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Relationship between alternative resuscitation strategies, host response and injury biomarkers, and outcome in septic shock: Analysis of the ProCESS study

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Abstract

Objective—The Protocol-based Care for Early Septic Shock (ProCESS) trial found no differences across alternative resuscitation strategies in all-cause mortality. A separate aim was to determine whether differences in resuscitation strategies affected trajectories of biomarkers of key pathways associated with downstream clinical outcomes of sepsis and whether there were differences in survival across treatment arms for patients with different baseline biomarker profiles.

Design—Secondary analysis of a large randomized clinical trial.

Setting—31 US hospitals.

Patients—628 patients with septic shock.

Interventions—Two resuscitation protocols versus usual care.

Measurements and Main Results—We measured a panel of biomarkers representing four pathophysiologic domains: *inflammation* (tumor necrosis factor, interleukin-6, and -10); *coagulation* (D-dimers, thrombin-anti-thrombin complex); *oxidative stress* (urine isoprostane) and *tissue hypoxia* (lactate) at 0, 6, 24 and 72 hours after treatment. We analyzed whether alternative resuscitation strategies affected biomarker trajectories over 72 hours and whether effects on 90-

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day hospital mortality varied by baseline (time 0) biomarker profiles—both using regression models with interaction terms for treatment arms.

For all baseline biomarkers, higher concentrations were associated with increased risk of death by 90 days. However, there was no significant effect of treatment assignment on subsequent biomarker trajectories. We did find evidence for heterogeneity of treatment effect of protocol-based care on mortality for patients with different baseline [IL-6] and [IL-6]•[IL-10] profiles wherein patients with the lowest quartiles fared better with protocol-based care (Odds Ratios 0.32 [0.13, 0.75] $p=0.01$ and 0.32 [0.14, 0.73] $p=0.01$ respectively).

Conclusions—In patients with septic shock, alterations in inflammation, coagulation, oxidative stress and tissue hypoxia are common and associated with adverse outcomes but are not influenced by protocol-based resuscitation compared to usual care. However, contrary to expectation, protocol-based resuscitation appeared to be superior in patients with lower concentrations of inflammatory biomarkers. The mechanisms responsible for this effect are unclear.

Keywords

Sepsis; septic shock; resuscitation; early goal-directed therapy; biomarkers; cytokines; lactate; oxidative stress; coagulation

Septic shock, hypotension or hypoperfusion secondary to infection, remains a difficult clinical problem with high morbidity and mortality. Indeed 90-day mortality ranged from 19–32% in recent trials (1–3). The mechanisms for tissue injury, organ failure and death in septic shock are not fully understood but appear to include inflammation, tissue hypoxia, oxidative stress and coagulation/thrombosis (4–6). Along with antibiotics and source-control, resuscitation is the main therapy for septic shock. Shock resuscitation involves the restoration of tissue perfusion, oxygen delivery and stabilization of hemodynamics. Shock resuscitation involves intravenous fluids and vasopressors but there is controversy as to whether additional measures such as vasodilators, inotropes, and blood transfusions are also helpful. Resuscitation is expected to modify some or all of the underlying pathophysiology of septic shock and therefore differences in resuscitation protocols will likely result in differences in the manifestations of inflammation, tissue hypoxia, oxidative stress and coagulation.

Protocol-based Care for Early Septic Shock (ProCESS) was a 31 center, randomized controlled trial that found no differences across alternative resuscitation strategies for septic shock on 60-, 90- or 365-day all cause hospital mortality (1). A separate aim of this study was to determine if the interventions affected a number of biomarkers known to be associated with pathophysiologic mechanisms of sepsis or whether there were differences in effect across patients with different baseline biomarker profiles. We analyzed the effects of alternative resuscitation strategies on biomarker trajectories and on 90-day all cause hospital mortality based on baseline biomarker profiles.

