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Duration and magnitude of hypotension and monocyte deactivation in patients with community-acquired pneumonia

Peter M. Simon¹, Russell L. Delude^{1,2}, MinJae Lee^{1,3}, Lan Kong^{1,3}, Lynda J. Guzik¹, David T. Huang^{1,4}, Derek C. Angus¹, and John A. Kellum¹ for the GenIMS Investigators

¹Clinical Research, Investigation, and Systems Modeling of Acute Illness (CRISMA) Center, Department of Critical Care Medicine; School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261

²Department of Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261

³Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15261

⁴Department of Emergency Medicine, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15261

Abstract

Purpose—To examine the relationship of duration and magnitude of arterial hypotension to subsequent cellular immune suppression and cytokinemia in patients hospitalized with community-acquired pneumonia (CAP).

Methods—We studied an observational cohort of 525 subjects hospitalized after presenting to the Emergency Department with radiographic and clinical signs of CAP. We compared the duration and magnitude of hypotension, using the cardiovascular Sequential Organ Failure Assessment subscore (CV SOFA), to day 3 monocyte expression of human leukocyte antigen-DR (mHLA-DR), a previously validated marker of cellular immune suppression.

Results—A significant association of CV SOFA with decreased mHLA-DR expression was present in univariate analysis ($p < 0.001$) and persisted after adjustment for illness severity and other covariates ($p = 0.01$). With CV SOFA separated into components of magnitude and duration, after covariate adjustment only duration was associated with day 3 mHLA-DR expression ($p = 0.03$). Levels of key pro- and anti-inflammatory cytokines (interleukin-6, interleukin-10, tumor necrosis factor) increased with hypotension exposure, and were also associated with mHLA-DR expression.

Conclusions—In patients admitted with CAP, arterial hypotension over the first three days is associated with markers of monocyte deactivation. The duration of exposure to hypotension may be more important than the magnitude, and monocyte deactivation correlates with interleukin-6 and interleukin-10 release. These results suggest that persistent hypotension might contribute to immunosuppression following septic shock.

Keywords

Septic shock; hypotension; SOFA; flow cytometry; immune suppression; monocyte deactivation; HLA-DR; cytokine

INTRODUCTION

Severe sepsis, organ dysfunction secondary to the systemic host response to infection, is both widely prevalent and highly fatal with an annual incidence of 750,000 cases/year in the United States and an associated mortality of approximately 30%¹. Accumulating evidence suggests that instead of being a unimodal pro-inflammatory state, the septic response is also characterized by functional cellular immune suppression, which may represent an impaired homeostatic response²⁻⁶. The clinical significance of this immune impairment is implied by the relationship of markers of immunosuppression with organ dysfunction and mortality⁷⁻⁹, though causal and mechanistic data are sparse.

Systemic arterial hypotension is one possible mechanism of immunosuppression in sepsis, and is of particular interest as it presents an opportunity for targeted therapy in the form of improved fluid resuscitation, vasopressor therapy or both. This role is suggested by animal models of hemorrhagic shock, in which there is considerable evidence linking the depth and also the duration of induced hypotension to functional assays of immunosuppression^{10,11}. This finding is consistent across several species and experimental conditions^{12,13}. A similar association between hypotension and subsequent immunosuppression has not been established in septic shock. This is in part because of the difficulty isolating the effect of hypotension in the presence of multiple covariates known to affect immune function¹⁴, which collectively preclude simple univariate analysis in either animal or human studies.

We therefore constructed the current study to evaluate the independent association of magnitude and duration of hypotension in patients hospitalized with community-acquired pneumonia (CAP), to monocyte surface expression of the activation marker human leukocyte antigen-DR (mHLA-DR). Decreased mHLA-DR expression has a strong, reproducible association with functional assays of immunosuppression^{7,15}, secondary nosocomial infection¹⁵, and adverse outcome from critical illness including sepsis^{7,9}. To determine whether hypotension and monocyte deactivation were also associated with the level of cytokinemia, we followed plasma levels of a subset of mediators - tumor necrosis factor (TNF), interleukin-6 (IL-6) and interleukin-10 (IL-10) – known to covary with other mediators and with sepsis severity¹⁶.

