The influence of early hemodynamic optimization on biomarker patterns of severe sepsis and septic shock*

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Background: **Despite abundant experimental studies of biomarker patterns in early severe sepsis and septic shock, human data are few. Further, the impact of the severity of global tissue hypoxia resulting from resuscitative strategies on these early biomarker patterns remains unknown.**

Methods: **The temporal patterns of interleukin-1 receptor antagonist, intercellular adhesion molecule-1, tumor necrosis factor-α, caspase-3, and interleukin-8 were serially examined over the first 72 hrs of hospitalization after early hemodynamic optimization strategies of early goal-directed vs. standard therapy for severe sepsis and septic shock patients. The relationship of these biomarker patterns to each hemodynamic optimization strategy, severity of global tissue hypoxia (reflected by lactate and central venous oxygen saturation), organ dysfunction, and mortality were examined.**

Results: **Abnormal biomarker levels were present upon hospital presentation and modulated to distinct patterns within 3 hrs based on the hemodynamic optimization strategy. The temporal expression of these patterns over 72 hrs was significantly associated with the severity of global tissue hypoxia, organ dysfunction, and mortality.**

Conclusion: **In early severe sepsis and septic shock, within the first 3 hrs of hospital presentation, distinct biomarker patterns emerge in response to hemodynamic optimization strategies. A significant association exists between temporal biomarker patterns in the first 72 hrs, severity of global tissue hypoxia, organ dysfunction, and mortality. These findings identify global tissue hypoxia as an important contributor to the early inflammatory response and support the role of hemodynamic optimization in supplementing other established therapies during this diagnostic and therapeutic "window of opportunity." (Crit Care Med 2007; 35:2016–2024)**

KEY WORDS: biomarkers; cytokines; early goal-directed therapy; inflammatory biomarkers; inflammation; hemodynamic optimization; lactate; multisystem organ failure; mortality; resuscitation; sepsis; severe sepsis; septic shock; venous oxygen saturation

Sepsis begins when an infectious agent stimulates the expression of proinflammatory, anti-inflammatory, and apoptotic biomarkers, leading a systemic inflammatory response. The transition to severe sepsis and septic shock is accompanied by imbalances between systemic oxygen supply and demand, which result in varying degrees of perfusion deficits (global tissue hypoxia), including overt circulatory shock (1). Global tissue hypoxia is both a product and a stimulus of this systemic inflammatory response (2). Biomarkers that represent the proinflammatory, anti-inflammatory, endothelial, and apoptotic aspects of systemic inflammation are interleukin-1 receptor antagonist (IL-1ra) (3), intercellular adhesion molecule-1 (ICAM-1) (4), tumor necrosis factor α (TNF-α) (5), caspase-3 (6), and interleukin-8 (IL-8) (7). Although biomarker patterns have been examined extensively in animal models and intensive care unit patients, data during the first few hours of hospital presentation are limited.

Early hemodynamic optimization strategies repeatedly have been shown

***See also p. 2206.**

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The early goal-directed therapy study was performed without industry or extramural support, and the catheters were purchased at full price from Edwards Lifesciences (Rivers E, Nguyen B, Havstad S, et al. *N Engl J Med* 2001; 345:1368–1377). A patent of central venous oximetry with modifications for its use during cardiac arrest was obtained and owned by Henry Ford Health Systems (HFHS) and listed under Dr. Rivers' name in 1994 (Rivers EP, Martin GB, Smithline H, et al. *Ann Emerg Med* 1992; 21:1094–1101). This patent was never commercialized by HFHS and was assigned to Edwards Lifesciences in return for unrestricted research funding as stated above. Dr. Rivers did not receive direct financial gain from this execution and has no intellectual properties or patents related to this research.

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to improve resuscitation end points, organ dysfunction, and mortality, and decrease health care resource consumption in patients with severe sepsis and septic shock (8–19). Because the early inflammatory response results from both infectious and hypoxic stimuli (2, 20), the relative pathogenic contribution of these two components remains unknown in the early phases. The purpose of this study is to determine whether there are associations between the magnitude and pattern of biomarker response and the: a) resuscitation strategy; b) severity of global tissue hypoxia; c) severity of organ dysfunction; and d) mortality of patients during the early phase of severe sepsis and septic shock.

MATERIALS AND METHODS

Study Design. This study is an examination of prospectively obtained biological samples during the Early Goal-Directed Therapy (EGDT) Collaborative Group therapeutic clinical study to examine the inflammatory patterns early severe sepsis and septic shock (9). These studies were conducted under the auspices of an independent, external safety and data monitoring committee with written informed consent and approved by the Henry Ford Hospital Institutional Review Board for Human Research.

