end of the ProCESS study. Finally, we recruited a sequential 300-patient validation set.

Demographics and Clinical Data Collection

We collected patient demographics, comorbid illnesses, causes of infection, and treatments.

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Blood Collection and Assays

We sampled blood on enrollment and at 6 and 24 h after the baseline sampling. After immediate centrifuge, we stored samples at -80°C . We assayed six different endothelial-related biomarkers comprising different components of endothelial permeability and hemostasis. We assayed endothelial permeability markers (VEGF [165 isoform], sFLT-1, Ang-2) using human Quantikine enzyme-linked immunoassay (ELISA) kits (R&D Systems) from plasma collected in tubes containing ethylene diamine tetraacetic acid, as well as hemostasis markers (vWF, TM, and tPA) from plasma collected in tubes containing citrate, following manufacturer recommendations for each kit. The vWF and TM measurements were made using human Quantikine ELISA kits (R&D Systems), whereas tPA was performed by hand ELISA

(Innovative Diagnostics). Since blood lactate is a sepsis marker commonly used in clinical practice, we include blood lactate in our prognostic models as a performance reference for the novel markers reported in this manuscript. Analyses of blood lactate concentrations were reported in detail as part of the original report of the trial¹⁰ and as part of a separate manuscript on sepsis mechanisms.¹²

Sample Size Calculation

We based our sample size estimate on VEGF levels over time. Assuming a two-sided type I error of 0.05 and power of 0.8, we estimated the need for a sample size of at least 600 subjects (e-Appendix 1 also presents the full statistical analysis plan).

Results

Overview

There were 605 subjects in the derivation set, with blood samples drawn over multiple time points (baseline and 6 and 24 h) and 305 patients in the validation set, with a baseline blood draw only. There were a total of 116 deaths (19.2% mortality) in the derivation set and 52 (17.0% mortality) in the validation set.

Patient Demographics

Patients in our study had a mean age of 62 years (SD, 15.7) (Table 1); 70% were white, 23% were African American, and the remaining were Asian or other. Hispanic ethnicity was 10.4%. Similar to prior studies, the prevalence of comorbid illness was high, including high rates of hypertension, diabetes, and cancer. The underlying cause of sepsis was most commonly pneumonia, and the baseline Sequential Organ Failure Assessment (SOFA) score was high (7.3; SD, 3.6). Demographics, comorbidities, and infection sources did not differ between our derivation and validation sets (Table 1). The patients were recruited from across the PROCESS study sites (e-Table 1).

Effect of Resuscitation Strategy on Patterns of Endothelial Cell Biomarkers

The first aim was to study whether different resuscitation strategies (EGDT, noninvasive protocolized resuscitation, or usual care) altered biomarker profiles over the first 24 h of care. With the background knowledge that there were no differences in clinical outcome between resuscitation strategies,¹⁰ there was similarly no difference in biomarker profiles of vascular permeability (sFLT-1, VEGF, Ang-2) or hemostasis (vWF, tPA, TM) for each time point between the three treatment arms (Table 2, e-Fig 1). Since there were no differences in biomarker levels between groups, we pooled the treatment groups for subsequent analyses.

Endothelial Biomarkers and Mortality

Univariate association over time: The endothelial permeability biomarkers (sFLT-1, VEGF, and Ang-2) were associated with increased mortality both at baseline and at each time point over 24 h (Fig 1, Table 3, e-Fig 2). The biomarkers sFLT-1 and Ang-2 had median levels at 0, 6, and 24 h that were higher in nonsurvivors compared with survivors, whereas the VEGF levels were significantly lower. The sFLT-1 and Ang-2 biomarkers started at higher levels and remained elevated in nonsurvivors, whereas survivors had lower levels that stayed lower. VEGF levels started lower and stayed lower in nonsurvivors compared with survivors (Fig 1, e-Fig 2). The hemostasis biomarkers displayed a pattern similar to that of sFLT-1 and Ang-2, with vWF, tPA, and TM also showing elevated median levels (Table 3) and log-transformed means in nonsurvivors compared with survivors over each of the time points (Fig 1).