Methods

Study design

Details of the ProCESS trial have been published (1). Briefly, ProCESS was a multicenter, randomized clinical trial testing alternative resuscitation strategies for patients presenting to emergency departments with septic shock. Eligible patients had suspected infection plus hypotension, hyperlactemia or both after an initial fluid bolus. One strategy, known as early goal-directed therapy (EGDT) (7), targeted fluids, vasoactive medication and blood transfusions to central venous oxygen saturation. Another strategy, termed protocol-based standard therapy (PST), used a simpler structured approach based on blood pressure and heart rate and the clinical assessment by the study team. The third arm of the trial was 'usual care', in which the clinical providers, not the study team, directed all care, with the study coordinator collecting data but not prompting any actions. As reported (1) these three strategies resulted in very different use of fluids, vasopressors, and blood transfusions.

Patients and study procedures

In ProCESS, we randomized 1341 patients 1:1:1 to EGDT, PST or usual-care within 2 hours of meeting entry criteria. The study protocol was approved by the University of Pittsburgh Investigational Review Board and all patients, or their legally authorized representatives, provided written informed consent. In this ancillary analysis we included 628 patients and collected blood and urine samples at baseline and 6, 24 and 72 hours after initiation of study treatment. The biomarker subset included patients from all participating sites and were enrolled in two time periods corresponding to the first and second half of the trial. In order to detect differences of 10–20% across various biomarkers (assuming 80% power) we intended to include at least 600 patients.

Laboratory analyses

We measured a panel of biomarkers representing four pathophysiologic domains: inflammation, coagulation, oxidative stress and tissue hypoxia. Each domain included a primary marker and up to 2 secondary markers. For *inflammation* we measured interleukin(IL)-6 (primary), tumor necrosis factor (TNF), and IL-10. For *coagulation* we measured thrombin-anti-thrombin complex (TAT) (primary) and D-dimers. For oxidative stress we measured urine isoprostane and for tissue hypoxia we measured lactate. We measured IL-6, IL-10, and TNF using an enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN). For D-dimers we used a latex agglutination assay (Diagnostica Stago, Parsippany, NJ) and for TAT we used an ELISA (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). We measured isoprostane in the urine using ELISA, (Oxford Biomedical, Oxford, UK) and plasma lactate was analyzed (enzymatic) using a DXC800 analyzer (Beckman Coulter, Brea CA). All assays were performed according to manufacturer specifications.

Outcome measures

To examine the effect of treatment on biologic pathway, we used each biomarker trajectory (log-transformed) as an outcome. For analyses of clinical outcomes our primary outcome was all-cause mortality 90 days after enrollment.

Statistical analysis

Characteristics of patients at baseline in the overall ProCESS cohort and in the biomarker subset were compared using means and standard deviations for continuous variables and counts and percentages for categorical variables. Linear and non-linear univariable associations with 90-day mortality were examined using the log transformed quartile biomarker ranking as class level and continuous predictors in a logistic regression model. The predictive ability of individual or combinations of markers (at baseline and 24 hours) with mortality were assessed using the area under the receiver operating characteristic area under the curve (ROC-AUC) using logistic regression based stepwise selection based models and (8) methods for comparing ROC-AUCs. We also created a parsimonious clinical regression model of baseline clinical covariates and added the biomarkers to see if they increased the predictive ability.

We analyzed the effects of alternative resuscitation strategies on biomarker trajectories using a linear mixed effects Tobit model (LMET) that accounts for left and right censoring due to assay sensitivity, and assumes missingness at random which is consistent with the intention to treat paradigm used for these analyses. We tested each outcome for differences sequentially by testing first whether protocol-based resuscitation (EGDT and PST pooled) was superior to usual care, and if so whether EGDT was superior to PST over time in an LMET interaction model of treatment with time. In separate sensitivity analyses we also fit a LMET interaction model after including the baseline biomarker level as a fixed covariate to assess whether baseline levels influenced the results of the aforementioned trajectory analyses.

Heterogeneity of treatment effects by baseline biomarker concentration on 90-day mortality was investigated using logistic regression based models with interaction between log-transformed biomarker quartile indicators and treatment with a conservative Bonferroni adjustment for multiple testing. Given the multiple comparisons involved and risk for false discovery we confined our analysis to only those markers or marker combinations that showed heterogeneity of treatment effect with p-values ≤ 0.01 . All statistical analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC).