Patient Population and Interventions. Eligible patients were adults presenting with severe sepsis and septic shock to the emergency department of an 850-bed, urban tertiary care facility. These patients or their surrogates consented to be randomized to receive standard or EGDT according to a previously published protocol and/or serial biomarker examinations (9).

Biomarker Samples, Physiologic Scoring, and Organ Dysfunction Measurements. Biological samples, clinical findings, and laboratory data were collected at hrs 0, 3, 6, 12, 24, 48, 60, and 72. Information required for the Acute Physiology and Chronic Health Evaluation II score (21), Simplified Acute Physiology Score II (22), Multiple Organ Dysfunction Score (23), and Sequential Organ Failure Assessment score (24) were obtained at each time point except hour 3. Patients were followed until hospital discharge.

Biomarker Immunoassays. Biomarker assays were performed independently by Biosite, San Diego, CA. Assays were performed using immunometric (sandwich) assays with NeutrAvidin-coated 384-well block microtiter plates (Pierce Biotechnology, Rockford, IL) and a Genesis RSP 200/8 Workstation (Tecan U.S., Durham, NC). Each sample was tested in duplicate. Before the assays, biotinylated primary antibody was diluted in assay buffer containing 10 mmol/L trishydroxymethylaminomethane HCl (pH 8.0), 150 mmol/L sodium chloride, 1 mmol/L magnesium chloride, 0.1 mmol/L zinc chloride, and 10 mL/L polyvinyl alcohol (9–10 kDa). The concentration of biotinylated antibody was predetermined by titration. The primary antibody (10 μL per well) was added to the plates and incubated. After washing, 10 g/L bovine serum albumin and 1 g/L sodium azide were added to the plate wells, which were then incubated at room temperature. Next, the plates were washed three times with borate-buffered saline containing 0.02% polyoxyethylene (20) sorbitan monolaurate (BBS-Tween).

For each sample, 10-μL aliquots were added to each plate well and the plates were incubated. Following this incubation, the plates were washed three times and alkaline phosphatase–conjugated antibody (10 μL per well) was added to each plate well and further incubated. The concentration of the alkaline phosphatase–conjugated antibody was predetermined to ensure a linear profile in the dynamic range of interest. After additional incubation, the plates were washed nine times with BBS-Tween. AttoPhos substrate (S1011, Promega, Madison, WI), a fluorescence-enhancing substrate previously diluted in AttoPhos buffer (S1021, Promega), was then added to aid in the measurement of the activity of antibody-conjugated alkaline phosphatase bound in each well. The plates were then scanned in a fluorometer (Tecan Spectrafluor, Tecan U.S.) using an excitation wavelength of 430 nm and an emission wavelength of 570 nm. Each well was scanned 6 times at 114-sec intervals, and the rate of fluorescence generation was calculated. Calibration curves were derived from eight points tested at multiple locations on the assay plate using a 4-parameter logistic fit, from which sample concentrations were subsequently calculated.

Each plate included calibration wells consisting of multiple analyte concentrations and

