The Meta-Genome of Sepsis: Host Genetics, Pathogens and the Acute Immune Response

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Key Words

Genetics · Sepsis · Gene expression · Meta-genome · Microbiome

Abstract

Severe infection and the patient response constitute sepsis. Here, we review the meta-genome (patient genetics, pathogen communities and host response) and its impact upon the outcome of severe sepsis. Patient genetics, both predisposition for infection and the subsequent response to infection are reviewed. The pathogen is discussed with particular emphasis upon the modern era of microbiome analysis and nucleic acid diagnostics. Finally, we discuss the host clinical and immune responses and present new data to suggest that the immune response is the key to understanding sepsis and improving a death rate of nearly 30%.

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The Clinical Presentation and Treatment of Sepsis

Sepsis is a sequela of bacterial infections that kills more than 200,000 people in North America annually. According to the Surviving Sepsis group [1], the number of cases

globally reaches 18 million/year (<http://www.surviving-sepsis.org/>), and with a mortality rate of 30–35% it is one of the leading causes of death worldwide. Sepsis is a clinical response to a suspected or proven infection and is defined by two or more of the following SIRS signs: tachypnea, tachycardia, leukocytosis or leukopenia, and hyperthermia or hypothermia [2]. Severe sepsis is sepsis plus a new organ dysfunction due to sepsis [2].

Clinical Presentation: Host Response

In early sepsis, a strong inflammatory response is triggered due to innate recognition of microbial signature molecules by pattern recognition receptors [3]. We have recently shown that at least 39 cytokines, chemokines and growth factors are highly regulated during this period [4, 5] (table 1). In cases of severe sepsis, the inflammation is more intense, and there are greater plasma levels of cytokines accompanied by a heightened physiologic response and secondary organ dysfunction [4, 5]. In the most extreme cases, a vicious cycle revolving around inflammation and coagulation leads to multiple organ failure and death within hours to days. The threat to organ function posed by the systemic hyperinflammatory response has

Table 1. Names and abbreviations of cytokines, chemokines and growth factors

EGF	epidermal growth factor
Eotaxin	eotaxin
FGF-2	basic fibroblast growth factor
Flt-3 ligand	Fms-related tyrosine kinase 3 ligand
Fractalkine	fractalkine
G-CSF	granulocyte colony-stimulating factor
GM-CSF	granulocyte-macrophage colony-stimulating factor
GRO	CXCL1
IFN- α_2	interferon- α_2
IFN- γ	interferon- γ
IL-10	interleukin-10
IL-12 (p40)	interleukin-12 (p40 subunit)
IL-12 (p70)	interleukin-12 (p70 subunit)
IL-13	interleukin-13
IL-1	interleukin-1
IL-17	interleukin-17
IL-1ra	interleukin-1 receptor antagonist
IL-1 α	interleukin-1 α
IL-1 β	interleukin-1 β
IL-2	interleukin-2
IL-3	interleukin-3
IL-4	interleukin-4
IL-5	interleukin-5
IL-6	interleukin-6
IL-7	interleukin-7
IL-8	interleukin-8
IL-9	interleukin-9
IP-10	CXCL10
MCP-1	CCL2
MCP-3	CCL7
MDC	CCL22
MIP-1 α , MIP-1	macrophage inflammatory protein-1 α
TGF- α	transforming growth factor- α
TNF- α	tumor necrosis factor- α
TNF- β	tumor necrosis factor- β
VEGF	vascular endothelial growth factor
sCD40L	soluble CD40-ligand
sIL-2R α	soluble IL-2 receptor- α

been thought to explain the subsequent shift towards an immune-suppressed state. This progression to an immunodepressed phenotype has been implicated as a cause of secondary infections. Characteristics of the immunosuppressive stage include anergy, lymphopenia, hypothermia and nosocomial infections. There is an increase in circulating anti-inflammatory cytokines such as IL-10, apoptosis of B and CD4+ T lymphocytes [6], an increased fraction of regulatory T lymphocytes [7], and markedly decreased expression of monocyte HLA-DR. This second phase of sepsis is thought to explain the high subacute

mortality associated with sepsis. Clinicians could potentially treat either the early hyperimmune organ failure or the later immunosuppressed state of patients with either potent immunosuppressants, such as corticosteroids, or selective immune adjuvants (or antibiotics), respectively. However, prevention of immunosuppression is not currently possible given uncertainties regarding accurate clinical and laboratory assessment of immune status.

