Understanding the Inflammatory Cytokine Response in Pneumonia and Sepsis

Results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study

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Background: Severe sepsis is common and frequently fatal, and community-acquired pneumonia (CAP) is the leading cause. Although severe sepsis is often attributed to uncontrolled and unbalanced inflammation, evidence from humans with infection syndromes across the breadth of disease is lacking. In this study we describe the systemic cytokine response to pneumonia and determine if specific patterns, including the balance of proinflammatory and anti-inflammatory markers, are associated with severe sepsis and death.

Methods: This is a cohort study of 1886 subjects hospitalized with CAP through the emergency departments in 28 US academic and community hospitals. We defined severe sepsis as CAP complicated by new-onset organ dysfunction, following international consensus conference criteria. We measured plasma tumor necrosis factor, IL-6 (interleukin 6), and IL-10 levels daily for the first week and weekly thereafter. Our main outcome measures were severe sepsis and 90-day mortality.

Results: A total of 583 patients developed severe sepsis (31%), of whom 149 died (26%). Systemic cytokine level elevation occurred in 82% of all subjects with CAP. Mean cytokine concentrations were highest at presentation, declined rapidly over the first few days, but remained elevated throughout the first week, beyond resolution of clinical signs of infection. Cytokine levels were highest in fatal severe sepsis and lowest in CAP with no severe sepsis. Unbalanced (high/low) cytokine patterns were unusual (4.6%) and not associated with decreased survival. Highest risk of death was with combined high levels of the proinflammatory IL-6 and anti-inflammatory IL-10 cytokine activity (hazard ratio, 20.5; 95% confidence interval, 10.8-39.0) ($P<.001$).

Conclusions: The circulating cytokine response to pneumonia is heterogeneous and continues for more than a week after presentation, with considerable overlap between those who do and do not develop severe sepsis. Unbalanced activation is uncommon, and mortality is highest when both proinflammatory and anti-inflammatory cytokine levels are high.

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SEVERE SEPSIS, A SYSTEMIC RESPONSE to infection complicated by acute organ dysfunction, develops in 900 000 people each year in the United States, a third of whom die.[1] Early models of sepsis using animals[2] and human volunteers injected with bacterial products[3] demonstrated a short, intense elevation of proinflammatory cytokine levels. Initially intended to control and eliminate infection, the cytokine activation became widespread and was presumed to precipitate organ failure characteristic of the clinical syndrome.[4] This theory is supported by the cytotoxic effects of various cytokines[5] and has underpinned many large-scale studies of interventions intended to blunt the proinflammatory response in sepsis.[6-21] Unfortunately, these trials generally failed.[22,23] One explanation is that death from sepsis may follow an excessive counterregulatory anti-inflammatory response.[24] However, both theories may be oversimplified, arising from an incomplete understanding of the complex pathophysiologic nature of human sepsis.

Typically in medicine, large-scale interventional trials are initiated only after the natural history of the target condition is well understood. The sepsis field is unusual in that there are surprisingly limited data on inflammatory cytokine responses in humans with infection and severe sepsis. The few clinical studies conducted thus far have often yielded results inconsistent with each other or with those of laboratory experiments and human volunteer studies. These clinical studies were often small, single-

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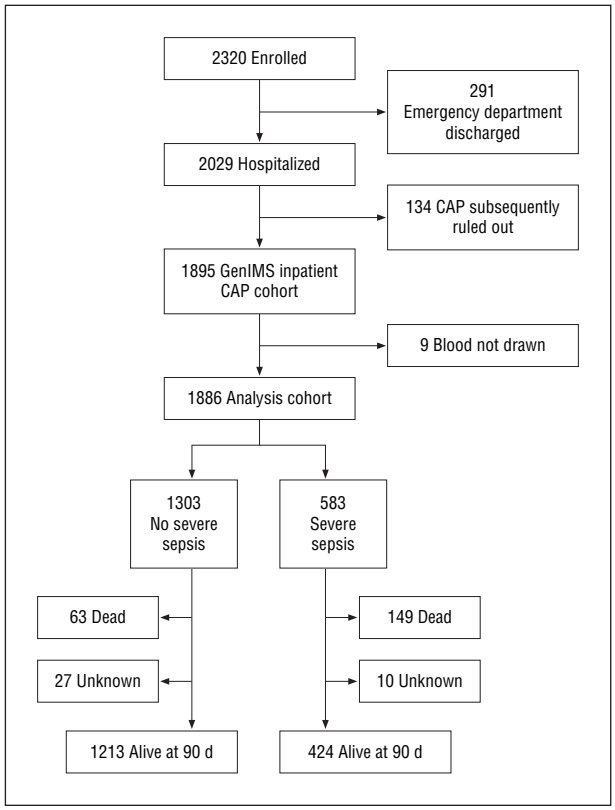


Figure 1. Subject disposition for the entire Genetic and Inflammatory Markers of Sepsis (GenIMS) cohort. CAP indicates community-acquired pneumonia.

center studies, which limited power and generalizability, and they often focused on subjects with established severe sepsis due to a myriad of infections. Also, comparators were often normal adults rather than infected but less ill subjects. These design limitations compromised the ability to study the timing of the response and to distinguish protective from harmful aspects.