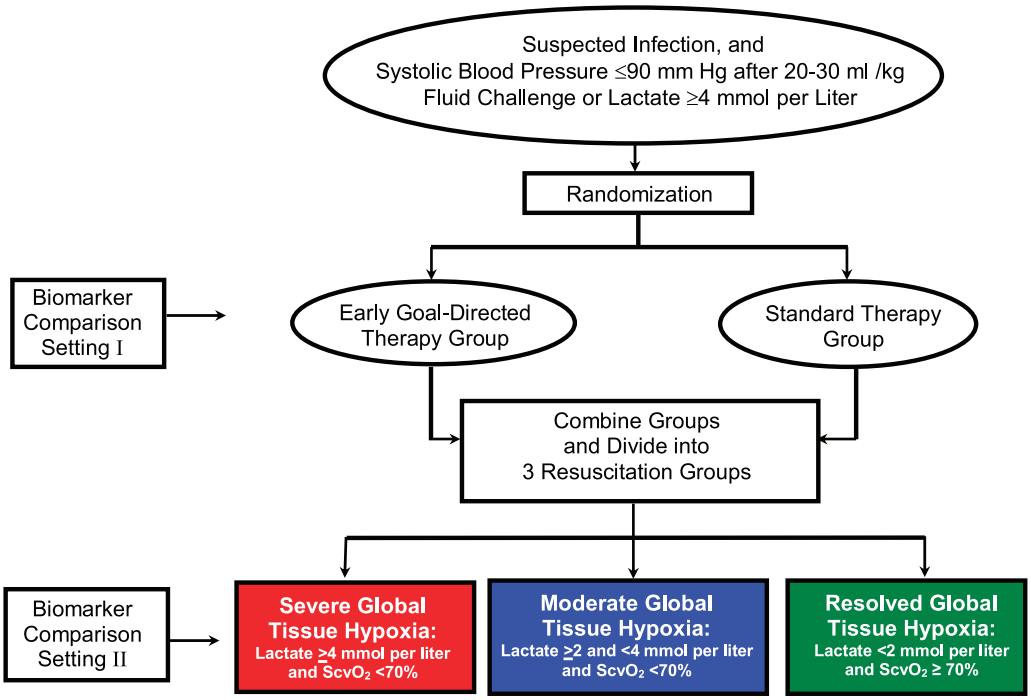

Figure 1. Study design and overview. The study algorithm compares the biomarker patterns of the early goal-directed and standard therapy groups (biomarker setting I) and groupings of global tissue hypoxia severity (biomarker setting II).

control samples. Calibration curves for each biomarker assay were generated for IL-1ra (150–30,000 pg/mL), ICAM-1 (2.5–900 ng/mL), TNF-α (20–2,000 pg/mL), caspase-3 (0.1–200 ng/mL), and IL-8 (15–3,000 pg/mL). Assay results were sent to the Henry Ford Hospital Department of Biostatistics and Epidemiology, which maintained the database and performed the statistical analyses independent of the study investigators.

Biomarker Response Comparison Group Methods. Biomarker patterns initially were compared between the standard and EGDT groups (comparison setting I, Fig. 1). Then, biomarker patterns were compared after stratification of all patients into three groups based on the severity of global tissue hypoxia at each time point (comparison setting II, Fig. 1). These groups represent the range from a state of oxygen supply dependency or hypodynamic to a normodynamic and hyperdynamic state. These three groups were subdivided into *severe global tissue hypoxia* (lactate level ≥4 mmol/L and central venous oxyhemoglobin saturation [$Scvo_2$] <70%), *moderate global tissue hypoxia* (lactate level, ≥2 to <4.0 mmol/L, and $Scvo_2$, <70%), and *resuscitated group* or *resolved global tissue hypoxia* (lactate level, <2 mmol/L, and $Scvo_2$, ≥70%) (11, 25–27).

Statistical Methods. The two-sample Student's *t*-test, Wilcoxon's rank-sum test, and chi-square test were employed to compare demographic, baseline clinical data, and organ dysfunction scores between the EGDT and the standard therapy patients. Two-way (one grouping factor and one repeated measures factor) analysis of covariance was used to examine overall biomarker differences between EGDT and standard therapy patients within specific time intervals of interest (3–72 hrs, 6–72 hrs, and 12–72 hrs), controlling for hour-0 mediator concentrations. To account for non-gaussian distributions, logarithmic transformations were performed on biomarker concentrations before executing the repeated measures analysis of covariance, with $p < .05$ accepted as statistically significant. The Kruskal-Wallis test was used to compare mediator concentrations at all individual time points among the three resuscitation groups, again controlling for the multiple testing by using the Bonferroni method of multiple comparison adjustment to reduce significance level for each test to $p < .006$.

Spearman's rank-correlation test (ρ) with the Bonferroni method of significance level adjustment was used to examine all individual time point correlations between inflammatory biomarker concentrations, resuscitation end points, and organ dysfunction scores, when appropriate. For each inflammatory mediator, repeated measures analysis of variance was used to test for overall differences between hospital survivors and nonsurvivors in a simultaneous evaluation of all nine time points, again using logarithmic transformations to account for non-gaussian distributions. The Kruskal-Wallis statistic was used to compare the baseline biomarker concentrations between patients who received vs. did not receive vasopressor support, red blood cell transfusions, and dobutamine therapy. Except as noted above, a two-tailed probability level of $p < .05$ was statistically significant.