Possibly due to this inability to determine real-time immune status in clinical settings, randomized controlled trials (RCTs) of both immune suppressants and immune adjuvants have been uniformly disappointing. For example, RCTs have been performed in which the approach was to render circulating immune-modulatory factors such as bacterial products invisible to the immune system using intravenous pooled immune globulin (IVIG). However, while numerous small studies of IVIG treatment in septic shock appeared to improve mortality [8], a large multicenter RCT did not show a mortality benefit in patients randomized to IVIG [9].

During the 1980s and before, the predominant organisms responsible for severe sepsis and septic shock were Gram-negative bacteria [10, 11]; thus, most therapies targeted cell wall components of these organisms. The first line of antiendotoxin therapy was a lipid A-specific antibody HA-1A. A multicenter RCT of HA-1A in septic shock showed no survival benefit in these very sick patients [12]. In 2000, 2 large RCTs of a murine antiendotoxin antibody to [13], and separately an antibody against Enterobacteriaceae common antigen [14] in patients in septic shock found no survival advantage to either therapy.

TNF- α can reproduce many of the key physiologic derangements seen in early sepsis, thus spurring numerous RCTs using neutralizing antibodies to TNF- α and its receptor [15–19]. Despite excellent neutralization of the biologic activity of TNF- α , none of these RCTs found a survival advantage with TNF- α neutralization treatment.

Steroids are the cornerstone of therapy for acute (non-specific) inflammation such as exacerbations of asthma. In the 1980s, 3 RCTs examined high-dose (30 mg/kg methylprednisolone) steroids in patients with septic shock [20–22]. All of these failed to show a survival benefit to those randomized to receive intravenous steroids, but did demonstrate a trend to faster resolution of shock (discontinuation of vasopressors) at the cost of increased secondary/nosocomial infections. More recent well-powered RCTs have used lower, less immunosuppressing doses of steroids (in part to treat acute adrenal insufficiency), and have provided conflicting results as to the

benefits associated with steroid therapy [23, 24]. The French RCT of Annane et al. [23] found that patients who had an abnormal adrenal axis (as assessed by ACTH stimulation test) who received corticosteroids (hydrocortisone plus fludrocortisone) had higher survival than patients on placebo. In distinct contrast, the later CORTICUS RCT found no difference in survival between corticosteroid and placebo-treated patients overall or in the patients who had an abnormal ACTH stimulation test [24]. The most recent Surviving Sepsis Guideline [25] suggests 'consideration' of corticosteroids only for patients with refractory septic shock.

Clinical Presentation: Infectious Organism

Our sepsis research group at UBC led one of the largest RCTs of septic shock, the Vasopressin in Septic Shock Trial (VASST) [26]. While the intervention tested was vasopressin plus norepinephrine versus norepinephrine alone, clinical and microbiological data were collected regarding the definite and presumably causative infectious organisms. In 778 patients admitted with septic shock at 27 centers across Canada, the USA and Australia in VASST, only 26% of blood cultures identified an infection in the VASST cohort, and only 57% of cultures from the primary site, e.g. sputum culture from pneumonia patients, grew an identifiable organism. In those for whom timed data were available (patients recruited at our institution), there was a median delay of 30 h from meeting shock criteria until preliminary standard microbiology report of cultures with identification of the generic organism type (e.g. Gram-positive cocci). A further delay of 24–48 h occurred in speciating the organism (e.g. *Staphylococcus aureus*), and determining antibiotic resistance (e.g. MRSA). In our view, this delay (24–48 h or more) and lack of accuracy in precise organism identification of infectious organism(s) in patients who have septic shock is completely inadequate in septic shock because inappropriate antibiotic selection increases the relative risk of dying by as much as 8% per hour [27–30]. Thus, technique(s) to rapidly and accurately identify the infecting organism(s) could lead to earlier more accurate antibiotic selection in septic shock. We emphasize that it is not only the delays to microbiology results reporting, but the very low sensitivity of traditional microbial culture, which thwarts accurate microbial diagnosis. In a disease with a stunning 15% mortality in the first 2 days and 25–35% by 28 days, the clinician receives no new diagnostic information throughout the critical very early