We conducted a large, multicenter inception cohort study of subjects presenting to the emergency department (ED) with community-acquired pneumonia (CAP), the leading cause of severe sepsis.[1] Our goals were to describe the systemic cytokine response to infection and to determine if there were specific patterns associated with severe sepsis and death.

METHODS

SITES AND SUBJECTS

The community-acquired Genetic and Inflammatory Markers of Sepsis (GenIMS) study enrolled subjects at 28 academic and community hospitals in southwestern Pennsylvania, Connecticut, southern Michigan, and western Tennessee. We included patients 18 years or older with a clinical and radiologic diagnosis of pneumonia, per the criteria of Fine et al,[25] namely, one or more symptoms suggestive of pneumonia and radiographic evidence of pneumonia within 24 hours of presentation. Exclusion criteria included transfer from another hospital; discharge from a hospital within the prior 10 days; an episode of pneumonia within the prior 30 days; chronic mechanical ventilation, cystic fibrosis, or active pulmonary tuberculosis; admission for palliative care; previous enrollment in the study; incarceration; and/or pregnancy. Participants or their proxies provided written consent. We obtained approval from the institutional review boards of the University of Pittsburgh and all participating sites.

STUDY PROCEDURES

All subjects were enrolled while still in the ED, if possible, or as soon after admission as could be accomplished. After enrollment, we gathered detailed baseline and sequential clinical information using structured subject or proxy interviews, bedside assessments, and medical record abstraction. We obtained blood for cytokine assays immediately following enrollment, daily for the first week, and weekly thereafter while subjects remained in hospital. We determined survival after discharge by telephone and a National Death Index search.

We tracked clinical data and blood samples using unique anonymized identification numbers, merging data only after assays had been completed. We observed strict data confidentiality and audited clinical data and assays for accuracy, including random chart audits, additional blood assays, and computer flags for inconsistencies.

CYTOKINE ASSAYS

We measured circulating levels of tumor necrosis factor (TNF) and IL-6 (interleukin 6) as markers of the proinflammatory and IL-10 as a marker of the anti-inflammatory cytokine response. Generally, day 1 blood samples were drawn at enrollment (day of ED presentation), and subsequent samples were drawn at 8 AM. We did not obtain day 1 samples from subjects presenting after 11 PM or on weekends or holidays for logistic reasons. Each blood sample was drawn into pyrogen-free vials containing heparin and within 1 hour the plasma was separated by centrifugation. The sample was then divided into four 1.5-mL tubes, frozen at –80°C, and batched and shipped on dry ice. We measured cytokine concentrations by chemiluminescent immunoassay using an automated analyzer (IMMULITE; Diagnostic Products Corp, Los Angeles, California), thawing samples only once before assay. We measured IL-6 and IL-10 levels in all samples. We measured TNF levels on day 1 samples for all subjects and on all samples for a subset (the first 1190 subjects).

CLINICAL DEFINITIONS

Subjects were assumed to have pneumonia if they met entry criteria and the primary diagnosis was not revised by the treating physicians in the subsequent 3 days. We defined severe sepsis as pneumonia plus acute organ dysfunction following the 2001 International Consensus Criteria.[26] We defined acute organ dysfunction as a new sepsis-related Organ Failure Assessment (SOFA)[27] score of 3 or higher in any of 6 organ systems, based on the recent international Sepsis Occurrence in the Acutely ill Patient (SOAP) study.[28] We used 90-day mortality as our primary measure of survival, based on end-point recommendations for sepsis trials from 2 recent international expert panels.[29,30] We defined systemic inflammatory response syndrome (SIRS) criteria following the 1992 American College of Chest Physicians/Society of Critical Care Medicine Sepsis Definitions Consensus criteria.[31]

STATISTICAL ANALYSIS

Statistical analyses were performed using SAS software, version 9.1 (SAS Institute, Cary, NC), with statistical significance set at $P<.05$. We assumed a log-normal distribution for cytokine concentrations and analyzed data in natural log scale. We constructed Tobit models to account for data that were truncated

Table 1. Clinical Characteristics at Baseline and During the Study[a]