Results

Patients

We measured biomarkers in 628 subjects (46.8%) enrolled in ProCESS. A comparison of baseline characteristics and outcomes between the overall ProCESS cohort and the Biomarker subset are shown in Table 1. The Biomarker subset was virtually identical to the overall ProCESS cohort. There was slightly greater minority representation but the distribution of underlying chronic conditions and sources of infection were very similar.

Likewise, severity of illness was the same by APACHE II score 21 ± 7.6 vs. 21 ± 7.8 and by the proportion with positive blood cultures (29.5% vs. 29.3%).

Baseline biomarker profiles and 90-day mortality

At baseline (on enrollment and prior to implementation of study treatment) median concentrations for all 7 biomarkers were elevated compared to normal ranges reported for each assay (as provided by the manufacturer) and in general were at their highest for the 72 hours we observed them (Figure 1). For example, plasma IL-6 concentrations were about 400–1100 pg/ml (6–7 Ln) at baseline decreasing to about 55 pg/ml (4 Ln) at 72 hours.

We observed higher mortality as biomarker concentrations were increased at baseline and these relationships were seen for all biomarkers (Table 2A). Despite these associations, none of the markers discriminated well for mortality on their own (AUCs <0.65). We next combined markers together to determine if a multi-marker panel would provide better prediction of mortality. We excluded urine isoprostane because of the smaller sample size for this marker. Even a full model including all six remaining biomarkers (Table S1) achieved an AUC of only 0.68 and was not very different from IL-6 or lactate alone as shown in table 2A. Overall, lactate was found to be the best performing single marker (highest score and AUC) and was not different from a model containing all biomarkers. This result was confirmed using a stepwise variable selection model with entry criteria of $p=0.2$ and retention criteria of 0.05. We also found no significant improvement in AUROC's when biomarkers were added to a parsimonious clinical regression model of baseline clinical covariates.

Biomarker profiles over time and by treatment arm

Median biomarker concentrations, except D-Dimer, were significantly lower at 24 hours (Figure 1) ($p<0.0001$ for all markers). Most markers were also associated with mortality when their 24 hour values were considered (Table 2B), exceptions being D-dimers and urine isoprostane. Median concentrations at each time point for all seven biomarkers across four domains grouped by treatment arm are shown in Figure 1. Although all biomarker concentrations decreased over time, the patterns observed were not different across treatment arms, as assessed by a lack of any significant time-by-treatment interaction terms in the model.

Heterogeneity of treatment effect on mortality by baseline biomarker concentrations

We next explored differences in treatment effect (protocol-based care vs. usual care) on 90-day all cause mortality stratified by baseline biomarker quartiles. We found limited evidence of heterogeneity of treatment effect for these biomarker-defined subgroups with significant interactions between treatment and baseline biomarkers: [IL-6] ($p=0.01$), [IL-10] ($p=0.04$), [IL-6]•[IL-10] ($p=0.01$), and [IL-6]•[IL-10]•[TNF] ($p=0.03$). Comparisons of treatment effect (odds ratios) for protocol-based care vs. usual care by log biomarker quartile are shown in Figure 2. For both [IL-6] and [IL-6]•[IL-10], protocol-based care was superior to usual care for patients with low baseline biomarkers (1st quartile) whereas no effect was seen for other patients. These results were consistent when individual protocols (EGDT and PST) were each compared to usual care and no differences were seen between EGDT and PST

(Figure S1). Given these results we also repeated the same analysis for [TNF] but found no effect (Figure S2).

Discussion

If resuscitation is effective, its efficacy probably stems from interruption of potentially injurious pathophysiological mechanisms prior to the onset of irreversible organ damage. If so, it is reasonable to hypothesize that more effective resuscitation strategies should induce larger salutary changes in markers associated with these pathways, and result in greater benefit in patients with more severe derangements. For example, Dorrenstein showed that simple fluid administration in humans challenged with endotoxin can attenuate the inflammatory response in as early as 3 hours (9).