MATERIALS AND METHODS

Study design

We studied 623 patients who had cell-surface markers measured by flow cytometry as part of the GenIMS (Genetic and Inflammatory Markers of Sepsis) study, a multicenter inception cohort of 2,320 patients presenting to 28 North American acute care hospitals with CAP (Figure 1). Details of the GenIMS cohort have been described elsewhere³. Briefly, subjects were identified in the Emergency Department (ED) with CAP by the criteria of Fine et al¹⁷ and followed daily by dedicated study coordinators. In the current study we excluded subjects with preexisting immunosuppression (i.e. metastatic cancer, HIV/AIDS, or immunosuppressive medication), and patients who were subsequently ruled out for CAP in the judgment of their primary clinicians. The study was approved by local institutional review boards at all participating centers.

We used the cardiovascular SOFA score to classify hypotension and recorded it daily. All analyses use data from only the first three days of hospitalization. This range was chosen due to censoring of roughly half the initial cohort by discharge or death between days 3 and 7, when cell-surface marker assays were performed, and incomplete SOFA subscale data after day 3. We tracked 90-day mortality, chosen based on consensus

recommendations^{18,19}, by hospital record review and a National Death Index query at study completion.

The primary readout of immunosuppression was day 3 circulating monocytic expression of the major histocompatibility complex type II molecule HLA-DR (mHLA-DR) by flow cytometry, representing down-regulation of exogenous antigen presentation to CD4⁺ T-cells. In addition, we followed plasma concentrations of TNF, IL-6, and IL-10³ to determine whether persistent hypotension was associated with cytokinemia.

Study procedures

Patients were enrolled either in the ED or as soon as possible after admission, and were then followed daily during hospitalization by structured interview and medical record abstraction. Cell-surface marker data were available for a subset of 623 subjects at hospitals within 60 miles of the coordinating center (University of Pittsburgh) at day 1, 3 and 7. In addition, patients had blood draws performed for cytokine analysis at enrollment, daily for the first week, and weekly thereafter while hospitalized.

Cytokine assays

Blood sampling and assay details have been described elsewhere³. Briefly, blood was drawn into heparinated vials, after which the plasma fraction was isolated by centrifugation, frozen to -80 °C and shipped on dry ice to the coordinating center. We measured cytokine concentrations by chemiluminescent immunoassay using an automated analyzer (IMMULITE; Diagnostic Products Corp, Los Angeles, California).

Flow cytometry

All reagents were obtained from Sigma-Aldrich Chemical Co. unless otherwise indicated. We used a phycoerythrin (PE)-conjugated anti-HLA-DR fluorescent antibody, with isotype and fluorophore matched controls used to confirm binding specificity (eBioscience, San Diego, CA). Whole blood samples were drawn from subjects directly into EDTA-containing vacutainer tubes (BD-Biosciences, San Jose, CA), and transferred to the coordinating center at room temperature for analysis. Sample processing and analysis were done within 48 hours. Our antigen panel was stable under these conditions for at least 56 hours prior to analysis (data not shown). Manufacturer recommended aliquots of antibody were reacted with 100 µl of whole blood for 15 min at 4 °C. Erythrocytes were lysed with 2 mL of lysis solution (4.13 g NH₄Cl, 0.5 g KHCO₃, and 0.019 g Na₄-EDTA in 500 ml, adjusted to pH 7.4 with NaOH) for 15 minutes. Cells were washed once with 2% bovine serum albumin in phosphate buffered saline, fixed in 1% paraformaldehyde, and stored at 4 °C prior to analysis.