phase of disease and treatment. Furthermore, in the era of the microbiome, it has become very obvious that only a fraction of potentially pathogenic organisms can be cultured in standard media [31–33], i.e. current clinical microbiology testing is likely very insensitive. This low sensitivity suggests that treating physicians often do not know the true cause(s) of severe infection. Furthermore, this lack of early accurate microbiologic diagnosis also impairs our evaluation and understanding of unique host factors that influence therapeutic success in septic shock. To restate, we suggest that better outcomes of septic shock could be achieved by (1) early accurate organism identification paired with (2) early accurate host genomic, immunity evaluation – the essence of the metagenomic approach.

Sepsis in the Era of the Meta-Genome

Host Genetics and Risk of Infection

Human host genetics may determine the risk of acquiring an infection (i.e. a relative immunodeficiency). While some disorders such as severe combined immunodeficiency reflect rare mendelian genetic mutations in the common gamma chain, adenosine deaminase or other more rare mutations, these mutations are invariably detected in childhood due to recurrent serious infections. In adults with no known immune-deficiency, it is more challenging to determine which (if any) genetic polymorphisms lead to an increased risk of acquiring infection, mainly due to the challenge of determining exposure to the pathogen(s). Despite this inherent limitation of delayed, insensitive organism identification, some polymorphisms appear to confer increased risk to specific pathogens. For example, polymorphisms in the known innate immune molecule Toll-like receptor 2 and the anti-infective cytokine IL-17A gene increase the risk of serious Gram-positive infections [34, 35]. A polymorphism in CD14, a key molecule in the innate immune response to Gram-negative endotoxin has been found to increase the risk of serious Gram-negative infections [34], while polymorphisms in the complement-activating mannose-binding lectin results in increased serious infections [34]. A functional polymorphism of IRAK4 increases the risk of Gram-positive infections in childhood and in adult septic shock [36].

The risk of acquiring specific pathogens also appears to be influenced by human host genetic variation. For instance, *Neisseria meningitidis* infection is detected more frequently in humans with polymorphisms in IL-1 and

TNF- α genes [37], while polymorphisms in the inflammasome gene CARD-8 increase the risk of mycobacterial infection in HIV+ patients [38].

Human host genetic variation may also influence the response to an infection, including the degree of immune activation and secondary organ failure. In the innate immune response, haplotype clades in the integrative inflammatory cytokine IL-6 increase the risk of organ failure and death in those who present with a severe infection [39], while polymorphisms in IL-8, protein C and NF κ B-inducing kinase are associated with increased and more severe organ failure complicating septic shock [40–43]. Because treatment of septic shock is largely supportive, aimed at early broad-spectrum (often nonspecific antibiotics), normalizing blood pressure and organ perfusion [25], polymorphisms in genes active in the vasopressor response are also associated with increased risk of organ dysfunction. A polymorphism in the beta-2 adrenergic receptor gene known to confer hyposensitivity to catecholamines necessitates higher doses of vasopressors and increases mortality [44]. A variant in the gene responsible for clearing vasopressin from plasma (LNPEP also known as vasopressinase) alters the pharmacokinetics of vasopressin infusion and increases the risk of death from septic shock [45]. Polymorphisms in the negative regulator of the angiotensin II receptor (AGTRAP) are also associated with increased mortality and blood pressure in patients with septic shock [46].

While these relatively common human host genetic variants (polymorphisms) influence both the susceptibility to and outcome from infection, there are to date no biomarkers that are predictive of response to drugs used in septic shock in part because of the paucity of novel therapeutics in severe sepsis and septic shock.