Mortality prediction using baseline and 24-h biomarker levels:

We compared the prognostic value of endothelial biomarkers from each domain by calculating the AUC for mortality. For the unadjusted AUCs, only sFLT-1 (0.74)—similar to lactate (0.74) and SOFA score (0.73)—had AUC values at baseline with a point estimate > 0.70 (Table 4). There was a positive association between the degree of biomarker elevation and mortality, as depicted by the increasing ORs for death by biomarker-level quartile. The diagnostic accuracy of the baseline biomarkers was relatively consistent in the 300-patient validation set for the biomarkers tested, with the exception of tPA, whose AUC was somewhat higher in the validation set (derivation cohort, 0.59; validation cohort, 0.69), and vWF, whose AUC was lower (derivation cohort, 0.70; validation cohort, 0.60) (Table 4). At 24 hours, sFLT-1 (0.79) had the highest AUC point estimate, whereas Ang-2, vWF, tPA, and TM were at 0.70 or higher,

TABLE 1] Demographics

Characteristic	Novel Endothelial Marker Derivation Group (n = 605)	Novel Endothelial Marker Validation Group (n = 305)
Mean age (SD), y	62 (15.7)	60.9 (16.1)
Male sex	335 (55.4)	177 (58.0)
Race, No. (%)		
White	424 (70.0)	232 (73.1)
African American	140 (23.1)	59 (18.6)
Asian	15 (2.5)	3 (0.95)
Other	21 (3.4)	20 (6.3)
Ethnicity, No. (%)		
Non-Hispanic	542 (89.6)	275 (86.8)
Hispanic	63 (10.4)	42 (13.3)
Domicile prior to admission, No. (%)		
Not nursing home	502 (82.9)	285 (89.9)
Nursing home	103 (17.0)	32 (10.1)
Chronic conditions, No. (%)		
Charlson comorbidity score, mean (SD)	2.8 (2.7)	2.6 (2.3)
Hypertension	358 (59.2)	190 (59.9)
Diabetes mellitus	203 (33.6)	94 (29.7)
Chronic respiratory disease	137 (22.6)	77 (24.3)
Cancer	109 (18)	60 (19.0)
Dialysis-dependent renal impairment	36 (6.0)	24 (7.6)
Congestive heart failure	78 (12.9)	45 (14.2)
Prior myocardial infarction	66 (10.9)	35 (11.0)
Cerebrovascular disease	71 (11.7)	25 (7.9)
Peripheral vascular disease	54 (8.9)	26 (8.2)
Chronic dementia	54 (8.9)	21 (6.6)
Hepatic cirrhosis	41 (6.8)	20 (6.3)
Peptic ulcer disease	28 (4.6)	16 (5.0)
AIDS and related syndromes	19 (3.1)	2 (0.63)

(Continued)

TABLE 1] (Continued)

Characteristic	Novel Endothelial Marker Derivation Group (n = 605)	Novel Endothelial Marker Validation Group (n = 305)
Source of sepsis, No. (%)		
Pneumonia	196 (32.4)	106 (33.4)
Urosepsis	128 (21.1)	80 (25.2)
Infection, source unknown	80 (13.2)	37 (11.7)
Intra-abdominal infection	76 (12.6)	40 (12.6)
Skin and soft-tissue infections	44 (7.3)	26 (8.2)
Catheter-related infection	18 (3.0)	9 (2.8)
Central nervous system	6 (1.0)	3 (0.95)
Endocarditis	5 (0.83)	1 (0.32)
Other	38 (6.3)	14 (4.42)
Considered after review not to have infection	14 (2.3)	1 (0.32)
Baseline SOFA score	7.3 (3.6)	7.0 (3.7)

SOFA = Sequential Organ Failure Assessment.

demonstrating improvement in prognostic accuracy with a later time point (Table 5). Similar to baseline values, the mortality risk for these markers at 24 h was positively associated with increasing levels by quartile.