Data were acquired on a BD FACSVantage SE cytometer with a minimum of 500 monocyte events acquired for each sample. Samples were gated via forward scatter (FSC) and side scatter (SCC) into lymphocyte, granulocyte and monocyte subpopulations²⁰, with percent-positivity for each marker determined by 1-color histogram. Representative results for patients with high and low HLA-DR expression are shown (Figure 2). The percent gated positive, based on fluorescence intensity relative to isotype controls, is reported as our primary outcome, as this measure has been reported to have better inter-trial agreement for monocytic HLA-DR expression than mean-fluorescence intensity²¹.

Clinical definitions

We assessed hypotension using the cardiovascular component of the Sequential Organ Failure Assessment (CV SOFA) score over the first three days of hospitalization^{22,23}. The magnitude of cardiovascular dysfunction in our study was operationalized as the highest

recorded CV SOFA score during the first three days (CV SOFA_{MAX}, range 0–4); and the duration of cardiovascular dysfunction was defined as total number of days during hospital days 1–3 that the CV SOFA score was >1 (CV SOFA_{DUR}, range 0–3). In addition, for purposes of analyzing the joint contribution of duration and magnitude of shock, we calculated a combined score of CV SOFA totaled over the first three days (CV SOFA_{TOT}, range 0–12). Definitions are shown in Table E1.

Noncardiovascular organ dysfunction (Total SOFA-CV SOFA) was assessed daily over the first three days and defined in an analogous manner, with separation into components of total (nonCV SOFA_{TOT}), magnitude (nonCV SOFA_{MAX}), and duration (nonCV SOFA_{DUR}) of organ dysfunction (Table E1).

Statistical analyses

For the baseline characteristics, we conducted univariate comparisons using Chi-squared tests, Student's t-tests, or their non-parametric equivalents, as appropriate. Unadjusted relationships between CV SOFA and mHLA-DR at day 3 were analyzed using one-way ANOVA. We used a linear regression model to test the relationship of CV SOFA to day 3 mHLA-DR with covariate adjustment. Two models were made for each biomarker outcome – one including CV SOFA_{TOT}, and the other with both CV SOFA_{MAX} and CV SOFA_{DUR} to test the independent contribution of these variables. These models each included terms for other factors potentially contributing to immunosuppression - noncardiovascular SOFA score (nonCV SOFA), age, race, gender, comorbidity at admission (Charlson score), mechanical ventilation use, ICU use and blood transfusion over the first three inpatient days²⁴. Model specification was evaluated using standard methods. The primary hypothesis, that CV SOFA_{TOT} is associated with day 3 mHLA-DR expression, was established a priori and not adjusted for multiple comparisons. Subanalyses were considered exploratory and not adjusted for error inflation.

There was a high prevalence of truncated cytokine data due to levels falling below assay detection limits (ranging from 27% to 71% of data). Therefore cytokine concentrations were either 1) dichotomized for analysis into low and high categories based on the manufacturer recommended upper normal range cutoffs (IL-6, 5.9 pg/mL; IL-10, 9.1 pg/mL; TNF, 8.1 pg/mL)³, or 2) when used as a continuous outcome, a Tobit model was used to account for censored data. Relationships between CV SOFA and cytokine levels (IL-6, IL-10, TNF) were tested using a repeated measures Tobit model, with adjustment for potential confounders using the same covariate panel as above. We used logistic regression to test the association of CV SOFA, nonCV SOFA and mHLA-DR with 90-day mortality outcome.

RESULTS

Of 623 patients who had cell-surface markers analyzed by flow cytometry 548 were subsequently hospitalized, had CAP confirmed, and had day 3 data available for analysis. We excluded 23 additional subjects because of preexisting immunosuppression (metastatic cancer, HIV, or immunosuppressive medications) for a final analysis cohort of 525 patients (Figure 1). Higher total CV SOFA score over the first three days (CV SOFA_{TOT}) was associated with several measures of illness severity, including noncardiovascular SOFA score, ICU admission, transfusion and requirement for mechanical ventilation (Table 1).