Characteristic	All (N = 1886)	CAP			Severe Sepsis		
		Severe Sepsis (n = 583)	No Severe Sepsis (n = 1303)	*P* Value	Dead at 90 d (n = 149)	Alive at 90 d (n = 424)	*P* Value
Age, y	67.8 (17) 72	71.5 (16) 76	66.2 (17) 70	<.001	78.4 (12) 81	69.4 (16) 73	<.001
Male sex	983 (52)	329 (56)	654 (50)	.01	82 (55)	241 (57)	.70
White	1524 (81)	493 (85)	1031 (79)	.006	134 (90)	354 (83)	.06
Underlying disease[b]							
Charlson Comorbidity Index >0	1367 (72)	447 (77)	920 (71)	.006	128 (86)	311 (73)	.002
Charlson Comorbidity Index	1.9 (2.2) 1	2.2 (2.3) 1	1.8 (2.2) 1	.001	2.8 (2.5) 2	2.0 (2.2) 1	<.001
Preexisting lung disease	493 (26)	159 (27)	334 (26)	.45	49 (33)	109 (26)	.09
Preexisting cardiovascular disease	487 (26)	163 (28)	324 (25)	.16	41 (28)	120 (28)	.86
Smoking history	1250 (66)	389 (67)	861 (66)	.78	94 (63)	290 (68)	.24
Admitted from a skilled nursing facility	116 (6)	69 (12)	47 (4)	<.001	33 (22)	34 (8)	<.001
PSI	88.0 (32) 86	105.0 (33) 102	80.4 (29) 79	<.001	123.1 (33) 118	99.3 (31) 99	<.001
PSI class				<.001			<.001
I and II	578 (31)	90 (15)	488 (37)		5 (3)	82 (19)	
III	475 (25)	104 (18)	371 (28)		17 (11)	84 (20)	
IV	629 (33)	260 (45)	369 (28)		72 (48)	184 (43)	
V	204 (11)	129 (22)	75 (6)		55 (37)	74 (17)	
APACHE III	56.2 (18) 55	65.9 (20) 65	51.9 (15) 52	<.001	76.4 (21) 75	62.5 (18) 62	<.001
Microbiologic characteristic				<.001			.34
Gram positive	236 (13)	87 (15)	149 (11)		28 (19)	59 (14)	
Gram negative	54 (3)	15 (3)	39 (3)		6 (4)	9 (2)	
Mixed	21 (1)	3 (1)	18 (1)		0 (0)	2 (0)	
Chlamydophila and *Legionella*	6 (0)	5 (1)	1 (0)		2 (1)	3 (1)	
Other	37 (2)	19 (3)	18 (1)		6 (4)	13 (3)	
Unknown	1532 (81)	454 (78)	1078 (83)		107 (72)	338 (80)	
Duration of symptoms prior to ED presentation, d	5.7 (19) 3	6.1 (23) 3	5.5 (16) 3	.08	6.5 (32) 2	5.7 (19) 3	.01
Duration of antibiotics course prior to ED presentation, d	0.8 (1.4) 0	0.8 (1.6) 0	0.8 (1.3) 0	.73	0.8 (2.1) 0	0.8 (1.2) 0	.07
Hospital LOS, d	7.3 (5.0) 6	10.3 (6.7) 8	6.0 (3.3) 5	<.001	11.1 (7.3) 9	10.0 (6.5) 8	.08
ICU use	302 (16)	224 (38)	78 (6)	<.001	75 (50)	148 (35)	.001
Mechanical ventilation use	132 (7)	132 (23)	0 (0)	<.001	52 (35)	79 (19)	<.001

Abbreviations: APACHE III, Acute Physiology and Chronic Health Evaluation III[39]; CAP, community-acquired pneumonia; ED, emergency department; LOS, length of stay; ICU, intensive care unit; PSI, Pneumonia Severity Index.[25]

[a] Unless otherwise indicated data are presented as mean (SD) median value or number (percentage) of subjects.

[b] According to the method of Charlson et al.[35]

because they fell below detection thresholds (ranging from 27% to 71% of data).[32,33] For data from single time points (eg, TNF concentrations on day 1), we used Tobit models to generate daily means and to compare the cytokine concentrations between groups.

For sequential data (eg, serial IL-6 measurements), we conducted regression analysis with mixed models that accounted for correlation of repeated measures over time,[34] incorporating Tobit models as necessary.[33] Models included linear, quadratic, and cubic terms to allow evaluation of trends. We determined differences across outcome groups by testing the significance of the regression coefficient in the models. We adjusted for potential confounders by expanding the models to include age, sex, race, and Charlson Comorbidity Index[35] as covariates. We also assessed the frequencies with which cytokine concentrations were elevated using the following upper limits of normal: IL-6, 5.9 pg/mL; IL-10, 9.1 pg/mL; and TNF, 8.1 pg/mL, per the manufacturer's specifications. We compared differences in the proportion of subjects with elevated concentrations using logistic regression based on generalized estimating equations.[36]

To explore whether discrete cytokine patterns existed, we used a group-based longitudinal analysis of IL-6 and IL-10 data to determine the likely number of informative subgroupings, the pattern of profiles in each subgroup, and the probability of subgroup membership for each individual.[37] This approach handles censored data directly.[38] For each cytokine, we generated 1, 2, 3, and 4 subgroup models, allowing different relationships (linear, quadratic, or cubic) for each subgroup, and selecting the best-fitting model based on the Bayesian Information Criterion.[37] We determined the frequency of different combinations of patterns for the 2 cytokines and, with Cox proportional hazards models, the potential association of these combinations with survival.

RESULTS

STUDY POPULATION AND OUTCOMES

We enrolled 2320 subjects, of whom 291 (13%) were discharged from the ED. Once admitted, 134 (6%) were excluded because their treating physicians subsequently revised their primary diagnosis, and 9 (0.4%) had inadequate blood specimens. The remaining 1886 subjects comprised the inpatient CAP cohort on whom we conducted our analyses (**Figure 1** and **Table 1**). In this

cohort, 583 subjects (31%) developed severe sepsis. In 47% of those developing severe sepsis (n=274), criteria were met on day 1; in 85% (n=496), criteria were met by day 4. The mortality rate rose steeply and was significantly higher for subjects who developed severe sepsis than for those who did not ($P<.001$) (**Figure 2**).