Adjusted analysis for biomarkers over time: We used logistic regression to model each biomarker at different time points and adjust for age, comorbid burden (Charlson comorbidity index score), and the presence of cancer, which were all associated with outcome (e-Table 2). We calibrated to a standardized beta estimate to allow comparison of changes in biomarker levels relative to their distribution; therefore, ORs are comparable. In adjusted analysis over all time points, the AUC for sFLT-1 was 0.80; Ang-2, and vWF had an AUC of 0.75 and 0.76, respectively, and lactate (included as a reference marker) had an AUC of 0.82 (e-Table 3).

Multimarker modeling: Finally, we examined how different combinations of endothelial permeability and hemostasis markers (baseline levels) could be used in combination to improve biomarker accuracy in predicting mortality. We ranked the top three models, grouped by the number of biomarkers in the model, for

TABLE 2] Biomarker Profiles by Treatment Group

Biomarker	Time Point	No. of Patients	Protocol-Based EGDT Arm Median (IQR)	No. of Patients	Protocol-Based Standard Therapy Arm Median (IQR)	No. of Patients	Usual Care Arm Median (IQR)	P Value
Vascular Permeability Markers								
sFLT-1	Baseline	189	342 (163-615)	211	256 (151-578)	205	275 (140-559)	.32
	6 h	190	279 (148-612)	209	261 (124-563)	199	300 (144-489)	.52
	24 h	176	220 (127-525)	197	230 (130-510)	184	248 (128-523)	.85
VEGF	Baseline	189	97.7 (27.0-219)	211	90.0 (33.3-196)	205	114 (42.6-225)	.35
	6 h	190	73.5 (28.2-184)	209	72.7 (21.4-198)	199	94.0 (39.6-198)	.09
	24 h	176	48.4 (15.4-110)	197	45.9 (23.5-139)	184	51.8 (15.4-119)	.70
Ang-2	Baseline	189	7,721 (3,121-15,914)	211	7,045 (3,441-15,165)	211	8,526 (4,208-18,119)	.28
	6 h	190	8,606 (4,092-20,332)	209	8,007 (4,080-16,743)	209	9,413 (4,433-20,152)	.56
	24 h	176	9,932 (4,084-26,369)	197	9,245 (4,570-20,603)	197	10,561 (4,790-24,922)	.60
Hemostasis Markers								
vWF	Baseline	189	3,190 (2,057-5,366)	211	2,971 (2,045-4,682)	205	3,570 (2,197-5,145)	.22
	6 h	190	3,276 (2,263-4,794)	209	2,981 (1,929-4,646)	199	3,399 (2,263-4,793)	.20
	24 h	176	3,205 (2,027-4,656)	197	2,807 (1,873-4,039)	184	3,640 (2,076-4,935)	.007
tPA	Baseline	189	2.6 (1.1-4.8)	211	2.4 (1.3-4.3)	205	2.3 (1.2-4.6)	.91
	6 h	190	1.9 (0.9-3.9)	209	1.9 (1.2-3.8)	199	2.0 (1.1-3.9)	.98
	24 h	176	1.3 (0.6-2.6)	197	1.4 (0.6-2.9)	184	1.3 (.7-2.6)	.74
TM	Baseline	189	5.1 (3.3-7.5)	211	4.6 (3.3-7.4)	205	4.8 (3.5-7.2)	.55
	6 h	190	5.3 (3.6-7.4)	209	4.7 (3.5-7.4)	199	5.1 (3.7-7.4)	.66
	24 h	176	5.2 (3.6-7.7)	197	4.9 (3.7-6.9)	184	5.0 (3.9-7.7)	.47

Ang-2 = angiotensin 2; EGDT = early goal-directed therapy; IQR = interquartile range; sFLT-1 = soluble fms-like tyrosine kinase 1; TM = thrombomodulin; tPA = tissue plasminogen activator; vWF = von Willebrand factor.