Although the precise pathways by which sepsis causes organ injury are not completely understood, infection triggers a complex inflammatory response in the host where both pro- and anti-inflammatory mediators are released. As part of the innate immune response, pro-inflammatory mediators orchestrate leukocyte trafficking and clearance of infection but may also cause tissue damage. Conversely, anti-inflammatory mediators check the pro-inflammatory response but can lead to alterations in immune function that impair the host response (4). We have previously shown that septic patients with higher circulating concentrations of both pro- and anti-inflammatory cytokines (namely, IL-6 and IL-10) have far worse survival compared to other patients even after controlling for baseline severity (5). Sepsis also leads to activation of intravascular coagulation and thrombosis pathways that may lead to organ injury. Both pathogen associated molecules like endotoxin and endogenous mediators such as TNF initiate coagulation through the induction of tissue factor expression, primarily on monocyte/macrophages and endothelial cells (10). Markers of thrombin generation, TAT and F1.2, were present in 95.5% and 77.5% of subjects at enrolment into PROWESS, a large study of rhaPC, and their corresponding blood concentrations over time were significantly higher in non-survivors (11, 12). Thus, microvascular thrombosis, may lead to tissue injury.

Arterial vasodilation, increased microvascular permeability, impaired left ventricular performance (acute cardiomyopathy) are also part of the septic shock syndrome. Unless rapidly corrected, the cumulative effects of these phenomena on tissue perfusion results in cellular hypoxia inevitably leading to cell dysfunction or death. Furthermore, during reperfusion, O₂ becomes available generating superoxide radical anion (O₂^{•-}) and other reactive oxygen species via injured mitochondria and other mechanisms (13). This “oxidative stress” may further damage cells and lead to organ injury and dysfunction. Importantly, these various pathophysiologic mechanisms are not mutually exclusive and interact in a complex manner, with numerous feedback loops (4).

Our expectation was that different resuscitation strategies would result in different rates of reversal of one or more of these pathways as evidenced by changes in circulating biomarkers linked to each pathway. Faster or more complete reversal of the underlying pathophysiologic process should lead to better survival. Evidence that these biomarkers and the pathways they represent, are associated with survival can be seen in table 2. Abnormalities in baseline

concentrations of all seven biomarkers, representing four pathways, were strongly associated with worse survival (Table 2a). For example, patients presenting with the highest quartile of any biomarker compared to those with the lowest, were 1.9 to 4.47 times as likely to die before day 90.

Despite the relationship between the extent of pathophysiology measured by each biomarker and 90 day survival, the different resuscitation strategies examined in the ProCESS trial did not result in different biomarker trajectories (Figure 1). Given that these strategies also failed to result in differences in survival (1) we might reasonably conclude that these interventions lacked differences in efficacy on these very same pathophysiological targets. One limitation to our analysis is that EGDT is a complex protocol and we could not dissect out the effects of individual components that could, theoretically, have opposing effects. Indeed we cannot address whether resuscitation affects inflammation, as it almost certainly does. It would not have been ethical to conduct a trial with a substandard resuscitation group. Instead, our study asks whether the differences in resuscitation practices under study in the ProCESS trial affected inflammation and other aspects of sepsis physiology. Furthermore, numerous ancillary therapies such as types of antibiotics, types and doses of vasopressors, and use of corticosteroids all have the potential to impact one or more of the physiologic pathways under study. We avoided post-hoc and post-randomization subgroup analyses instead leveraging randomization in the trial to control for such effects since their distribution should be random.

It is also notable that in the first trial of EGDT (7), a subsequent report also investigated the effect of different resuscitation strategies on inflammation (14). Although the authors did not report on IL-6 or IL-10, TNF resolved faster with EGDT compared to control ($p=0.03$). Thus, in a randomized trial finding differences in survival with differing resuscitation strategies, differences in biomarkers were also seen while in ProCESS neither signal was apparent.