CYTOKINE CONCENTRATIONS AT PRESENTATION

We obtained blood for assay of cytokine concentrations on ED presentation from 1429 of the 1886 subjects (76%) (**Table 2**). Not surprisingly, mean cytokine concentrations were elevated, although 17%, 64%, and 63% of subjects had normal concentrations of IL-6, TNF, and IL-10, respectively. For all 3 cytokines, day 1 concentrations were higher in those with severe sepsis than in those without severe sepsis ($P<.001$) and in nonsurvivors than in survivors ($P=.01$). In addition, among the 1221 subjects who presented without severe sepsis (85%), IL-6 concentrations were higher for those who subsequently developed severe sepsis than for those who did not ($P=.03$).

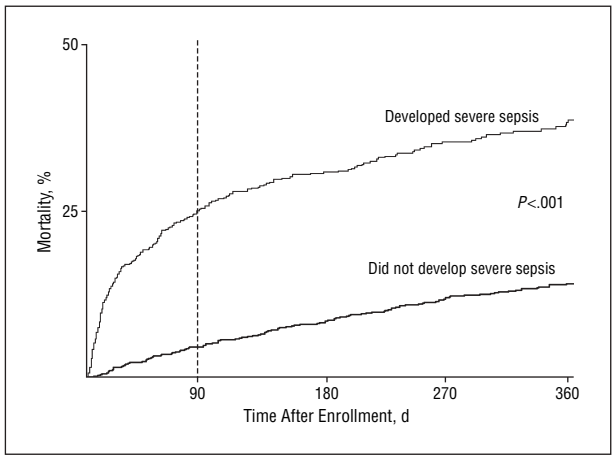


Figure 2. Mortality rates for subjects with community-acquired pneumonia with and without sepsis. Severe sepsis was defined as sepsis with organ failure (Sepsis-related Organ Failure Assessment[27] score ≥3 in any organ system).

Similarly, among subjects who developed severe sepsis at any point, nonsurvivors had higher concentrations of IL-6 and IL-10 than survivors ($P=.01$). Day 1 cytokine concentrations were also available on 285 of the 291 ED discharges (98%) and 101 of the 134 subjects (75%) who were excluded for having a change in primary diagnosis. The differences in day 1 concentrations between survivors and nonsurvivors remained significant when these additional subjects were included in the analysis ($P<.001$).

CYTOKINE CONCENTRATIONS OVER TIME

Cytokine concentrations over time are presented in **Figure 3**. The mean IL-6 concentration fell rapidly from day 1 over the subsequent 2 days but remained elevated throughout the first week. Mean IL-6 concentrations (Figure 3A, top) and the proportion of subjects with elevated concentrations (Figure 3B, top) were higher for those who developed severe sepsis (survivors and nonsurvivors) compared with those who did not ($P<.001$ and $P<.001$) and for those who died following severe sepsis compared with those who survived ($P<.003$). Of note, although mean levels were elevated, a number of subjects had normal concentrations on any given day and 12% of survivors (n=198) and 4% of nonsurvivors (n=8) had normal levels throughout.

The mean TNF concentrations were generally lower than those observed for IL-6, with a large number of subjects displaying normal levels (53% of nonsurvivors and 61% of survivors). Otherwise, the pattern was similar to that seen with IL-6, where levels fell over the first 2 days and remained mildly but persistently elevated thereafter, with higher levels seen among those with severe sepsis (survivors and nonsurvivors) vs those without severe sepsis ($P=.01$ and $P<.001$).

Concentrations of IL-10 were also lower than those observed for IL-6, with more noticeable decay over the first 2 days. A very high proportion of subjects had normal concentrations (64% of survivors and 42% of nonsurvivors had normal levels throughout), but higher levels were associated with survivors with severe sepsis

Table 2. Cytokine Concentrations at Presentation to the Emergency Department[a]

	All			No Severe Sepsis on Day 1			Severe Sepsis During Hospitalization		
Cytokine	Severe Sepsis on Day 1	No Severe Sepsis on Day 1	*P* Value	Developed Subsequent Severe Sepsis	Never Developed Severe Sepsis	*P* Value	Dead at 90 d	Alive at 90 d	*P* Value
IL-6 (n = 1426)									
Level	98.7	38.7	<.001	51.4	36.5	.03	109.4	59.6	.01
Elevated	89.9	82.3	.01	87.4	81.1	.03	92.2	87.1	<.001
IL-10 (n = 1423)									
Level	10.4	5.1	<.001	5.1	5.0	.91	10.7	6.5	.01
Elevated	55.1	34.5	<.001	33.6	34.7	.75	53.0	41.1	.03
TNF (n = 1424)									
Level	7.5	5.5	<.001	6.0	5.5	.20	7.3	6.3	.22
Elevated	45.9	34.3	.001	35.9	33.9	.58	38.4	47.0	.11

Abbreviations: IL, interleukin; TNF, tumor necrosis factor.
[a]Cytokine level data are reported as geometric mean values, which roughly approximate medians, in picograms per milliliter estimated from the Tobit models; elevated levels are reported as percentage of subjects.