The Roles of the Infecting Pathogen

The true ecology at the site of infection and circulating bacterial ‘load’ has yet to be defined in sepsis. The current use of a binary approach to culture (positive vs. negative) does not quantitate the bacterial density or provide evidence of all organisms present in the presumably infected materials [47]. Bacterial growth in cultures, if it happens, typically does not occur for several hours to days, and later sensitivity reporting does not occur until 24–48 h into the course of severe sepsis and septic shock [48]. Moreover, blood cultures in particular lack sensitivity and are positive only 30% of the time in cases of sepsis [47]. Molecular diagnostics of the infect-

ing pathogens represent a novel method to assess and diagnose the breadth of specific bacterial species and their density (often also called ‘bacterial load’ or ‘burden’).

Pathogen Density in Circulating Blood

Higher bacterial loads predict worse outcome in infections including Gram-negative sepsis [49], methicillin-resistant *S. aureus* bacteremia [50] and bacterial meningitis [51, 52]. Thus, bacterial load could be conceived as and indeed is used as a prognostic biomarker. At this stage, there is equipoise as to which method (traditional culture or molecular diagnostics) is most representative of the biological ‘truth’. Regarding molecular diagnostic approaches, there appears to be a threshold level below which infecting organism nucleic acid detection cannot detect bacterial DNA. PCR of bacterial DNA appears most sensitive currently. However, PCR fails to detect organisms identified by standard microbial culture between 20 and 30% of the time [47]. In another cohort study, a universal bacterial detection method using 16S PCR was positive in only 50% of positive blood cultures [53]. A later prospective cohort study compared the diagnostic accuracy of 16S rDNA detection in bacterial meningitis, early-onset neonatal sepsis and spontaneous bacterial peritonitis [54]. The molecular strategy was more accurate than conventional cultures for bacterial meningitis and neonatal sepsis but gave notably worse results (i.e. decreased sensitivity) for the peritoneal fluid of patients with possible spontaneous bacterial peritonitis. Thus, most clinicians do not appreciate that PCR has a lower rate of contamination than blood cultures likely because of the need for a higher bacterial load for 16S rDNA detection than conventional blood culture [55]. Recently, the potential for quantitative PCR testing in organisms which are difficult to culture (e.g. *Acinetobacter baumannii*) was highlighted in a prospective cohort study that used bacterial load measurements to modify ongoing antibiotic treatment in critically ill patients with *A. baumannii* bacteremia [56]. Bacterial loads in this study were correlated with successful bacterial clearance defined by clinical laboratory microbiology and informed the treating team when it was appropriate to discontinue antibiotics. Thus, it appears that if an alternate technology with higher sensitivity to PCR to detect a pan-pathogen nucleic acid region were available, there could be rapid adoption of such testing to be used as a predictive biomarker to guide antibiotic therapy.

The Microbiome at the Site of Infection and in Blood

Current PCR-based techniques are limited in identifying unique pathogens in blood due to the relatively low abundance of bacteria. Commercially available multiplex PCR microbial detection kits are licensed to detect pathogen DNA directly from blood and are approved for clinical use in Europe [57]. Implementation of multiplex PCR microbial detection kits yields earlier pathogen detection as well as pathogen detection despite negative conventional microbial cultures [58–60]. However, results of multiplex PCR tests are not unequivocally better than blood cultures because of difficulty in assigning the ‘gold standard of infection’ [29–31]. In a study of 245 patients with possible sepsis [32], a multiplex PCR kit provided results in 24 h compared to 68 h for blood culture, and had a significantly higher positive microbial detection rate than blood cultures (30 vs. 14%). However, regarding specificity, only 17 of 53 positive blood cultures were reproduced as positive by PCR. Further clouding the validity of these molecular approaches is the notion that pathogen identification from blood (by culture or molecular methods) almost certainly underestimates the true breadth of the microbial community (microbiome) at the site of infection. For instance, it is now known that gut microbiome contains hundreds of species of bacteria [61–63]. In patients who had perforated colon in the VASST study of septic shock, the median number of cultured organisms in both blood and peritoneal fluid was just 2 [26]. At most, 4 organisms were identified through bacterial culture. Traditional culture clearly underestimates the biodiversity of the infecting bacterial community.