Figure 1 – Mean log-transformed biomarker levels with 95% CIs stratified by survivors and nonsurvivors (60-day in-hospital mortality). Ang = angiopoietin; sFLT-1 = soluble fms-like tyrosine kinase 1; TMB = thrombomodulin; tPA = tissue plasminogen activator; vWF = von Willebrand factor.

each combination of markers (e-Table 4). The best single-marker model was sFLT-1 (AUC, 0.74); the best two-marker model was sFLT-1 and vWF (AUC, 0.76), and the model with all five markers was only minimally

better (AUC, 0.77). Biomarker panel predictive ability degraded in the validation cohort, although multimarker panels still conferred a mild prognostic advantage (e-Table 4).

TABLE 3] Biomarker Profiles by 60-Day In-Hospital Mortality Status

Biomarker	Time Point	No. of Patients	Lived Median [IQR]	No. of Patients	Died ^a Median (IQR)	P Value
Vascular Permeability Markers						
sFLT-1	Baseline	489	242 (141-457)	116	663 (307-1,064)	< .001
	6 h	489	243 (122-438)	109	588 (326-1,197)	< .0001
	24 h	466	203 (118-385)	91	684 (299-1,198)	< .0001
VEGF	Baseline	489	109.0 (39.2-229.3)	116	78.5 (2.3-166.1)	< .002
	6 h	489	83.4 (33.3-202.7)	109	69.4 (13.3-174.7)	.08
	24 h	466	51.2 (19.7-120.1)	91	32.1 (.0-122.2)	< .003
Ang-2	Baseline	489	6,570 (3,146-13,945)	116	12,859 (6,808-28,135)	< .0001
	6 h	489	7,290 (3,833-15,738)	109	17,526 (7,972-29,777)	< .0001
	24 h	466	8,357 (4,042-20,361)	91	22,517 (9,832-45,631)	< .0001
Hemostasis Markers						
vWF	Baseline	489	2,955 (1,946-4,734)	116	4,894 (3,296-6,517)	< .0001
	6 h	489	3,006 (1,977-4,393)	109	4,667 (3,095-5,743)	< .0001
	24 h	466	2,974 (1,863-4,217)	91	4,632 (3,390-5,869)	< .0001
tPA	Baseline	489	2.22 (1.11-4.32)	116	3.01 (1.67-5.35)	< .003
	6 h	489	1.82 (1.01-3.61)	109	2.65 (1.42-5.69)	< .0001
	24 h	466	1.19 (0.60-2.57)	91	2.03 (1.01-3.72)	< .005
TM	Baseline	489	4.56 (3.20-6.65)	116	6.69 (4.52-9.96)	< .0001
	6 h	489	4.76 (3.38-6.80)	109	6.99 (4.81-1.0)	< .0001
	24 h	466	4.80 (3.59-6.68)	91	7.19 (5.38-1.3)	< .0001

See Table 2 legend for expansion of abbreviations.

^aDied = 60-day in-hospital mortality.

Discussion

We demonstrate the following key findings: (1) the alternative resuscitation strategies, which failed to alter outcomes in the parent ProCESS trial, also had no effect on endothelial cell biomarker profiles of vascular permeability or hemostasis; (2) increased circulating levels of the endothelial biomarkers sFLT-1, Ang-2, vWF, tPA, and TM were associated with increasing mortality both at baseline and over time points within the first 24 hours, whereas lower VEGF levels at baseline and over time were associated with higher mortality; (3) sFLT-1 had the highest point estimate for prognostic accuracy; (4) in an adjusted analysis, the aforementioned biomarkers retained their significance both at baseline and over time; and (5) there was a modest increase in prognostic accuracy when multiple endothelial-related biomarkers were combined as part of a biomarker panel. The strengths of the present study include the demonstration that a subset of endothelial-related biomarkers was associated with mortality both on presentation and over the early course of sepsis and the simultaneous measurement of multiple markers in a very large study cohort to allow for direct comparison of

biomarker combinations to predict mortality. Our data provide additional evidence in support of the endothelium as an important diagnostic and therapeutic target in sepsis, with particular emphasis on restoring mechanisms of vascular permeability and hemostasis.