Total cardiovascular SOFA score over the first three days is associated with decreased day 3 monocyte HLA-DR expression

In univariate analysis, CV SOFA_{TOT} correlated with our main outcome measure of day 3 mHLA-DR expression (CV SOFA_{TOT} = 0: 76.6%±16.0; CV SOFA_{TOT} = 1: 75.0%±16.6;

CV SOFA_{TOT} ≥ 2 : 67.2% $\pm$ 20.0; $p < 0.001$) (Figure 3). In multivariate analysis with adjustment for potential confounders (Table 2, Model 1) CV SOFA_{TOT} remained significantly associated with day 3 mHLA-DR ($p = 0.015$). Association between CV SOFA_{TOT} and day 3 mHLA-DR persisted with addition of a second illness severity measure, day 1 APACHE score (Table 2, Model 5, $p = 0.013$ for CV SOFA_{TOT}).

Decreased monocyte HLA-DR expression in sepsis is related to hypotension with patients given vasopressors excluded from analysis

Given the known immunomodulatory effects of vasopressor drugs²⁵, and the conflation of physiology and therapy in the SOFA cardiovascular subscale (Table E1), we performed a sensitivity analysis excluding patients who received vasopressors ($n = 19/525$). This demonstrated a persistent association between CV SOFA_{TOT} and day 3 mHLA-DR ($p = 0.005$) (Table 2, Model 4) in patients who did not receive any vasopressors.

The duration of cardiovascular dysfunction and the magnitude of noncardiovascular dysfunction are associated with day 3 monocytic HLA-DR expression

In a multivariate model combining magnitude and duration of cardiovascular and noncardiovascular dysfunction, after adjustment only the duration of cardiovascular dysfunction and the magnitude of noncardiovascular dysfunction were associated with day 3 mHLA-DR (Table 2 - CV SOFA_{DUR}, $p = 0.033$, nonCV SOFA_{MAX}, $p = 0.005$). Interaction terms for the SOFA-derived variables were found to be nonsignificant, and collinearity was not evident (highest variance inflation factor = 3.83).

CV SOFA is related to the level, but not the trajectory, of mHLA-DR expression

To understand whether the relationship between cardiovascular dysfunction and mHLA-DR expression was established before presentation to the ED and study enrollment, or occurred during the initial hospitalization, we analyzed the relationship of CV SOFA_{TOT} to day 1 mHLA-DR and the change in mHLA-DR expression from day 1 to 3 (Table 3). These results show that CV SOFA_{TOT} was associated with day 1 mHLA-DR expression, but not with the change in mHLA-DR from day 1 to 3 in either univariate or multivariate analysis (Table 3). Similar relationships were seen in a repeated measures (generalized linear) model of the effect of CV SOFA_{TOT} on mHLA-DR expression over time. Again we noted an association of this exposure to the level of mHLA-DR expression at days 1 and 3, but no effect of the time component or a time-CV SOFA interaction.

Hypotension in septic shock is associated with increases in IL-6, IL-10 and TNF, but these do not completely explain the relationship between hypotension and immune suppression

There was a high level of truncation (level below assay detection threshold) of cytokine data, so data are presented as above or below normal range. Increasing duration and magnitude of hypotension was associated with elevation in plasma IL-6, TNF and IL-10 (Figure E1), and persisted after adjustment for noncardiovascular SOFA in a repeated measures Tobit model (Table E2). This is consistent with previous data that increasing sepsis severity is associated with elevation of both pro- and anti-inflammatory cytokines (9). In a mediator model combining CV SOFA, nonCV SOFA, IL-10 and IL-6 all were independently associated with day 3 mHLA-DR (Table E3), suggesting that any relationship between hypotension and mHLA-DR expression is partially but not entirely mediated by immunomodulatory cytokine release.