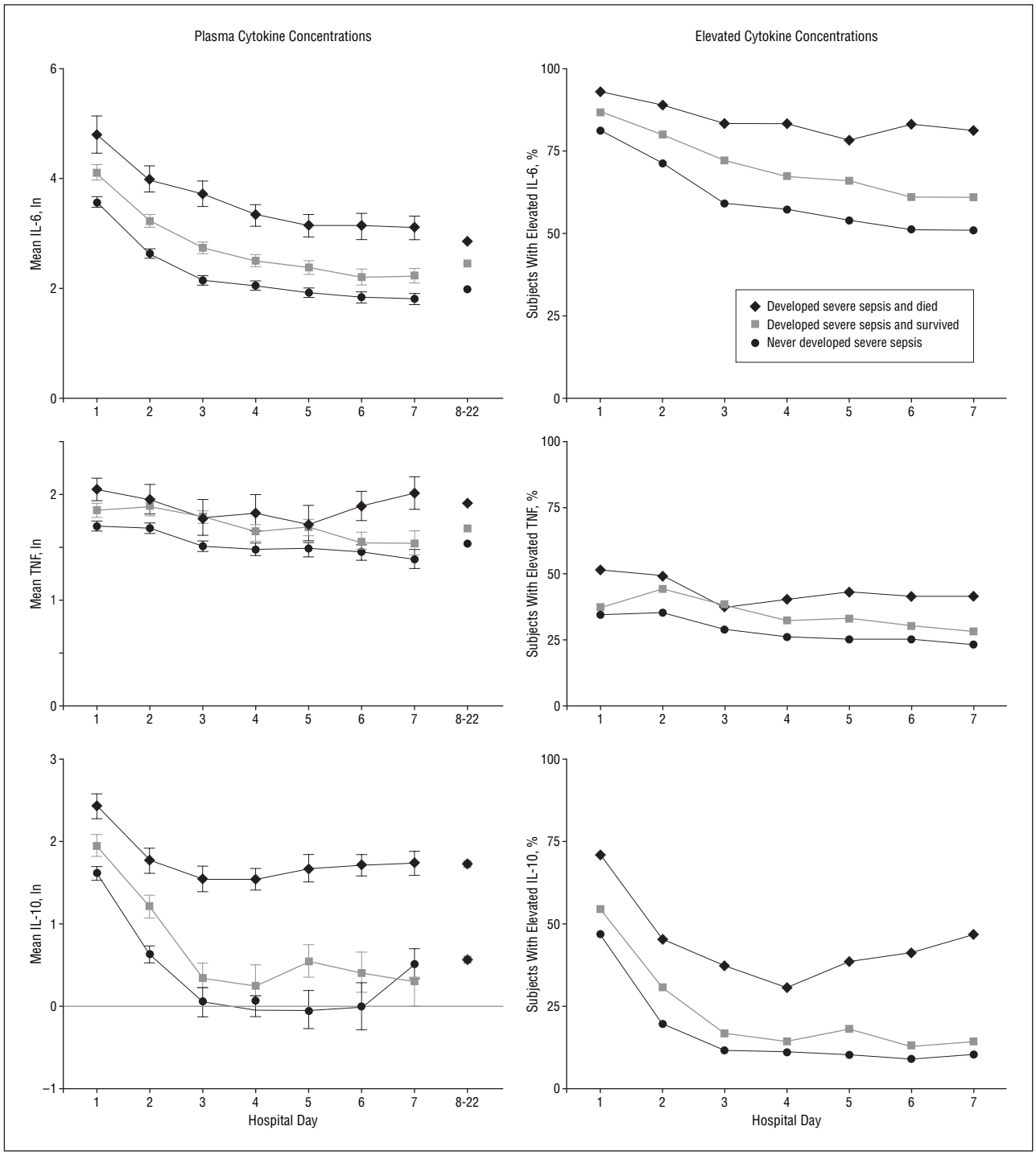
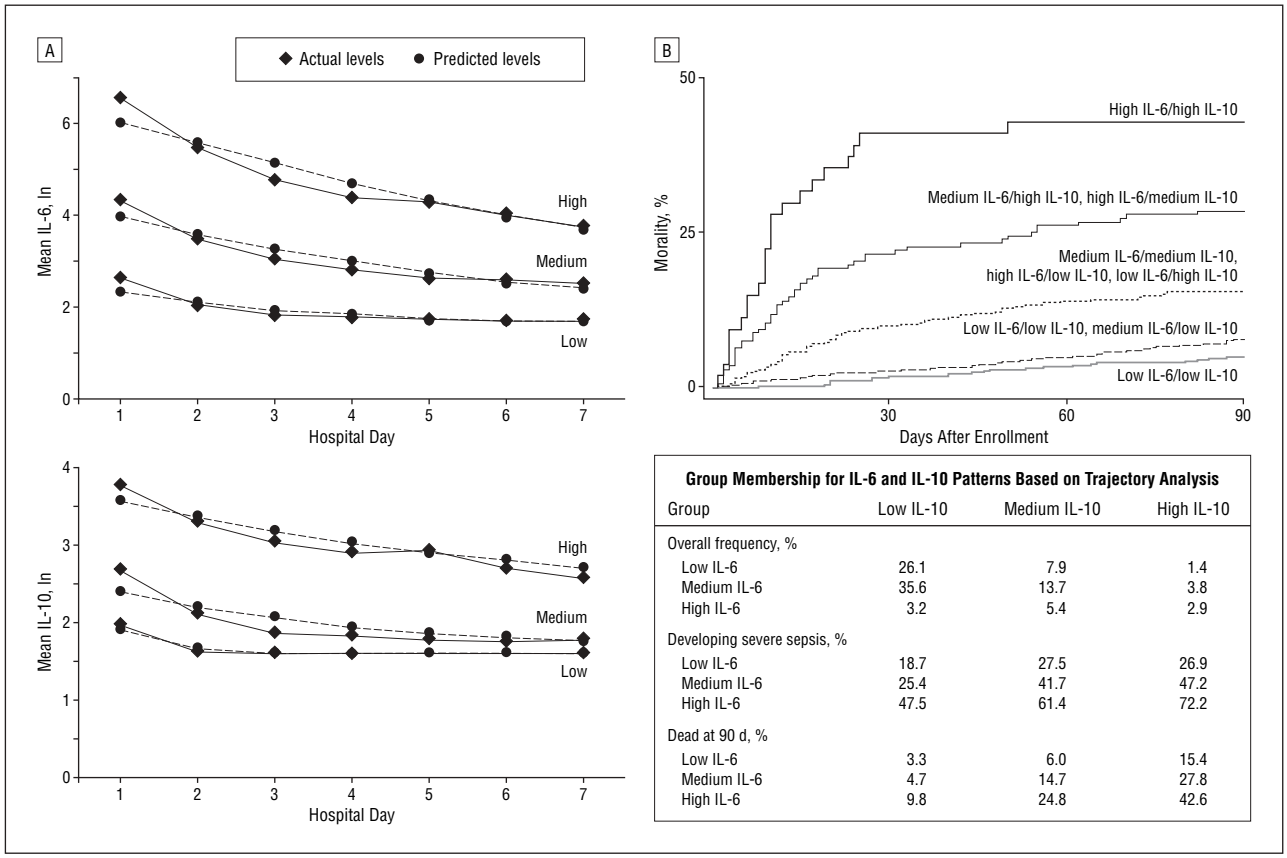