A simple explanation for these differing results could be that more severely inflamed patients were enrolled in the earlier trial compared to our study. Such an explanation could serve to reconcile both differences in clinical and biochemical outcomes. If this scenario were true, patients with more severe abnormalities in inflammation in the ProCESS trial would benefit from protocol-based care whereas lack of benefit or even harm might be seen in patients with less severe abnormalities. However, our results do not support this hypothesis. We examined this question by comparing treatment effects across quartiles of [IL-6], [IL-6]•[IL-10] and [TNF]. As can be seen in figure 2 (and also broken out by all three groups in supplemental figure S1) protocol-based care was not superior to usual care in patients with higher concentrations of inflammatory biomarkers. In fact, our analysis suggests that the opposite is true—any potential benefit from protocol-based resuscitation was confined to those patients with the lowest concentrations of biomarkers. We caution however, that although these results are statistically significant, our *a priori* hypothesis was that protocol-based resuscitation would benefit patients with higher (not lower) levels of inflammation and our results do not permit us to reject the null hypothesis.

Finally, it should be stated that multiple differences in patient care likely occurred between the ProCESS trial (1) and the first description of EGDT (7)—as noted by many commentators (15) and by simple fact that overall survival has markedly improved over the intervening years (2, 3, 16). While EGDT itself may have been similar, both the care of control patients and background care for all patients may have been different. Such differences, while entirely expected between trials conducted nearly 15 years apart, may account for some of the differences in outcomes.

In conclusion, in a subset of 628 patients enrolled in a large multicenter randomized trial, protocol-based resuscitation did not result in faster resolution of any of the indicators of four pathophysiologic mechanisms of sepsis-associated organ injury. Furthermore, protocol-based resuscitation was not any more effective for patients with extreme derangements in pathologic indicators even though these indicators were associated with decreased survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Median concentrations of biomarkers over time stratified by treatment arm
A: IL-6; B: IL-10; C: TNF; D: TAT; E: D-dimer; F: Lactate; G: urine Isoprostane.

Figure 2. Treatment effect by baseline biomarker profiles. Top Panel: Quartiles of log [IL-6]. Bottom Panel: Quartiles of log [IL-6]*log [IL-10]
 Protocol-based care (both EGDT and PST arms combined). Protocol-based care resulted in lower OR for mortality as baseline [IL-6] decreased (omnibus text for interaction ($p=0.002$);

the same was seen for [IL-6]•[IL-10] ($p=0.01$). Treatment differences were observed in the lowest quartiles (Q1) of [IL-6] ($p=0.01$) or [IL-6]•[IL-10] ($p=0.01$).

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Table 1Characteristics of patients at baseline in ProCESS and biomarker subset^a

Characteristic	ProCESS (N=1341)	ProCESS Biomarkers (N=628)
Age – year ^b	61±16.1	60±15.9
Male sex	748 (55.8)	366 (58.8)
Race		
White	916 (68.3)	433 (69.6)
Black or African American	333 (24.8)	140(22.5)
Asian	26 (1.9)	15(2.41)
Other	66 (4.9)	34(5.47)
Ethnicity ^c		
Non-Hispanic	1196 (89.3)	538 (86.5)
Hispanic	143 (10.7)	84 (13.5)
Domicile prior to admission ^d		
Non-nursing home	1128 (84.4)	536 (86.5)
Nursing home ^e	209 (15.6)	84 (13.6)
Chronic conditions ^f		
Charlson comorbidity score	2.7±2.6	2.7± 2.6
Hypertension	789 (58.8)	372(59.8)
Diabetes mellitus	458 (34.2)	228 (36.7)
Chronic respiratory disease	298 (22.2)	144 (23.3)
Cancer	234 (17.5)	112 (18.0)
Renal impairment	213 (15.9)	90 (14.5)
Congestive heart failure	161 (12.0)	80 (12.9)
Prior myocardial infarction	143 (10.7)	67 (10.7)
Cerebral vascular disease	126 (9.4)	65 (10.5)
Peripheral vascular disease	110 (8.2)	50 (8.0)
Chronic dementia	100 (7.5)	44 (7.1)
Hepatic cirrhosis	87 (6.5)	38 (6.1)
Peptic ulcer disease	72 (5.4)	30 (4.8)
AIDS and related syndromes	38 (2.8)	16 (2.6)
Source of sepsis		
Pneumonia	443 (33.0)	212 (34.1)
Urosepsis	284 (21.2)	142 (22.8)
Intra-abdominal infection	177 (13.2)	68 (10.9)
Infected, source unknown	170 (12.7)	82 (13.2)
Skin and soft-tissue infections	96 (7.2)	43 (6.9)
Catheter-related infection	38 (2.8)	18 (2.9)
Central nervous system	10 (0.8)	5 (0.8)