CV SOFA does not predict mortality in a logistic regression model including other markers of illness severity

Table 4 shows results of a logistic regression model of the relative mortality contribution of CV and nonCV SOFA, done to ensure that the association between CV SOFA and mHLA-DR does not just reflect illness severity. After covariate adjustment, CV SOFA_{TOT} no longer correlated with 90-day mortality (OR 0.95, 0.77–1.17, $p=0.65$), however nonCV SOFA_{TOT} remained independently predictive (OR 1.12, 1.05–1.20, $p<0.001$) as did mechanical ventilation use, Charlson score, age and transfusion. Of note, higher day 1 mHLA-DR expression predicted survival at 90 days (OR for death 0.083, $p<0.01$, data not shown), consistent with prior data on the importance of cellular immune suppression to sepsis outcome²⁶.

DISCUSSION

The main finding of our study is that in septic shock the duration of hypotension, measured by SOFA, is independently associated with monocyte HLA-DR expression, an important measure of cellular immune suppression. Immune suppression is increasingly recognized as a pathogenic mechanism for late mortality from sepsis^{8,9}. Multiple immune derangements have been reported in patients with sepsis including decreased monocytic surface expression of HLA-DR⁹, decreased in vitro cytokine release in response to LPS or TNF simulation²⁷, attenuated neutrophil oxidative burst²⁸, cytomegalovirus viremia²⁴ and apoptosis with massive loss of lymphocytes, monocytes and dendritic cells²⁹. The resulting functional immune suppression is readily observed by the clinician as an increased incidence of nosocomial infection with organisms that are normally easily cleared by the healthy host. Our study, conducted in a large multicenter cohort of patients presenting to the ED with CAP, explored one possible contributor to the severity of immune suppression following infection--the magnitude and duration of systemic arterial hypotension – and to our knowledge is the first report to evaluate this relationship in human sepsis.

In a secondary analysis plasma levels of markers of anti-inflammation (IL-10) and pro-inflammation (IL-6) were related to CV SOFA (Table E2), and also independently to mHLA-DR expression (Table E3). This does not suggest that the association between CV SOFA and mHLA-DR is mediated by these particular cytokines. However, since pro- and anti-inflammatory mediators are almost universally elevated in patients with sepsis, and covary with each other and illness severity, we include this subset as covariates to estimate the magnitude and duration of the systemic inflammatory response^{3,16,30}. Notably, IL-6 and IL-10 are not known to themselves induce hypotension or capillary leak³¹. The mechanism of monocyte deactivation from hypotension is unclear, though tissue hypoxia has pleiotropic effects on monocytes through both hypoxia-inducible factor-1 α dependent and independent pathways^{32,33}. Immune suppression following hemorrhagic shock is attenuated by chloroquine and prolactin administration^{34,35}.

Perhaps the most serious threat to the validity of our study is whether we may just be capturing illness severity despite covariate adjustment, as nonsurvivors are known to have lower levels of mHLA-DR^{4,6,9}. To explore this possibility, we tested the association of our covariate panel with 90-day mortality outcome (Table 4), and found that noncardiovascular SOFA score and other severity indicators were independently associated with risk for death, whereas CV SOFA_{TOT} did not remain predictive in multivariate analysis. This suggests that our models adequately account for the effect of illness severity on immunosuppression, in effect isolating hypotension as a physiologic quantity rather than a severity marker, and persistent association with hypotension can be taken to be independent of illness severity. However in this observational trial we cannot state causality for the association noted between hypotension and cellular immune suppression.

An additional limitation is that for ethical reasons we cannot extricate the effect of hypotension on immune function from the impact of vasopressor use, a class of drugs which have complex immunomodulatory effects²⁵. However, a sensitivity analysis using our multivariate model of CV SOFA_{TOT} vs. day 3 mHLA-DR but excluding patients given vasopressors (n=19/525, Table 2) showed minimal effect on significance testing (p=0.005 in the most parsimonious model). Other therapies delivered to critically ill patients are also potentially immunomodulatory, including mechanical ventilation and transfusion. Our multivariate models included these interventions, but it is possible there are important unobserved predictors not related to those in the model.