Table 1. Demographic, baseline physiologic, and clinical data for the standard and early goal-directed therapy (EGDT) groups[a]

	Standard Therapy	Early Goal-Directed Therapy
No. of patients	119	124
Demographics		
Age, yrs	64 ± 17	68 ± 17
Sex, % male	50	50.8
Study entry time		
Emergency department arrival to study entry, hrs	1.5 ± 1.9	1.3 ± 1.5
Entry criteria variables		
Temperature, °C	36.6 ± 2.2	35.9 ± 3.2
Heart rate, beats/min	115 ± 27	117 ± 32
Systolic blood pressure, mm Hg	109 ± 35	107 ± 36
Respiratory rate, breaths/min	30 ± 11	32 ± 11
$Paco_2$, mm Hg	31 ± 16	32 ± 16
White blood cell count, K/μL	14.3 ± 9.9	13.8 ± 8.4
Blood lactate, mmol/L	6.9 ± 4.5	7.8 ± 4.7
Selected baseline laboratory tests		
Serum anion gap, mEq/L	22 ± 9	22 ± 7
Serum creatinine, mg/dL	2.6 ± 2.1	2.6 ± 2.0
Serum urea nitrogen, mg/dL	45 ± 33	46 ± 31
Serum glucose, mg/dL	240 ± 295	293 ± 368
Serum total bilirubin, mg/dL	1.7 ± 2.4	1.2 ± 1.7
Serum γ-glutamyl transpeptidase, U/L	113 ± 220	118 ± 160
Serum albumin, g/dL	2.9 ± 0.7	2.9 ± 0.7
Arterial blood pH	7.32 ± 0.19	7.30 ± 0.17
Central venous oxygen saturation, %	49.5 ± 14	48.6 ± 12
Sepsis definitions and categories, %		
Severe sepsis without shock	47	45
Septic shock	53	55
Culture positive—all cultures	77	75
Positive blood culture	36	35
Antibiotic therapy		
Antibiotics in first 6 hrs, %	92	89
Adequate antibiotics, %	94	97
Duration, days	12.8 ± 18.8	11.1 ± 15.9
Baseline physiologic or organ dysfunction scores		
APACHE II score	20 ± 8	22 ± 7
SAPS II	48 ± 11	51 ± 11
MODS	7 ± 3	8 ± 3
SOFA score	7 ± 3	6 ± 3
Pao_2/Fio_2 ratio, mm Hg	304 ± 172	287 ± 157

APACHE, Acute Physiology and Chronic Health Evaluation; SAPS, Simplified Acute Physiology Score; MODS, Multiple Organ Dysfunction Score; SOFA, Sequential Organ Failure Assessment.

[a]Values are mean ± SD unless otherwise indicated. There was no significant difference between standard care and EGDT for all variables. The values in this table will differ from the original study of EGDT as comparison settings I and II reflect a subset analysis of this study (9).

RESULTS

There were no statistically significant differences among the demographic, baseline clinical data, antibiotic therapy, or baseline organ dysfunction scores between the standard and EGDT groups (Table 1). There were no significant differences in hour-0 biomarker concentrations between the standard and EGDT groups (comparison setting I, Fig. 2*A*). EGDT resulted in lower levels of IL-1ra ($p = .026$) and ICAM-1 ($p = .033$) from 3 hrs to 72 hrs, TNF-α ($p = .031$) and caspase-3 ($p = .024$) from 6 hrs to 72 hrs, and IL-8 ($p = .049$) from 12 hrs to 72 hrs (Fig. 2*A*). The peak biomarker concentrations also were significantly lower in the EGDT compared with the control resuscitation group over 72 hrs (Table 2).

There were no significant differences in baseline biomarker concentrations among the three patient groups stratified by tissue hypoxia (comparison setting II, Fig. 2*B*). The most severe global tissue hypoxia group had significantly higher concentrations than the moderate global