Figure 3. Mean plasma cytokine concentrations (A) and proportion of subjects with community-acquired pneumonia (CAP) with elevated plasma concentrations (B). IL-6 indicates interleukin 6; ln, natural log; and TNF, tumor necrosis factor. A, Mean (ln) plasma cytokine concentrations for subjects with CAP by outcome and over time. B, Percentage of subjects with CAP with elevated concentrations compared with the normal range for each molecule by outcome. Elevated was defined for IL-6 as greater than 5.9 pg/mL; for IL-10, greater than 9.1 pg/mL; and for TNF, greater than 8.1 pg/mL.

compared with survivors without severe sepsis ($P<.001$) and nonsurvivors ($P<.001$) and for nonsurvivors with sepsis vs survivors with sepsis ($P=.002$).

For all 3 cytokines, differences between groups remained significant after adjusting for differences in baseline patient characteristics. Restricting analysis to the subset of subjects who developed severe sepsis after day 1 (n=309), no rise in concentrations or spike occurred for any cytokine immediately prior to or on the day that severe sepsis developed. Of note, although cytokine measurements were often normal, only 4 of the 149 fatal cases of severe sepsis (2.7%) exhibited no cytokine concentration elevation. On the other hand, some amount of systemic cytokine concentration elevation was also present in those who fared well. For example, in the 364 of the 1886 patients who survived (19%) with virtually no or-



Group Membership for IL-6 and IL-10 Patterns Based on Trajectory Analysis

Group	Low IL-10	Medium IL-10	High IL-10
Overall frequency, %			
Low IL-6	26.1	7.9	1.4
Medium IL-6	35.6	13.7	3.8
High IL-6	3.2	5.4	2.9
Developing severe sepsis, %			
Low IL-6	18.7	27.5	26.9
Medium IL-6	25.4	41.7	47.2
High IL-6	47.5	61.4	72.2
Dead at 90 d, %			
Low IL-6	3.3	6.0	15.4
Medium IL-6	4.7	14.7	27.8
High IL-6	9.8	24.8	42.6

Figure 4. Patterns of cytokine response to infection and resulting patterns of outcomes. A, Trajectory analysis of IL-6 (interleukin 6) and IL-10 concentration data suggests 3 distinct patterns of cytokine response during the first 7 days in the hospital. Combinations of 1, 2, 3, or 4 groups with quadratic, cubic, or linear trends of the cytokine levels, irrespective of outcome, were analyzed. For both IL-6 and IL-10, 3-group models (high, medium, and low cytokine concentration) with a quadratic trend for each group exhibited the best fit. In Indicates natural log. B (top), The Kaplan-Meier analysis includes survival curves that represent different combinations of the IL-6/IL-10 cytokine responses. Although there are 9 such combinations, some groups have been collapsed together because of similar survival rates. Five distinct patterns of outcome were observed. The numbers of subjects in each group are as follows: low IL-6, n=667 (35.4%); medium IL-6, n=1003 (53.2%); high IL-6, n=216 (11.4%); low IL-10, n=1255 (65%); medium IL-10, n=509 (27%); and high IL-10, n=152 (8%).

gan dysfunction (sum of worst SOFA score for all organs <1), 79% had elevated cytokine levels on day 1, and 74% after day 1 (days 2-30).