In conclusion, these data support the hypothesis that immunosuppression resulting from community-acquired pneumonia is, at least in part, related to the duration of systemic arterial hypotension. Further research is needed to explore the mechanisms of this relationship, and whether immunosuppression from septic shock may be attenuated by therapy to reduce the duration of hypotension.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Subject disposition for the entire GenIMS cohort and the study cohort

For logistical reasons, cell-surface markers were only measured for subjects within 60 miles of the coordinating center.

Abbreviations: SOFA, Sequential Organ Failure Assessment Score; CAP, Community-acquired pneumonia; mHLA-DR, monocyte human leukocyte antigen-DR

Figure 2. Representative flow cytometry output for patients with high and low monocytic HLA-DR expression

Forward- and side-scatter dotplots and single channel histograms for representative patients with high (**top row**) and low (**bottom row**) monocytic HLA-DR expression. Shaded histograms show mean channel fluorescence for anti-HLA-DR PE, unshaded histograms are isotype control results from the same sample. All histograms are from monocyte gate only (based on characteristic light scatter). Abbreviations: SSC, side scatter; FSC, forward scatter; HLA-DR, human leukocyte antigen-DR; Gran, granulocyte gate; MO, monocyte gate; Ly, lymphocyte gate; PE, phycoerythrin

Figure 3. Univariate association of cardiovascular SOFA with day 3 monocyte HLA-DR expression

Box-and-whisker plots of total (CV SOFA_{TOT}), duration (CV SOFA_{DUR}) and magnitude (CV SOFA_{MAX}) of cardiovascular dysfunction versus day 3 monocyte HLA-DR expression. Expression level is in percent of monocytes gated positive based on position relative to isotype control.

Table 1

Characteristics of the GenIMS flow cytometry study cohort by total cardiovascular SOFA score over 1st three days^a

Variable	Total CV SOFA score over 1 st three days			Overall	p-value
	0	1	2+		
Number of patients	321 (61.1)	118 (22.5)	86 (16.4)	525 (100)	
Median age (IQ range)	71 (55–80)	72 (57–80)	75 (59–82)	72 (56–80)	0.40
Caucasian race	284 (88.5)	113 (95.8)	85 (98.8)	482 (91.8)	0.0016
Female sex	141 (43.9)	63 (53.4)	40 (46.5)	244 (46.5)	0.21
History of smoking	236 (73.5)	82 (69.5)	74 (86.1)	392 (74.7)	0.0278
History of alcohol use	99 (30.8)	42 (35.6)	22 (25.6)	163 (31.1)	0.81
Charlson comorbidity score	1.9 (2.1)	2.5 (2.7)	2.2 (2.3)	2.1 (2.3)	0.086
Appropriate antibiotics in 8 hours	306 (95.3)	112 (94.9)	85 (98.8)	503 (95.8)	0.3173
Units PRBC transfused ^b	0.01 (0.1)	0.1 (0.5)	0.08 (0.4)	0.04 (0.3)	0.0013
ICU use ^b	21 (6.5)	18 (15.3)	39 (45.4)	78 (14.9)	<0.001
Mechanical ventilation use ^b	6 (1.9)	6 (5.1)	10 (11.6)	22 (4.2)	<0.001
APACHE III, day 1	52 (16)	56 (17)	66 (23)	55 (18)	<0.001
PSI, day 1	94 (35)	101 (38)	124 (48)	121 (47)	<0.001
CV SOFA, day 1	0.02 (0.1)	0.41 (0.5)	0.8 (0.9)	0.23 (0.5)	<0.001
nonCV SOFA, day 1	1.8 (1.4)	2.1 (1.6)	2.8 (2.4)	2.0 (1.7)	0.0083
90 day mortality	33 (10.3)	16 (13.6)	22 (25.6)	71 (13.5)	0.0011

Baseline characteristics and selected outcomes/exposures for GenIMS flow cytometry study cohort.