Thus, our review of the literature and our exploration of the VASST study lead to our overarching hypothesis that 16S bacterial rRNA sequencing might have a role in accurately defining bacterial species at the site of infection where the density of pathogens is much higher than in blood.

The Microbiome of Community-Acquired Pneumonia

Accordingly, from 2011 to 2013, we enrolled 40 patients with severe community-acquired pneumonia and 14 noninfected elective preoperative control patients from whom we obtained sputum and tracheal aspirates (at the time of intubation), respectively, in a prospective cohort study approved by the Providence Health Care Research Ethics Board. 10 ml of sterile saline was instilled

into the endotracheal tube at intubation and 5 ml aspirated into a sterile container. Standard curves for RT-qPCR were constructed from DNA extracted from *Escherichia coli* DH5 α bacteria grown in LB broth, using DNeasy kit (QIAGEN, 69506) comparing cycle threshold versus copy number in which DNA is diluted 10-fold from 9.92×10^{-7} to 9.92×10^{-1} ng/ μ l (corresponding to 1,000,000 to 1 bacterial genomes). Real-time PCR (qPCR) was performed on the ViiA7 system (Applied Biosystems) for a 466-bp fragment of the bacterial 16S ribosomal DNA that was amplified using the forward primer 5'-TCCTACGGGAGGCAGCAGT-3', and 5'-GGAC-TACCAGGGTATCTAATCCTGTT-3' as the reverse primer, as originally described by Nadkarni et al. [64]. Quantification and detection of the amplified products were measured using a DNA-binding dye (SYBR green), rather than the original TaqMan probe. Bacterial copy number was derived from the *E. coli* DH5 α -derived standard curve detailed above. We compared the pan-bacterial copy number per ml of aspirate with our ability to generate adequate Roche 454FLX 16S rRNA libraries. We utilized the NIH protocols (www.hmpdacc.org) developed for the Human Microbiome Project by performing Roche 454 sequencing of PCR-amplified regions of the V3–V5 region of the 16S rRNA, and defined success as a visually discernable gel band of bar-coded amplicon and by the generation of at least 1,000 reads greater than 250 bp per pooled 1/96th plate. Figure 1 demonstrates the cut-off values for rRNA 454 library generation. The lowest density of bacterial genomes we were able to successfully amplify was 250,000 copies/ml. All 40 patients and 4 of 14 healthy controls had successful microbiome sequencing. Thus, this technology, in conjunction with a measure of bacterial load, appears to hold great promise for improved diagnosis of the respiratory pathogens responsible for severe pneumonia.

Acute Immune Response to Infection

The immune response to infection is a complex amalgam of the dose and duration of exposure to pathogen-associated molecular patterns (PAMPs), the patient's underlying immune status and medical interventions. Calvano et al. [65] assessed a simple model of infection: the change over 24 h in genome-wide expression levels of circulating leukocytes after a single exposure of 2 ng per kg of the PAMP lipopolysaccharide (fig. 2). While over 1,400 genes were significantly regulated with a peak at 4 h, by 24 h the subjects' leukocyte gene expression had returned

Fig. 1. Bacterial copy number per ml of tracheal aspirate vs. total number of mapped sequences using Roche 454 sequencing of the V3–V5 region of the bacterial rRNA genome in patients with pneumonia. The lowest bacterial density that resulted in a successful amplification and microbiome analysis was 250,000 copies/ml.

to baseline and the subjects suffered no adverse dysfunction. Consider in distinct contrast patients with severe sepsis in whom the ongoing stimulus of an active infection is characterized by a prolonged hyperinflammatory state that often leads to multiple organ dysfunction. We evaluated the cytokine, chemokine and growth factor response in 362 patients with septic shock. Cytokine concentrations were measured on a Luminex platform and converted to molar values using published sizes for cytokine molecular weights. At median times of 12 and 36 h following the onset of septic shock, we defined unique patterns of the 39 cytokines, chemokines and growth factors; in many cases, the values were 500 times normal values [4] (fig. 3). In these patients, we sought to understand whether the alteration in cytokines was simply a marker of the severity of underlying illness and chronic health, or whether some component of the immune response was predictive of subsequent mortality independent of clinical variables. Therefore, we assessed common clinical variables (table 2) and the aforementioned cytokines, chemokines and growth factor protein levels at baseline and 24 h after onset of septic shock in patients ($n = 362$) from the VASST study.