Although previous studies quantified endothelial biomarker levels in sepsis, the vast majority involved small sample sizes (eg, most were < 100 patients) or a limited number of markers measured in each investigation.¹³ Our results build on prior efforts by allowing for simultaneous assessment of several biomarkers in a large patient population. We did confirm an association between our biomarker targets and mortality both initially and over the first 24 h. Our findings are largely consistent with prior studies that have identified an association between elevated circulating of biomarkers and increased severity or mortality (or both) for sFLT-1,^{3,7,14} Ang-2,^{9,15-21} vWF,^{22,23} tPA, and TM.²⁴⁻²⁶ However, there have been other studies of sFLT-1, Ang-2,^{6,27,28} vWF,²⁹ tPA, and TM that failed to find an association between elevated levels of these biomarkers and outcomes. Interestingly,

TABLE 4] The Association of Baseline Biomarker Levels With Mortality

Biomarker	Continuous Value OR ^a (95% CI)	Quartile 2 OR (95% CI)	Quartile 3 OR (95% CI)	Quartile 4 OR (95% CI)	Derivation AUC (95% CI)	Validation AUC (95% CI)
sFLT-1	2.1 (1.7-2.5)	1.2 (0.5-2.6)	2.5 (1.2-5.2)	7.9 (4.0-15.6)	0.74 (0.68-0.79)	0.70 (0.61-0.78)
VEGF	0.91 (0.72-1.1)	0.55 (0.32-0.94)	0.35 (0.19-0.63)	0.44 (0.25-0.77)	0.59 (0.42-0.66)	0.56 (0.47-0.66)
Ang-2	1.5 (1.2-1.8)	2.7 (1.2-5.8)	3.5 (1.6-7.4)	7.0 (3.4-14.5)	0.68 (0.62-0.73)	0.67 (0.58-0.76)
VWF	2.0 (1.7-2.5)	1.2 (0.5-2.5)	3.0 (1.5-5.9)	5.9 (3.1-11.5)	0.70 (0.64-0.75)	0.60 (0.51-0.69)
tPA	1.3 (1.1-1.6)	1.6 (0.8-3.0)	2.1 (1.1-4.0)	2.3 (1.2-4.3)	0.59 (0.53-0.64)	0.69 (0.61-0.77)
TM	1.5 (1.3-1.8)	1.7 (0.8-3.5)	3.1 (1.5-6.1)	4.8 (2.5-9.4)	0.68 (0.62-0.72)	0.69 (0.60-0.77)
Lactate	2.4 (1.8-3.2)	6.2 (1.7-21.9)	6.6 (1.9-23.2)	19.9 (5.9-67)	0.74 (0.66-0.82)	0.71 (0.58-0.78)

This table reports the results of logistic regression models for the association between baseline biomarker quartiles and mortality, using the first quartile as the reference group. We show the AUC values in both the derivation and validation sets for the actual continuous biomarkers values. AUC = area under the curve. See Table 2 legend for expansion of other abbreviations.

^aStandardized estimate; unit is normalized to the SD of each biomarker.

studies of VEGF levels have revealed discordant findings, with some investigations showing an association between elevated levels of VEGF and mortality,⁸ others between lower levels of VEGF and mortality,³⁰ and a third group finding no association between VEGF and mortality.¹⁴ Variability in measurement techniques and inconsistent association with outcomes when assessing VEGF has been previously described³¹; thus, discordant findings are not completely surprising. We found an association between lower VEGF levels and higher mortality; however, one must note that in our study, the diagnostic accuracy of the VEGF models was relatively low, demonstrating a weaker association with outcome than some of the other biomarkers. Overall, our results not only provide further data regarding the biomarker-mortality relationship but also allow comparison of individual biomarker performance.

The ProCESS investigation found that there was no difference in clinical outcomes between resuscitation strategies. Consistent with these data, we found no association between resuscitation strategy assignment and profiles of endothelial cell biomarkers. Given the association between multiple domains of endothelial cell signaling and mortality, we hypothesize that the failure of alternative resuscitation protocols to improve mortality may be explained, at least in part, by the inability of these strategies to reduce endothelial cell dysfunction, an important determinant of mortality in sepsis. Alternatively, each of the ProCESS care approaches may be at the same interventional threshold for these markers, and different therapeutic approaches (either quantitative or qualitative) could create a more graded biomarker response.

