

COMMENTARY

Sepsis in transit: from clinical to molecular classification

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See related research by Maslove *et al.*, <http://ccforum.com/content/16/5/R183>

Abstract

In the previous issue of *Critical Care*, Maslove and colleagues studied circulating neutrophil transcriptional expression to discover and validate a molecular subclassification of adult patients with sepsis. The authors divided patients into small derivation ($n = 55$) and validation ($n = 71$) cohorts. Their complex methodology included partitioning around medoid and hierarchical clustering methods to define two transcriptionally distinct subtypes of sepsis. Pathway analysis found that chemokine and cytokine pathways as well as Toll-like receptor signaling were enhanced. Investigation of specific drug target genes relevant to sepsis found significantly different expression levels between the two molecular subtypes. Interestingly, most patient characteristics did not differ between groups, except for an increase in the proportion of severe sepsis in molecular subtype 1. Possible confounders of this study were the small sample size, population stratification, and lack of information regarding drug interventions, all of which support the need for more studies with larger cohorts that include transcriptional profiles. This thought-provoking hypothesis-generating study could lead to a new neutrophil expression-based molecular classification of adult sepsis.

Clinically defined sepsis, severe sepsis, and septic shock encompass a highly heterogeneous patient population clinically because of the very complex pathophysiology underlying the host immune response. Owing to this complexity, a reductionist approach has led to numerous studies of single molecules and their pathways in elegant

preclinical research, yet randomized controlled trials (RCTs) generally have not demonstrated drug efficacy in sepsis. In the search for a novel approach that parallels very successful discoveries in cancer, subclassification of septic patients on the basis of molecular signatures combined with relevant clinical features is a promising strategy in sepsis.

In the previous issue of *Critical Care*, Maslove and colleagues [1] combined neutrophil gene expression microarray data of septic patients from two previous prospective studies of critically ill and septic patients [2,3] and then divided patients randomly into derivation ($n = 55$) and validation ($n = 71$) cohorts. The authors' statistical and mathematical methodology is complex. With the derivation cohort, they began using partitioning around medoids (PAM) clustering based on Euclidean distance on all data to create two clusters, from which they derived a list of differentially expressed genes. In parallel, a GenBank search was used to create a list of candidate genes related to sepsis. A series of enrichment steps, including PAM and significance analysis of microarrays, were applied to the cross-reference of the two lists in order to find the most discriminatory genes, the optimal k value, and silhouette value. K is the number of clusters, and silhouette values describe how well defined the clusters are. They settled on a k value of 2 and a silhouette value of 0.3, which allowed successful class labeling of the derivation patients.

To increase the robustness of the cluster identification, the original data were also analyzed by using hierarchical clustering based on Manhattan distance, resulting in the same clustering of the patients with clear bimodal distribution after principal component analysis.

These complex methods were then repeated in the validation cohort. The authors report a final silhouette width of 0.26, suggesting that the methods applied well to the validation cohort. Satisfied with the clusters, Maslove and colleagues used hierarchical clustering with pathway analysis for both derivation and validation cohorts to determine co-expressed genes distinct to the clusters. Perhaps not surprisingly, chemokine and cytokine pathways as well as Toll-like receptor signaling were at

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the top of the pathway analysis. Interestingly, some pathways considered novel in sepsis (cell cycle, cancer (p53), and Parkinson's disease pathways) were also identified.

Of note, there were no significant differences in patient characteristics between the two clusters, except for an increased proportion of patients who had severe sepsis in molecular subtype 1. Despite differences in severe sepsis rates, the proportions of septic shock and mortality rates were not different between groups, thus highlighting the potential for new classes of patients with sepsis.

Maslove and colleagues also used the Pharmacogenomics Knowledge Base and GeneMania to identify sepsis drug gene targets for drotrecogin alpha (activated protein C), vasopressin, hydrocortisone, and norepinephrine. They found significant differences in fold changes of expression of many of these target genes between sepsis subtypes 1 and 2. This is encouraging for the design of future clinical trials that could include circulating neutrophil expression to classify patients according to predicted response to drugs (that is, a predictive biomarker).

The work of Maslove and colleagues is similar to recent advances in transcriptional subclassification of cancer [4], pediatric sepsis [5], and myocardial dysfunction in septic shock [6]. Overall, the authors found two distinct sepsis subtypes based on a molecular signature that was otherwise unidentified based on classic clinical characteristics.

Points to be considered for improvement in future studies are the lack of information about patient ethnicity and the specific interventions the patients received in the study by Maslove and colleagues. Genetic variation alters inter-individual expression of inflammatory mediators [7-11]; hence, caution should be exercised to reduce the risk of false-positive, spurious associations due to population stratification. We suggest that future studies report ethnicity and relevant patient genotypes and evaluate larger sample sizes to optimize statistical power and clinical external validity. To improve the understanding of drug efficacy and safety, more information is needed regarding drug treatments because, for example, glucocorticoids alter expression of many targets of inflammatory pathways [12,13], yet data on glucocorticoid treatment of patients were not included in the analyses by Maslove and colleagues. Indeed, stratification by drug treatment to examine the interaction of the gene expression profiles of the relevant pharmacogenomic genes and response to these drugs would be very interesting and could explain the low signal-to-noise ratio in many RCTs in sepsis.

In summary, Maslove and colleagues draw attention to the existence of transcriptionally based clusters of patients which could lead to a very useful novel approach to clinical trial design and ultimately treatment of sepsis [14,15]. The lack of predictive biomarkers in previous

clinical trials may indeed be contributing to their limited success [15]. Replication of findings such as those of Maslove and colleagues in larger cohorts with genotypic and pharmacologic intervention data is imperative to further the field and perhaps increase our ability to discover and validate effective treatments for sepsis.