We used a sophisticated analytic approach to this complex dataset. In brief, the rpart R package was used for decision tree analysis (R version 2.12). For classification models (parameter method = class), decision trees were constructed for association with survival at 28 days. Values for complexity parameter and minsplit were based on minimizing the 10× cross-validated error. Weightings were applied to negative cases (death) as inverse proportion of fraction dead to bias models toward predicting death at the cost of accuracy for predicting positive cases (survival). Weightings for negative cases were 3 for 28-day survival. Survival (rate) analysis was performed using rpart (parameter method = exp) for patient survival, and days alive and free of renal dysfunction. Statistical differences of survival curves were assessed with the survdiff method of R package survival using a G-rho rank test. Correlation analysis between clinical features and cytokine levels was performed with R cor.test method from the core stats package, using Kendall tau rank correlation method. The rpart R package was used for decision tree analysis (R version 2.12). Using classification models (parameter method = 'class'), decision trees were constructed for survival outcome at 28 days. Values for complexity parameter and minsplit were based on maximizing the

Fig. 2. Samples from 8 healthy volunteers were tested at baseline (0 h) and 2, 4, 6, 9 and 24 h after intravenous administration of 2 ng/kg endotoxin (4 subjects) or vehicle (4 subjects). Significant (false discovery rate of <0.1%) probe sets (5,093) were subjected to

K-means clustering into 10 bins (0–9). Probe sets for which the abundance was above the mean are shown in red, below the mean are shown in blue, and equivalent to the mean are in white. Reproduced with permission from Nadkarni et al. [64].

10× cross-validated specificity done outside the rpart execution to allow for weightings on survival/nonsurvival classes. For 28-day survival, nonsurvivors comprised ~1/3 of patients and weighting on nonsurvivor cases was 3; similarly, day-5 survival and late survival was approximately 1/10, and weighting on nonsurvivor cases was 10. For comparison, we used random forest to determine whether performance improvements could be made with more complex models. Survival analysis was performed using rpart (parameter method = ‘exp’) for patient survival, and days alive and free of renal dysfunction. For survival analysis, the complexity parameter was chosen as the minimum that gave a cross-validated error clearly exceeding the minimum obtained error. A tree analysis was

run with $cp = 0.001$; from this rpart model, the minimum error (‘xerror’) was added to the variance in xerror (‘xstd’); the minimum value of cp giving less xerror than this was chosen. Statistical differences of survival curves were assessed with the ‘survdif’ method of R package ‘survival’ using a G-rho rank test.

Correlation analysis between clinical features and cytokine levels was performed with R cor.test method from stats package, using Kendall tau rank correlation method.

Survival rate survival tree analysis of time of death versus cytokines consists of only three splits; IL-8 measured at 24 h, the difference between CSF2 measured at 24 h and baseline, and IL-1B at baseline (fig. 4). The resulting four groups of patients had dramatically different mortality: 79

Fig. 3. Cytokine cluster analysis of baseline plasma cytokine, chemokines and growth factor levels in patients who had septic shock. Patient subgrouping, survival and features are indicated on the top colored rows. The baseline groups are the low (green), medium (yellow) and high (red) subgroups; they were identified based on plasma cytokines measured at a median of 12 h following the onset

of severe infection (septic shock). For the most part, one can predict low and medium cytokine subgroups, referred to on the upper track as baseline groups (by markers) through partitioning based upon IL-2 and CSF2, while the high cytokine group requires additional cytokine information. Reproduced with permission from Fjell et al. [4].

and 81% (leaves 3 and 11), and 26 and 28% (leaves 4 and 10). The survival rate tree survival analysis using all clinical features and cytokine measurements produced a tree with three splits and four leaves. Only one clinical feature (age) could predict survival as well as cytokine levels.