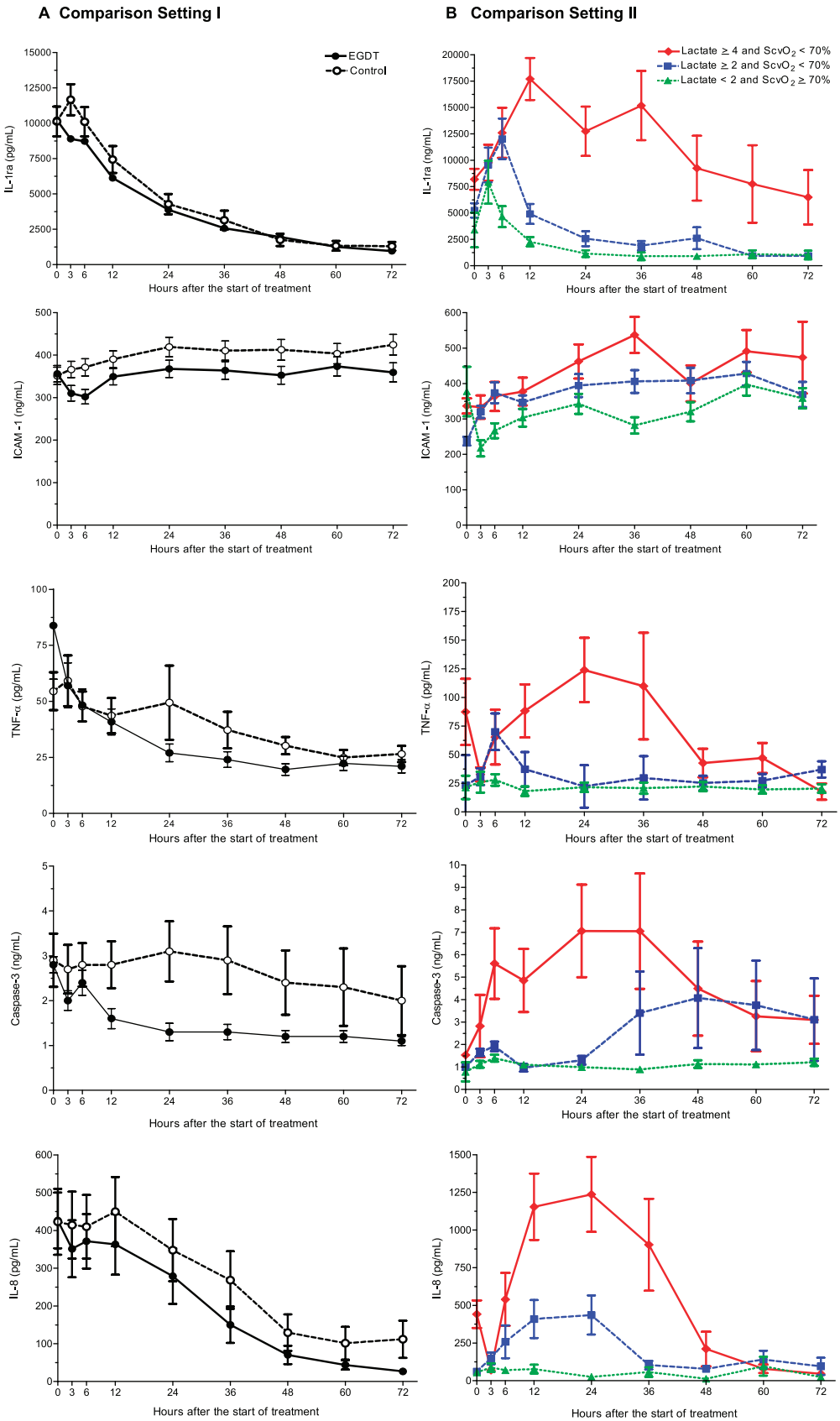

Figure 2. *A*, comparison setting I of biomarker patterns between the standard and early goal-directed therapy (*EGDT*) resuscitation groups for the first 72 hrs. *Open circles* represent standard therapy patients and *closed circles* represent EGDT patients. Data are shown as mean values and standard deviations. There were no significant differences in hour-0 biomarker concentrations between the standard and EGDT groups. Statistically significant group differences were detected for interleukin (*IL*)-1ra ($p = .026$) from 0–72 hrs; intercellular adhesion molecule (*ICAM*)-1 ($p = .033$) from 3–72 hrs; tumor necrosis factor

Table 2. Peak biomarker concentrations during 72 hrs in comparison settings I and II

Biomarker	Comparison Setting I[a]		Comparison Setting II[a]		
	Control	EGDT	Lactate ≥4 mmol/L and $Scvo_2$ <70%	Lactate ≥2 mmol/L to <4.0 mmol/L and $Scvo_2$ <70%	Lactate <2 mmol/L and $Scvo_2$ ≥70%
IL-1ra, pg/mL	11,668.7 ± 11,433.5	8,903.5 ± 10,668.3	17,695.2 ± 11,072.2	12,021.4 ± 12,558.6	7,908.6 ± 8,382.7
ICAM-1, ng/mL	424.64 ± 227.02	373.51 ± 221.68	537.22 ± 197.4	427.95 ± 214.45	396.91 ± 208.38
TNF-α, pg/mL	59.2 ± 119.1	57.1 ± 110.9	123.87 ± 143.12	69.9 ± 104.75	27.88 ± 31.78
Caspase-3, ng/mL	3.1 ± 6.9	2.4 ± 3.1	7.06 ± 10.52	4.07 ± 13.16	1.39 ± 0.98
IL-8, pg/mL	449.64 ± 892.78	371.30 ± 740.90	1236.98 ± 1269.31	435.82 ± 964.55	96.09 ± 418.24