Cytokine levels remained elevated much longer than clinical signs. For example SIRS criteria resolved quickly, with fewer than 50% of subjects having more than 2 SIRS criteria after day 2, whereas more than 50% of subjects continued to have elevated IL-6 levels by day 7.

FREQUENCY AND OUTCOME OF DISTINCT CYTOKINE PATTERNS

Trajectory analyses suggested very distinct cytokine patterns. For both IL-6 and IL-10, 3-group models (high, medium, and low concentrations) with a quadratic trend for each group exhibited the best fit compared with all other combinations of 1-, 2-, or 4-group models with quadratic, cubic, or linear trends (**Figure 4**A). For IL-6, the mean day 1 concentration of the high group was 50-fold higher than that of the low group. Model selection and group membership were similar when the analyses were repeated with inclusion of baseline characteristics as covariates.

Figure 4B shows the distribution and outcomes of the cohort across the combined IL-6 and IL-10 concentration patterns. Of the 9 possible combinations of concentration, 3 accounted for more than 75% of subjects: medium IL-6/low IL-10, 36% (n=672); low IL-6/low IL-10, n=492 (26%); and medium IL-6/medium IL-10, n=259 (14%). Both severe sepsis and mortality rates varied by combination, with the highest rates observed for combinations of higher cytokine expression (**Table 3** and Figure 4B). These findings persisted in Cox proportional hazards models (**Table 4** and **Figure 5**) that also controlled for age, sex, race, and comorbidity. Patterns of relative overexpression or underexpression of IL-6 with respect to IL-10 were uncommon and not obviously associated with very high or very low adverse outcomes.

COMMENT

We found that several of the past interpretations of the inflammatory response to sepsis—at least in patients with CAP—may be incorrect or misleading, with important implications for the development of future therapies. First, although systemic cytokine activation was common, it was not universal. Second, although we initiated our study at the time of presentation in the ED, cytokine concentrations had already peaked, and very few subjects had markedly elevated levels later, suggesting the classic cyto-

Table 3. Cytokine Levels Over Time[a]

Cytokine	Enrollment Day 1	2	3	4	5	6	7	>7
IL-6 (n = 1426)								
Developed severe sepsis and died	4.7	3.8	3.6	3.3	3.1	3.1	3.0	2.9
Developed severe sepsis and survived	4.1	3.3	2.8	2.5	2.4	2.3	2.3	2.4
Did not develop severe sepsis and survived	3.6	2.6	2.1	2.0	1.9	1.8	1.8	1.9
IL-10 (n = 1423)								
Developed severe sepsis and died	2.4	1.6	1.3	1.4	1.5	1.5	1.6	1.8
Developed severe sepsis and survived	1.8	1.3	0.5	0.3	0.7	0.7	0.4	0.6
Did not develop severe sepsis and survived	1.6	0.6	−0.0	−0.1	−0.2	−0.2	0.5	0.3
TNF (n = 1424)								
Developed severe sepsis and died	2.0	1.9	1.7	1.7	1.7	1.8	1.8	1.9
Developed severe sepsis and survived	1.8	1.8	1.8	1.6	1.7	1.6	1.6	1.6
Did not develop severe sepsis and survived	1.7	1.7	1.5	1.5	1.5	1.5	1.4	1.5

Abbreviations: IL, interleukin; TNF, tumor necrosis factor.
[a]All levels are expressed as natural log means. Mortality was measured at 90 days.

Table 4. Cox Proportional Hazards Model for Mortality Including IL-6 and IL-10 Patterns

Variable	Hazard Ratio (95% Confidence Interval)	*P* Value
Age[a]	1.61 (1.41-1.84)	<.001
CCI >0	1.77 (1.17-2.69)	.007
White	1.16 (0.69-1.98)	.57
Female	0.78 (0.57-1.06)	.11
IL-6/IL-10 concentration[b]		
Low/medium	1.79 (0.79-4.05)	.16
Medium/low	1.17 (0.63-2.17)	.61
Low/high	4.27 (1.43-12.78)	.001
High/low	2.94 (1.15-7.53)	.02
Medium/medium	4.45 (2.48-7.99)	<.001
Medium/high	7.55 (3.90-14.61)	<.001
High/medium	9.53 (5.08-17.91)	<.001
High/high	20.52 (10.79-39.04)	<.001

Abbreviations: CCI, Charlson Comorbidity Index[35]; IL, interleukin.
[a]For 10-year increments.
[b]The IL-6/IL-10 patterns were obtained from the trajectory analysis; group membership was assigned based on highest probability; the low/low group was used as the referent.