Abbreviations

PAM, partitioning around medoids; RCT, randomized controlled trial.

Authors' contributions

SAT executed the literature review, contributed to the critical analysis, and composed the manuscript. JAR developed the initial critical analysis and edited the manuscript.

Competing interests

SAT declares that she has no competing interests. JAR holds stock in and is on the Board (June 2012 to the present) of Sirius Genomics Inc. (Vancouver, BC, Canada), which has submitted patents that are owned by the University of British Columbia (UBC) and licensed to Sirius Genomics Inc. and that are related to the genetics of sepsis and its treatment; UBC has also submitted a patent related to the use of vasopressin in septic shock. He is an inventor on these patents. He has received consulting fees from Ferring Pharmaceuticals (Parsippany, NJ, USA), which manufactures vasopressin and is developing selepressin; from AstraZeneca (London, UK), which is developing anti-tumor necrosis factor- α ; from BioCritica (Indianapolis, IN, USA), which used to sell activated protein C in the US; from MedImmune (Gaithersburg, MD, USA); from Grifols (Barcelona, Spain), which sells albumin; and from Sirius Genomics Inc. He has received grant support from Sirius Genomics, Ferring Pharmaceuticals, AstraZeneca, and Eli Lilly and Company (Indianapolis, IN, USA) that is provided to and administered by UBC. He has received speaking honoraria from Pfizer Inc (New York, NY, USA) and Eli Lilly and Company.

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References

1. Maslove DM, Tang BM, McLean AS: **Identification of sepsis subtypes in critically ill adults using gene expression profiling.** *Crit Care* 2012, **16**:R183.
2. Tang BM, McLean AS, Dawes IW, Huang SJ, Cowley MJ, Lin RC: **Gene-expression profiling of gram-positive and gram-negative sepsis in critically ill patients.** *Crit Care Med* 2008, **36**:1125-1128.
3. Tang BM, McLean AS, Dawes IW, Huang SJ, Lin RC: **The use of gene-expression profiling to identify candidate genes in human sepsis.** *Am J Respir Crit Care Med* 2007, **176**:676-684.
4. Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, Coller H, Loh ML, Downing JR, Caligiuri MA, Bloomfield CD, Lander ES: **Molecular classification of cancer: class discovery and class prediction by gene expression monitoring.** *Science* 1999, **286**:531-537.
5. Wong HR, Cvijanovich NZ, Allen GL, Thomas NJ, Freisztat RJ, Anas N, Meyer K, Checchia PA, Lin R, Shanley TP, Bigham MT, Wheeler DS, Doughty LA, Tegtmeyer K, Poynter SE, Kaplan JM, Chima RS, Stalets E, Basu RK, Varisco BM, Barr FE: **Validation of a gene expression-based subclassification strategy for pediatric septic shock.** *Crit Care Med* 2011, **39**:2511-2517.
6. Pathan N, Hemingway CA, Alizadeh AA, Stephens AC, Boldrick JC, Oragui EE, McCabe C, Welch SB, Whitney A, O'Gara P, Nadel S, Relman DA, Harding SE, Levin M: **Role of interleukin 6 in myocardial dysfunction of meningococcal septic shock.** *Lancet* 2004, **363**:203-209.
7. Nakada TA, Russell JA, Boyd JH, Aguirre-Hernandez R, Thain KR, Thair SA, Nakada E, McConechy M, Walley KR: **beta2-Adrenergic receptor gene polymorphism is associated with mortality in septic shock.** *Am J Respir Crit*

- Care Med* 2010, **181**:143-149.
8. Russell JA, Wellman H, Walley KR: **Protein C rs2069912 C allele is associated with increased mortality from severe sepsis in North Americans of East Asian ancestry.** *Hum Genet* 2008, **123**:661-663.
 9. Thair SA, Walley KR, Nakada TA, McConechy MK, Boyd JH, Wellman H, Russell JA: **A single nucleotide polymorphism in NF-kappaB inducing kinase is associated with mortality in septic shock.** *J Immunol* 2011, **186**:2321-2328.
 10. Walley KR, Russell JA: **Protein C -1641 AA is associated with decreased survival and more organ dysfunction in severe sepsis.** *Crit Care Med* 2007, **35**:12-17.
 11. Wurfel MM, Gordon AC, Holden TD, Radella F, Strout J, Kajikawa O, Ruzinski JT, Rona G, Black RA, Stratton S, Jarvik GP, Hajjar AM, Nickerson DA, Rieder M, Sevransky J, Maloney JP, Moss M, Martin G, Shanholtz C, Garcia JG, Gao L, Brower R, Barnes KC, Walley KR, Russell JA, Martin TR: **Toll-like receptor 1 polymorphisms affect innate immune responses and outcomes in sepsis.** *Am J Respir Crit Care Med* 2008, **178**:710-720.
 12. Axelrod J, Reisine TD: **Stress hormones: their interaction and regulation.** *Science* 1984, **224**:452-459.
 13. Ledderose C, Mohnle P, Limbeck E, Schutz S, Weis F, Rink J, Briegel J, Kreth S: **Corticosteroid resistance in sepsis is influenced by microRNA-124-induced downregulation of glucocorticoid receptor-alpha.** *Crit Care Med* 2012, **40**:2745-2753.
 14. Cohen J, Opal S, Calandra T: **Sepsis studies need new direction.** *Lancet Infect Dis* 2012, **12**:503-505.
 15. Marshall JC: **Sepsis: rethinking the approach to clinical research.** *J Leukoc Biol* 2008, **83**:471-482.

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