Abbreviations: CV SOFA, Cardiovascular Sequential Organ Failure Assessment score; PRBC, Packed red blood cells; ICU, Intensive care unit; APACHE III, Acute Physiology and Chronic Health Evaluation III

^a unless otherwise indicated data are presented as mean (SD) or number (percentage) of subjects.

^b over first three hospital days

Table 2

Association of CV SOFA/nonCV SOFA over first 3 inpatient days with day 3 monocytic cell-surface expression of HLA-DR

Variable	Day 3 mHLA-DR	
	Coefficient ^a	p-value
<i>Unadjusted (univariate)</i>		
CV SOFA _{TOT}	-0.028	<0.001
<i>Model 1 - SOFA total model^b</i>		
CV SOFA _{TOT}	-0.015	0.015
nonCV SOFA _{TOT}	-0.0049	0.016
Age	0.00062	0.21
Caucasian race	0.030	0.28
Female sex	0.0030	0.84
Charlson score		0.35
PRBC transfusion	-0.020	0.44
Mechanical ventilation	-0.026	0.56
Intensive care unit use	-0.058	0.023
<i>Model 2 - Combined SOFA magnitude/duration model^b</i>		
CV SOFA _{DUR}	-0.032	0.033
nonCV SOFA _{DUR}	0.016	0.073
CV SOFA _{MAX}	0.0048	0.82
nonCV SOFA _{MAX}	-0.015	0.0054
<i>Model 3 - Bivariable model (standardized β-coefficients)^c</i>		
CV SOFA _{TOT}	-0.16	<0.001
nonCV SOFA _{TOT}	-0.19	<0.001
<i>Model 4 - Bivariable model, excluding patients given vasopressors^c</i>		
CVSOFA _{TOT}	-0.024	0.005
nonCV SOFA _{TOT}	-0.0083	<0.001
<i>Model 5 - SOFA model with APACHE (standardized β-coefficients)^b</i>		
CV SOFA _{TOT}	-0.12	0.013
nonCV SOFA _{TOT}	-0.086	0.079
APACHE II score	-0.16	0.003

Relationship of hypotension, operationalized as CV SOFA score, to day 3 monocyte HLA-DR expression (% positive). All variables calculated based on first three days of hospital admission.

^a Unless otherwise specified, coefficients are unadjusted and reflect change in % of monocytes which are HLA-DR⁺ for each 1 point increment in scale or presence/absence of factor. Where specified, standardized β -coefficient used to estimate relative SOFA subscale contribution to mHLA-DR

^b Models 1, 2 and 5 show SOFA organ failure subscales adjusted for age, race, sex, Charlson comorbidity score, transfusion, mechanical ventilation use, and ICU use by multiple regression (all variables only shown for Model 1).

^c Model 3 and 4 are most parsimonious models using only organ failure subscores.

Abbreviations: CV SOFA, Cardiovascular Sequential Organ Failure Assessment score; nonCV SOFA, noncardiovascular SOFA (total SOFA - CV SOFA); CV SOFA_{TOT}/CVSOFA_{DUR}/CV SOFA_{MAX}, total/duration/maximum CV SOFA over first three days (see text); PRBC, Packed red blood cells; ICU, Intensive care unit; HLA-DR, Human leukocyte antigen-DR

Table 3

Association of CV SOFA/nonCV SOFA over first 3 inpatient days with day 1 monocytic HLA-DR expression and change in monocyte HLA-DR expression from days 1–3