EGDT, early goal-directed therapy; IL, interleukin; ra, receptor antagonist; ICAM, intercellular adhesion molecule; TNF, tumor necrosis factor.
[a]Peak concentration after hour 0.

tissue hypoxia or resolved global tissue hypoxia groups at one or more time points for all of the biomarkers. The $p < .006$ level of significance was attained at 12 hrs through 72 hrs for IL-1ra, 36 hrs for ICAM-1, 12 hrs for TNF-α, 12 hrs and 36 hrs for caspase-3, and at 12 hrs through 48 hrs for IL-8. Peak concentrations of biomarkers also were significantly higher in the severe global tissue hypoxia groups compared with the moderate and resolved global tissue hypoxia groups over 72 hrs (Table 2).

With both treatment groups combined, there were significant relationships between biomarker concentrations and organ dysfunction scores at each time point. The maximum ρ between biomarker concentration and Acute Physiology and Chronic Health Evaluation II, Simplified Acute Physiology Score II, and Multiple Organ Dysfunction Score, respectively, occurred at the same time point for each biomarker, all $p < .003$. The maximum ρ with IL-1ra was 0.55, 0.45, and 0.62 at 36 hrs, respectively. The maximum ρ with ICAM-1 was 0.30, 0.22, and 0.52 at 72 hrs, respectively. The maximum ρ with TNF-α was 0.22, 0.24, and 0.28 at 6 hrs, respectively The maximum ρ with caspase-3 was 0.29, 0.30, and 0.47 at 12 hrs, respectively. The maximum ρ with IL-8 was 0.40, 0.35, and 0.56 at 36 hrs, respectively. The Sequential Organ Failure Assessment score performed differently. The maximal ρ (0.68 for IL-1ra, 0.51 for ICAM-1, 0.33 for TNF-α, 0.41 for caspase-3, and 0.53 for IL-8) between the Sequential Organ Failure Assessment score and biomarkers was at hrs 24, 36, 12, 12, and 12, respectively, all $p < .001$. Overall, the Sequential Organ Failure Assessment score and Multiple Organ Dysfunction Score demonstrated the highest ρ to individual biomarker concentrations. Mean biomarker levels over 72 hrs were significantly greater in hospital nonsurvivors than survivors for all examined (IL-1ra, $p < .001$; ICAM-1, $p = .001$; TNF-α, $p = .007$; caspase-3, $p < .001$; IL-8, $p < .001$).

In all treatment groups, patients who received vasopressor support over the first 72 hrs had significantly higher baseline IL-1ra ($p = .010$) and IL-8 ($p = .002$) than those who did not. Similarly, patients who received red blood cell transfusions had significantly higher baseline IL-1ra ($p = .031$) and IL-8 ($p = .042$) concentrations than those who did not.

DISCUSSION

The transition from severe sepsis to septic shock is accompanied by circulatory insufficiency ranging from oxygen supply dependency or a hypodynamic state to a hyperdynamic state. This state depends on the stage of disease presentation, host cardiovascular reserve, extent of hemodynamic optimization provided, and other factors (11, 28, 29). It is a prevailing hypothesis that the persistence of oxygen supply dependency leads to global tissue hypoxia, accumulation of oxygen debt, inflammation, organ dysfunction, and increased mortality (20, 27, 30–32). It is from this hypothesis that early titrated hemodynamic optimization is one of the integral components in altering the pathogenesis and outcomes of this disease.

This study examined the temporal evolution of biomarker activity in the more proximal aspects of disease presentation, which is a distinguishing feature compared with previous studies (33). Significant decreases in biomarker levels were observed as early as 3 hrs for IL-1ra and ICAM-1, 6 hrs for TNF-α and caspase-3, and 12 hrs for IL-8 as a result of hemodynamic optimization strategies (Fig. 2*A*). Significantly higher levels of biomarkers were seen with increasing severity of global tissue hypoxia as peak levels of IL-1ra were observed at 12 hrs; TNF-α, caspase-3, and IL-8 at 24 hrs; and ICAM-1 at 36 hrs (Fig. 2*B*) (Table 2). These findings were largely observed in patients presenting during a hypodynamic state (mean $Scvo_2$ ranging from 48.6 ± 12% to 49.5 ± 14% and mean lactate ranging from 6.9 ± 4.5 to 7.8 ± 4.7 mmol/L). Dr. Boulos and colleagues (20) found that the level of mitochondrial respiration in endothelial cells exposed to septic human serum was significantly impaired. They also observed a significant correlation between the level of mitochondrial respiration, cardiac output ($r =$