Characteristic	ProCESS (N=1341)	ProCESS Biomarkers (N=628)
Endocarditis	7 (0.5)	4 (0.6)
Other	85 (6.3)	37 (6.0)
Considered after review not to be infected	31 (2.3)	11(1.8)
Blood culture positive	396 (29.5)	182 (29.3)
APACHE II score	21±7.6	21±7.8
Entry criteria		
Refractory hypotension	727 (54.2)	359 (57.7)
Hyperlactatemia	800 (60.0)	355 (57.1)
Physiological variables		
Systolic blood pressure - mmHg	100.7±28.8	102±29.7
Serum lactate – mmol/L	4.9±3.2	4.7±3.1
Time to randomization ^g		
From ED arrival - min	187±109	189±103
From meeting entry criteria - min	69±56	70±40

^aValues indicated with ± are means ± SD. Values indicated with N (n) are number of subjects (%).

^bExcludes one subject with missing age.

^cExcludes two subjects with missing ethnicity.

^dExcludes four subjects with missing domicile prior to admission.

^eNursing home population includes personal care homes, skilled or unskilled assisted living, or extended care facilities.

^fChronic conditions defined as per Charlson comorbidity index.^{20, 21}

^gNot all subjects were eligible at time of ED arrival.

Table 2A

Odds Ratios for 90-Day Mortality by Baseline Biomarker Quartiles

Biomarker	Quartile 2	Quartile 3	Quartile 4	P-value	AUC
IL-6	2.42 (1.41–4.16)	1.83 (1.06–3.18)	2.67 (1.56–4.57)	0.002 ^G	0.60
IL-10	2.12 (1.11–4.03)	1.40 (0.88–2.20)	1.90 (1.19–2.90)	0.011 ^T	0.56
TNF	.	1.19 (0.59–2.41)	2.19 (1.48–3.25)	0.0002 ^T	0.58
TAT	1.34 (0.79–2.28)	1.87 (1.12–3.13)	1.95 (1.17–3.25)	0.005 ^T	0.57
D-Dimer	.	1.32 (0.84–2.08)	2.20 (1.41–3.43)	0.001 ^T	0.57
Urine Isoprostane	2.04 (0.88–4.72)	0.98 (0.40–2.41)	2.96 (1.29–6.77)	0.02 ^G	0.62
Lactate	1.76 (1.00–3.10)	1.99 (1.14–3.48)	4.47 (2.62–7.63)	<0.0001 ^T	0.64

Table 2B

Odds Ratios for 90-Day Mortality by 24 hour Biomarker Quartiles

Biomarker	Quartile 2	Quartile 3	Quartile 4	P-value	AUC
IL-6	2.18 (1.16–4.07)	2.25 (1.24–4.09)	4.56 (2.58–8.07)	<0.0001 ^T	0.64
IL-10	1.00 (0.60–1.66)	.	2.76 (1.61–4.73)	<0.0001 ^T	0.60
TNF	0.83 (0.52–1.33)	.	1.60 (0.91–2.79)	0.03 ^G	0.56
TAT	0.66 (0.26–1.19)	1.07 (0.62–1.86)	2.12 (1.26–3.58)	0.0003 ^G	0.61
D-Dimer	.	1.25 (0.78–2.01)	1.49 (0.91–2.42)	0.25 ^G	0.54
Urine Isoprostane	0.98 (0.41–2.35)	0.88 (0.36–2.15)	2.08 (0.92–4.71)	0.13 ^G	0.59
Lactate	1.55 (0.87–2.78)	0.95 (0.52–1.72)	3.24 (1.92–5.45)	<0.0001 ^T	0.63

Shown are Odds Ratios and 95% Confidence Intervals. Quartile 1 was used as reference in both models. Test of trend^T across quartiles reported if significant and model lack of fit test > 0.05 otherwise a type III test of general quartile association with endpoint^G was reported. AUC, area under the receiver operating characteristic curve. Empty cells represent overlapping quartiles.