We were also able to show a modest improvement in mortality prediction by simultaneously incorporating multiple biomarkers into a single diagnostic panel. In view of the complexity of the pathophysiology of sepsis, we submit that multimarker panels are a promising way forward in sepsis prognosis. In retrospect, one shortcoming of our multimarker approach was that our selected biomarkers were, by design, all related to endothelial cell signaling. Similar to prior work of ours, the incorporation of biomarkers from different pathophysiological domains may have yielded a more prognostic panel.³² In addition, we propose that diagnostic panels may ultimately be combined with future therapies to facilitate a theragnostic approach, whereby novel therapies are tailored to patients who

TABLE 5] ORs for 60-Day Mortality by 24-Hour Biomarker Quartiles

Biomarker	Continuous Value ^a OR (95% CI)	Quartile 2 OR (95% CI)	Quartile 3 OR (95% CI)	Quartile 4 OR (95% CI)	AUC
sFLT-1	2.2 (1.8-2.7)	2.1 (0.76-5.8)	2.5 (0.93-6.7)	14.9 (6.1-36)	0.78 (0.71-0.83)
VEGF	1.0 (0.82-1.3)	0.45 (0.23-0.85)	0.39 (0.20-0.75)	0.60 (0.33-1.1)	0.49 (0.41-0.65)
Ang-2	1.6 (1.3-1.9)	2.3 (0.90-5.8)	4.0 (1.6-9.5)	8.0 (3.4-18.6)	0.72 (0.66-0.77)
vWF	2.0 (1.6-2.4)	1.4 (0.55-3.3)	3.0 (1.3-6.7)	6.7 (3.1-14.4)	0.70 (0.64-0.76)
tPA	1.3 (1.1-1.6)	1.2 (0.55-2.5)	2.4 (1.2-4.7)	2.5 (1.2-4.9)	0.62 (0.55-0.68)
TM	1.7 (1.4-2.1)	1.8 (0.68-4.6)	5.0 (2.1-11.8)	7.9 (3.4-18.4)	0.72 (0.66-0.77)
Lactate	2.9 (2.0-4.3)	2.7 (0.90-8.2)	2.2 (0.81-6.0)	8.8 (3.7-21)	0.75 (0.64-0.84)

This table reports the results of logistic regression models for the association between baseline biomarker quartiles and mortality, using the first quartile as the reference group. We show the AUC values in both the derivation and validation sets for the actual continuous biomarkers values. See [Table 2](#) and [3](#) legends for expansion of abbreviations.

^aStandardized estimate; unit is normalized to the SD of each biomarker.

manifest the pathophysiological defect that is being targeted.

There are limitations to our trial. First, it is possible that we selected the wrong markers and that other endothelial biomarkers would have been even more informative. Second, with the exception of TM, the expression of our biomarkers is not restricted to endothelial cells; elevated circulating levels reflect their synthesis and release from both endothelial and nonendothelial cells. Third, circulating biomarker levels in venous blood reflect the sum of expression/release from multiple vascular beds and thus do not provide information about individual endothelial cell populations. Moreover, since the endothelium demonstrates vascular bed-specific responses to activation markers, a singular circulating readout may not represent key vital changes in one or another organ. Fourth, there were missing values, especially at

the 24-h time point, due to deaths or missed blood draws, which may have altered the results. Finally, we assumed that missing blood draws were random events, whereas they may have been prone to selection bias.

Conclusions

We found no relationship between different resuscitation strategies in sepsis, but we did identify a number of key endothelial biomarkers associated with mortality in sepsis. Endothelial biomarkers of permeability (sFLT-1 and Ang-2) and hemostasis (vWF, tPA, and TM) were associated with increased mortality both at baseline and over the first 24 hours, whereas decreased VEGF levels both at baseline and over the first 24 hours were associated with mortality. The biomarker sFLT-1 remains a particularly promising prognostic biomarker in sepsis.

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