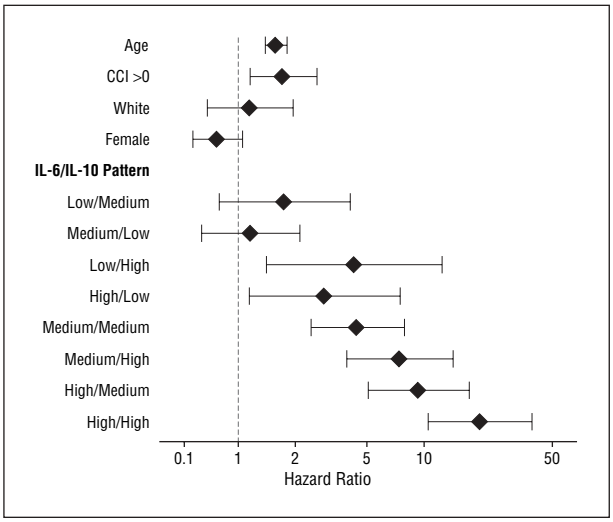


Figure 5. Risk of death among patients with community-acquired pneumonia according to baseline characteristics and plasma cytokine concentrations. Results are from a Cox proportional hazards model for mortality. Point estimates are shown along with their 95% confidence intervals. While race and sex did not seem to contribute to the risk of death, age, comorbidity, and high concentrations of IL-6 (interleukin 6) or IL-10, even combined with low levels of the other cytokine, were associated with death. The combination of medium-level IL-6 and IL-10 also increased the risk of death severalfold, while high levels of both cytokines increased the risk of death by more than 20 times. Age indicates increase in age by 10 years; CCI, Charlson Comorbidity Index[35]; in all low/medium/high concentration combinations, the first term designates IL-6 groups and the second term IL-10 groups, with low/low used as the referent for the hazard ratio of the rest.

kine cascade was already fully activated by the time patients sought hospital care. Third, although cytokines may be released secondary to organ damage or dysfunction,[40,41] we did not see an increase in cytokine concentrations with the onset of organ dysfunction. Fourth, although concentrations were higher in those who fared worse, differences between groups with different outcomes were modest. Furthermore, systemic activation was a prominent feature in many subjects who mounted a successful response to infection, suggesting such systemic activation may be neither overly exuberant nor necessarily deleterious.

Fifth, for many subjects, cytokine activation persisted throughout the hospital course, far longer than traditional models would suggest, and far longer than the typical courses of anticytokine strategies in prior negative trials. For example, Halm et al[42] showed that clinical signs of sepsis (tachycardia, tachypnea, and fever) resolve within 2 to 3 days after admission for CAP, and in both failed anti-TNF antibody studies,[6,9] a drug was administered for only 3 days. The duration of cytokine activation was also longer than clinical signs such as SIRS criteria. Finally, although different subjects had different patterns of cytokine response, these patterns could best be described as high, medium, and low generalized activation. *Immunologic dissonance*, defined as a preponderance of either proinflammatory or anti-inflammatory cytokine activation,[43] was neither common nor obviously associated with poor outcome.

Although our findings are somewhat discordant with conventional thinking, they reinforce the findings of 3 small single-center studies of serial cytokine concentrations in severe sepsis.[44-46] These studies reported modest cytokine elevations with an absence of a spike and continued activation over several days. More recently, Kinasewitz et al[47] reported the cytokine patterns in subjects enrolled in the PROWESS trial (Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis).[47] These data were also similar, with low levels of IL-10 and persistent, slowly decaying IL-6 concentrations.

The current effort to delineate the natural history of sepsis provides insight into the potential reasons for failure in prior anticytokine strategies and guidance for future therapies. First, treatments designed to manipulate early cytokine activation are at best impractical, especially for community-acquired sepsis, since the classic cascade seen in human volunteer and animal studies either does not occur or occurs before presentation. Second, a "1 size fits all" anticytokine approach is likely ineffective and potentially dangerous because not all patients mount a cytokine response, and many patients who mount a cytokine response fare well. Third, the highest risk of death was seen in patients who expressed high levels of both proinflammatory and anti-inflammatory cytokines, suggesting that therapy targeted at individual cytokines might not be useful. Finally, typical dosing over 2 or 3 days may be too short, since cytokine activation is often persistent. It is notable that successful trials of corticosteroids for CAP[48] and septic shock[49] used a 1-week course.

There are important caveats and limitations to our work. First, limiting our study to CAP reduced unwanted heterogeneity but may affect the generalizability of our findings. Extrapolating our findings to severe sepsis arising from other types of infection is speculative. Second, recruiting a large number of patients from multiple centers provided power to explore different patterns but placed logistic constraints on our blood sampling frame. It was impractical to draw blood samples more frequently than daily, which limited our ability to explore whether more time-sensitive patterns existed. It was also impractical to draw blood samples after hospital discharge. However, we did not find evidence of discharge-related informative censoring affecting our estimates of the serial patterns. Third, we relied on statistical inference to determine the presence of discrete cytokine patterns, which necessarily is limited by power to find rare patterns. Fourth, we relied on the circulating concentrations of just 3 cytokines as biomarkers of the innate immune response. There may well be important patterns at the local or tissue level, within the cellular component of the immune response, or within other acute response cascades, which are not reflected by changes in these biomarkers. Furthermore, these cytokines themselves have multiple, context-specific effects and are not easily classified as proinflammatory or anti-inflammatory.

In conclusion, we have, for the first time to our knowledge, described the systemic cytokine response to infection and severe sepsis in a large, multicenter inception cohort of subjects presenting to the ED with CAP. Our results show that this response is much longer in duration and much more heterogeneous than suggested by previous studies. Nevertheless, individuals with high circulating levels of both proinflammatory and anti-inflammatory cytokines had a markedly increased risk of severe sepsis and death.

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