This decision tree analysis incorporating clinical variables and early plasma cytokine, chemokine and growth

factor levels in patients with septic shock highlights the importance of the immune response that complements the clinical predictors to model survival (Apache II score) and organ derangement (e.g. acute renal dysfunction). Of all clinical features and cytokine, chemokine and growth factor levels, the strongest distinguishing characteristic was plasma IL-8 measured at 24 h (the top split in fig. 5).

Fig. 4. Decision tree for 28-day survival using cytokine data in patients who had septic shock based on measurement and decision tree analysis plasma levels taken at baseline and at 24 h of 39 cytokines, chemokines and growth factors. The three numbers in each box are the estimated rates of death scaled to the overall rate, and the number of deaths over the number of patients in the group (e.g. for leaf 3, the number of deaths per time was 3.3 times the overall rate, and 41 of 52 patients died). Splits higher on the tree reflect more important (prominent) factors impacting survival.

Fig. 5. Decision tree for 28-day survival using baseline clinical variables and cytokine data in patients who had septic shock based on measurement and decision tree analysis plasma levels taken at baseline and at 24 h of 39 cytokines, chemokines and growth factors. As in figure 4, the three numbers in each box are the estimated rates of death scaled to the overall rate, and the number of deaths over the number of patients in the group. Splits higher on the tree reflect more important (prominent) factors impacting survival.

Table 2. Clinical variables that were used in outcome modeling combined with plasma levels of 39 cytokines, chemokines and growth factors in patients who had septic shock

Category	Variable
Demographics	age gender body mass index ethnicity height weight
Preexisting medical problems	alcohol abuse cancer CHF CNS pathology COPD diabetes end-stage renal disease immunocompromised ischemic heart disease cirrhosis previous organ transplant previous steroid use APACHEII first 24 h most abnormal arterial pressure lowest cardiac output (thermo-dilution) central venous pressure most abnormal temperature urine output
Physiology (daily over 28 days)	white blood cell count bilirubin baseline creatinine lactate PaO ₂ /FiO ₂ platelets HCO ₃ potassium sodium anaerobic organism on culture any positive microbiology culture fungi on culture arterial pH
Laboratory at presentation	PEEP (ventilation) pressure control level (ventilation) phenylephrine milrinone fluid administration (ml) epinephrine dialysis dobutamine dopamine activate protein C administered dose of norepinephrine
Therapies	

Using a plasma protein survey, which is large by cytokine standards but small given the scope of genomic changes, we were able to define IL-8, CSF2, CCL-11 and IL-1B as important immune regulators of outcome in a prototypical very severe infection, i.e. septic shock.

Currently, RNA sequencing techniques deliver for log scale greater dynamic range than microarray and can identify even single molecules present in a transcriptome, i.e. incredibly high sensitivity. Using this new technology to accurately and quickly identify infecting pathogens and to probe and obtain signatures of the host immune response to infection will likely result in the identification of novel critical regulators of immune function and secondary organ dysfunction and earlier more accurate treatment decisions in severe infections such as septic shock.

Conclusions

The clinical presentation and outcomes of infection are the result of the complex interplay of host genetics, individual pathogen type and load and finally the genome-wide expression response to infection in circulating leukocytes. The inherited (genetic) component of the

meta-genome includes the susceptibility to infection (i.e. that could one day yield diagnostic biomarkers), the pace and extent of the immune response, the risk of later organ dysfunction and death (i.e. prognostic biomarkers) and ability to respond to therapies (i.e. predictive biomarkers). Pathogen load and speciation is a concept not yet used in everyday clinical medicine, but it appears that new nucleic acid technologies will usher in a new era of first treating to rapidly and accurately suppress known pathogens, and second, identification of new and emerging pathogens. The levels and fluctuations of key regulators of acute inflammation (such as IL-8, CSF2, CCL-11 and IL-1B) predict subsequent organ dysfunction and death from severe infection. We anticipate that new targets for therapy in severe sepsis and septic shock will emerge from unbiased genome-wide assessment of the immune response.

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