Figure 2—*Continued*. (*TNF*)-α ($p = .031$) and caspase-3 ($p = .024$) from 6–72 hrs, and IL-8 ($p = .049$) from 12–72 hrs. *B*, comparison setting II of biomarker patterns between global tissue hypoxia groups for the first 72 hrs. The severe global tissue hypoxia group (lactate concentrations ≥4 mmol/L and $Scvo_2$ <70%) are *diamonds*, the moderate global tissue hypoxia group (lactate level of ≥2 and <4.0 mmol/L and $Scvo_2$ <70%) are *squares*, and the resolved global tissue hypoxia group (lactate level <2 mmol/L and $Scvo_2$ ≥70%) are *triangles*. Data are shown as mean values and standard deviations. There were no significant differences in baseline levels. The severe global tissue hypoxia group had significantly higher concentrations than the moderate or resolved global tissue hypoxia groups at one or more time points for all of the biomarkers. The $p < .006$ level of significance was attained at hrs 12–72 for IL-1ra, hr 36 for ICAM-1, hr 12 for TNF-α, hrs 12 and 36 for caspase-3, and at hrs 12, 24, 36, and 48 for IL-8.

.52; $p < .05$), and mixed venous oxygen saturation ($r = .61$; $p < .05$), suggesting that tissue hypoperfusion or a hypodynamic state augments the release of inflammatory mediators. This hypodynamic state is generally earlier then the hyperdynamic phase and/or pathologic supply independency described in previous studies (26, 28, 34–41). During the later phase of oxygen supply independency, microcirculatory dysfunction and cytopathic tissue hypoxia may be a more predominant pathogenic mechanism, leading to the development of organ dysfunction and mortality (20, 42). Thus, the impact of resuscitation on biomarker activity may be related to the hemodynamic stage and severity of disease presentation.

There are bench-to-bedside correlates to these biomarker observations. TNF-α–induced caspase-3 activation, in particular, has been shown to cause an early apoptotic cascade of myocardial dysfunction and cardiovascular insufficiency within 3 hrs in animal models (6, 43). From a therapeutic standpoint, timely resolution of this cardiovascular insufficiency has significant outcome implications (44). Dr. Levy and colleagues (45) have shown a significant association between the duration of cardiovascular insufficiency, vasopressor use (particularly within the first 24 hrs), and outcome. In this study, there was a significant association between increased IL-1ra levels and paralleling vasopressor use. The attenuation of these biomarker concentrations and vasopressor use with EGDT provides supportive evidence why a meta-analysis by Dr. Kern and colleagues (46) revealed that hemodynamic optimization appears to be most effective when patients are treated within 8 hrs to 12 hrs after the disease insult and before organ dysfunction (47).

The ρ between biomarker levels, organ dysfunction, and mortality has been examined in intensive care unit models of sepsis. In this study, the maximum ρ between biomarker concentration and organ dysfunction was 0.68 for IL-1ra, 0.52 for ICAM-1, 0.33 for TNF-α, 0.47 for caspase-3, and 0.56 for IL-8, which compares favorably to (if not improves upon) previous studies (48–51). Most notable is that there is a much earlier temporal relationship between inflammation and the onset of organ dysfunction. Mean biomarker levels during 72 hrs were significantly greater in hospital nonsurvivors than survivors for all examined, supporting increasing evidence that the initial inflammatory response directly correlates to early but not late sepsis mortality (52, 53). For future sepsis trials in particular, these findings suggest that immunomodulatory agents such as anti–TNF-α antibodies (54) and IL-1ra antagonists (55) should be therapeutically targeted to biomarker levels that were observed to peak at an earlier disease stage of disease presentation. This may partly explain the lack of outcome efficacy in prior trials for these very important adjuncts in the treatment of this disease.

Previous studies have shown that the sepsis-triggered biomarker activity is multimodal, which was observed when comparing the peak concentrations of comparison setting I and II. The time to peak biomarker concentrations in comparison setting II in those patients with the greater degrees of global tissue hypoxia was generally later than that observed in comparison setting I, suggesting a "second hit" phenomenon (56). Delayed peaks in IL-1ra, ICAM-1, and IL-8 were observed 12 hrs later in patients with the greater degrees of global tissue hypoxia and were of greater magnitude than the earlier peak concentrations in comparison setting I. These delayed peaks or second hits have been described previously and associated with increased organ dysfunction *in vitro* (57, 58) and *in vivo* (31, 32, 59, 60).