Variable	Day 1 mHLA-DR		Delta mHLA-DR day 1 to 3		mHLA-DR 1–3 generalized linear mixed model ^a			
	B-Coeff.	p-value	B-Coeff.	p-value	Effect of exposure		Time-exposure interaction	
					B-Coeff.	p-value	B-Coeff.	p-value
CV SOFA _{TOT}	-0.028	<0.001	-0.012	0.85	<i>Unadjusted (univariate)</i>			
					-0.031	<0.001	0.00063	0.83
CV SOFA _{TOT}	-0.020	0.0086	0.0034	0.62	<i>Model 1 - SOFA total model</i>			
					-0.019	0.011	0.00077	0.80
nonCV SOFA _{TOT}	-0.0044	0.043	-0.0014	0.94	<i>Model 2 - SOFA magnitude/duration model</i>			
					-0.0042	0.062	-0.00043	0.59
CV SOFA _{DUR}	-0.026	0.12	-0.0034	0.81	<i>Model 1 - SOFA total model</i>			
					-0.027	0.044	-0.0022	0.75
nonCV SOFA _{DUR}	0.0026	0.79	0.0098	0.26	<i>Model 2 - SOFA magnitude/duration model</i>			
					0.0088	0.26	0.0082	0.046
CV SOFA _{MAX}	-0.0061	0.79	0.0099	0.64	<i>Model 1 - SOFA total model</i>			
					-0.0041	0.82	0.0053	0.60
nonCV SOFA _{MAX}	-0.012	0.044	0.00072	0.89	<i>Model 2 - SOFA magnitude/duration model</i>			
					-0.015	0.0025	-0.0018	0.40

Association of hypotension exposure to monocyte HLA-DR expression (% positive) on day 1 and from days 1 to 3.

^a Generalized linear model results shown as main effect of exposure to hypotension (CV SOFA), adjusted for illness severity (nonCV SOFA). The time*exposure interaction term tests for differences in mHLA-DR trajectory over days 1–3 in subjects exposed to increasing duration and magnitude of hypotension.

Abbreviations: CV SOFA, Cardiovascular Sequential Organ Failure Assessment score; nonCV SOFA, noncardiovascular SOFA (total SOFA - CV SOFA); CV SOFA_{TOT}/CVSOFA_{DUR}/CV SOFA_{MAX}: total/duration/maximum CV SOFA over first three days (see text)

Table 4

Relationship of CV SOFA with 90-day mortality

Variable	OR for 90-day mortality ^a (95% CI)	p-value
<i>Unadjusted (univariate)</i>		
CV SOFA _{TOT} only	1.28 (1.10–1.48) ^a	0.0014
nonCV SOFA _{TOT} only	1.18 (1.12– 1.24) ^a	<0.001
<i>Model 1 – Total CV SOFA over first three days</i>		
CV SOFA _{TOT}	0.95 (0.77–1.17) ^a	0.65
nonCV SOFA _{TOT}	1.12 (1.05–1.20) ^a	<0.001
Age	1.04 (1.02–1.07)	<0.001
Caucasian race	0.81 (0.24–2.75)	0.73
Female sex	0.86 (0.48–1.53)	0.61
Charlson score		0.019
PRBC transfusion	1.66 (0.87–3.16)	0.12
Mech. ventilation	1.18 (0.32–4.38)	0.80
ICU use	3.19 (0.15–6.97)	0.0037
<i>Model 2 - CV SOFA/nonCV SOFA duration and magnitude</i>		
CV SOFA _{DUR}	1.73 (1.07–2.81) ^a	0.027
nonCV SOFA _{DUR}	1.01 (0.68–1.50) ^a	0.943
CV SOFA _{MAX}	0.54 (0.27–1.08) ^a	0.0814
nonCV SOFA _{MAX}	1.40 (1.17–1.67) ^a	0.0003

Logistic regression model of CV SOFA and other covariates with 90 day all-cause mortality.

Abbreviations: CV SOFA, Cardiovascular Sequential Organ Failure Assessment score; nonCV SOFA, noncardiovascular SOFA (total SOFA - CV SOFA); CV SOFA_{TOT}/CVSOFA_{DUR}/CV SOFA_{MAX}, total/duration/maximum CV SOFA over first three days (see text); PRBC, Packed red blood cells; ICU, Intensive care unit

^a odds ratios are for 1 point increments in scale