There is evidence of a significant interaction between volume therapy (which was significantly greater in the EGDT group during the first 6 hrs) and inflammation. Dr. Dorresteijn and colleagues (61) demonstrated that isomolar volume loading before the administration of endotoxin in the human model resulted in significantly lower and delayed rise in IL-8, IL-1β, and TNF-α concentrations compared with nonhydrated controls. Clinical signs of the systemic inflammatory response syndrome also were significantly reduced. These findings may add further understanding, not only to pathogenic mechanisms, but also in the appropriate and timely introduction of conservative vs. liberal fluid management strategies in the resuscitation of severe sepsis and septic shock patients complicated by acute lung injury (62, 63).

The cardiopulmonary complications pathogenically associated with IL-8, in particular, provide further clinical insight to the second hit or delayed peak observed at 24 hrs or 12 hrs after the first peak. Similar to findings by Dr. Hack and colleagues (7), we found a moderate but significant correlation between IL-8, mean arterial pressure ($\rho = -0.511$), lactate levels ($\rho = 0.612$), and respiratory failure/Pao_2/Fio_2 ($\rho = -0.493$) (all $p < .001$) from 12 to 72 hrs (64). This is consistent with Dr. Estenssoro and colleagues' (65) observation that the presence of shock during the first 24 hrs of admission carries the greatest prognostic predictor for prolonged mechanical ventilation. This is consistent with a 15% increase in rate of mechanical ventilation ($p = .02$) and 11% higher or two-fold increase in death rate from sudden cardiopulmonary deterioration ($p = .02$) over 72 hrs in patients receiving standard therapy in the EGDT study (9). It is also plausible that the higher use of mechanical ventilation, which has been shown to increase inflammation, also may impact IL-8 activity (66).

An evolution in the early pathogenic understanding of other acute life-threatening disorders—such as trauma, acute myocardial infarction, and stroke—has changed the pathogenic landscape of these diseases and resulted in the development of pivotal therapeutic interventions within those "golden hours." In the case of acute myocardial infarction, the substantial logistic challenges of rapid diagnostic, pharmacologic, and catheter-based reperfusion interventions largely have been overcome and have led to significant improvement in outcomes for this deadly disease. Similarly, in the management of sepsis, significant gaps associated with increased mortality have existed between evidence-based discovery and timely implementation (67, 68). However, in recent years, multiple studies and observations have confirmed that early application of standard operating procedures providing early hemodynamic optimization consistently has shown significant outcome benefit and cost-effectiveness similar to EGDT (8–10, 12–17, 19, 39, 69–77).

Limitations and Additional Considerations. Adequate samples were obtained on 92.3% of eligible patients, equally distributed between control and EGDT. Complete sampling could not be obtained owing to the presence of severe anemia; patient, family, or clinician concerns about additional blood draws; inadequate or insufficient samples for analysis; or patient mortality. The determination of adequacy for assay was independent of the investigators. The control group and its biomarker activity is not totally representative of "wild type or no care," because

these patients received a comparably higher level of care than in most emergency departments (78). As a result, the magnitude of biomarker levels between treatment groups may be dampened.

The mitigation of global tissue hypoxia and prevention of related complications appears to be of greater benefit despite the risks and concerns of significantly more intravenous fluid, red blood cell units, and inotropes titrated to objective end points during the first 6 hrs in the EGDT group (62, 79–81). The association of vasoactive agents, such as dobutamine effects on IL-8 and fluid type on immunomodulation, is well established (82, 83). Dobutamine use was significantly greater in the EGDT group compared with the control group. However, this study was not powered to determine how vasoactive agents and fluids modulate inflammation. This study was performed before and in parallel to the trials examining the efficacy of recombinant human activated protein C (drotrecogin alpha), intensive insulin therapy, and corticosteroids, so these therapies were not part of the sepsis treatment protocol (69, 84, 85).

CONCLUSION

In early severe sepsis and septic shock, within the first 3 hrs of hospital presentation, distinct biomarker patterns emerge in response to hemodynamic optimization strategies. A significant association exists between temporal biomarker patterns in the first 72 hrs, severity of global tissue hypoxia, organ dysfunction, and mortality. These findings identify global tissue hypoxia as an important contributor to the early inflammatory response, and support the role of hemodynamic optimization in supplementing other established therapies during this window of opportunity.

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