Intensive Care Med (2018) 44:509–511
https://doi.org/10.1007/s00134-018-5122-z

EDITORIAL

Using multiple 'omics strategies for novel therapies in sepsis

James A. Russell[1*], Peter Spronk[2] and Keith R. Walley[1,2]

Sepsis is life-threatening organ dysfunction caused by dysregulated host response to infection [1]. Treatment is complicated because sepsis is heterogeneous, explaining the lack of effective drugs. Sepsis treatment includes broad-spectrum antibiotics, vasopressors, ventilation and dialysis. A limitation of antibiotics is that they do not directly remove the bacterial endotoxins and exotoxins, which may cause organ failure.

Exotoxins are potent immunologic stimulators—very low concentrations stimulate deleterious immunologic responses. Most exotoxins function as if they are superantigens [that bind to T cell receptors, activate T cells (especially T helper cells) and stimulate cytokine release].

Blocking endotoxin effects by blocking the TLR4 receptor with eritoran (a TLR4 blocker) was unsuccessful in severe sepsis [2]. This pivotal trial may have been negative because the timing was inadequate, the patients had severe sepsis rather than just septic shock, patients were not sick enough (placebo mortality 56% in the prior phase II trial but only 27% in the pivotal trial), eritoran may have worsened outcomes in gram-positive sepsis (46% of patients) (mortality rates: eritoran 34% vs. placebo 25%) or other causes.

There are no novel drugs available to treat sepsis. We propose a new drug discovery strategy that focuses on (1) the early infectious stage, (2) multiple 'omics and (3) an inverted drug discovery sequence to increase the chances of success.

Why focus on early sepsis?

Prior drug discoveries in sepsis that focused on the host inflammatory responses failed. Early antibiotics remain the only effective treatment [3], so we focus on the early infectious phase. "Early" is difficult to define for sepsis because determining 'time-zero' in human sepsis is impossible. Herein, we define early as inclusion within the first 24 h after emergency department arrival.

Antibiotics are recommended within 1 h of presentation [4] because each 1-h delay is associated with 4–6% decreased survival [5]. However, antibiotics do not directly remove bacterial endotoxins that stimulate immune, inflammatory, apoptotic and coagulation pathways causing organ failure and death [6].

Why multi-'omics?

Most sepsis drugs were developed by understanding the disease mechanism and targeting a relevant pathway. An 'omics association is typically an unbiased discovery that points to a possible mechanistic pathway. We define multi-'omics as measurement and examination of associations of at least two types of 'omics variables, from genomics, lipidomics, proteomics to metabolomics. Multi-'omics confirmation refines mechanistic understanding so that high probability drug targets can be identified.

Death due to infection is more heritable than death due to cancer or heart disease [7]. More recently, it has been proposed that environmental influences in early life may override genetic influences [8]. However, there is great value in evaluating the associations of genetic variations with impaired endotoxin clearance, organ dysfunction and death to facilitate drug discovery.

As an example, the endotoxin clearance cascade is a strong candidate pathway for study. Variation of endotoxin clearance cascade genes could alter endotoxin clearance, inflammation, bacterial load and survival. Key aspects of endotoxin cascade neutralization include binding to HDL, modulation by proprotein convertase subtilisin/kexin type 9 (PCSK9), transfer to LDL, LDL/

*Correspondence: Jim.Russell@hli.ubc.ca
[1] Division of Critical Care Medicine, Centre for Heart Lung Innovation, St. Paul's Hospital, 1081 Burrard Street, Vancouver, BC V6Z 1Y6, Canada
Full author information is available at the end of the article

endotoxin clearance via the hepatic LDL receptor, VLDL binding of endotoxin and the role of VLDL receptors in adipose tissue and transfer proteins (e.g., cholesterylester transfer protein).

Using a candidate gene approach, we discovered that PCSK9 inhibition acts as a broad-spectrum adjunct to all antibiotics in severe infection. We evaluated *PCSK9* because PCSK9 inhibitors were developed to lower cholesterol [9–12] and because endotoxins are lipid rich. LPS bound to LDL is cleared via hepatic LDL receptors and then excretion in bile. PCSK9 impedes LPS clearance by decreasing LDL receptor density [9]. Septic patients with *PCSK9* loss-of-function (LOF) genotypes have higher survival and lower plasma cytokine concentrations than wild type and patients carrying gain-of-function polymorphisms (GOF) [9].

The most common single-nucleotide polymorphisms of PCSK9 [13] are missense LOF variants rs11591147 (R46L), rs11583680 (A53V) and rs562556 (V474I); the most common missense GOF variant is rs505151 (G670E). The minor allele frequencies in sepsis patients are: rs11591147: 0.6–1.2%, rs11583680: 11–13%, rs562556: 16–17% and rs505151: 4–5% [9] similar to the general population. These PCSK9 mutations are pleiotropic [14]; the degree of cardiovascular protection is greater than expected by the LDL reduction perhaps because of other aspects of lipoprotein metabolism, inflammation, thrombosis, immune function (anti-viral and -malarial properties) and PCSK9 function in non-hepatic tissues.

Why an inverted drug discovery sequence?

Previous sepsis drugs arose from classic drug discovery: researchers identified mechanism(s) of sepsis in animal models and then did trials in humans. This strategy does not account for genetic heterogeneity of microorganisms and the host. We propose inverting (as in our PCSK9 discovery) the standard drug discovery sequence by starting with human 'omics, confirm mechanisms in models and then make go/no-go decisions for potential targets for clinical development.

One could extend our PCSK9 genomics-based approach, by adding multi-'omics to discover other novel targets. First, sequence genes of a relevant pathway (e.g., 32 endotoxin clearance cascade genes) and determine associations with 28-day survival. Then, measure multi-'omics in the same sepsis cohorts to determine associations of variants with multi-'omics in those cohorts. Next, examine associations of gene variants with multi-'omics in human volunteers administered low-dose lipopolysaccharide to select candidate targets meeting three criteria: variants with (1) significantly decreased survival, (2) significantly different level(s) of multi-'omics and (3) significantly different multi-'omics in the human lipopolysaccharide infusion cohort. Selected candidate targets would be evaluated for mechanisms in (1) human hepatocytes (because the liver clears endotoxins) and (2) murine gene knock-out models (e.g., peritonitis). Targets with mechanisms of action are taken to drug synthesis (antibody and small molecules). We did such a feasibility study of multi-'omics in 24 septic shock patients and 99 heathy controls and found significantly lower levels of

Table 1 Associations of PCSK9 genotype (wild type vs. loss of function) with protein, lipid and metabolite concentrations in patients with septic shock ($n = 24$)

Metabolites and lipids	PCSK9 genotype wild type ($n = 13$)	PCSK9 genotype LOF ($n = 11$)	p
Citrulline	18.5 (15.9–21.3)	21.9 (20.0–24.1)	0.026
Glutamic acid	37.3 (24.5–44.7)	38.9 (35.4–54.7)	0.043
Lysophosphatidylcholine C18:2	2.2 (1.5–7.3)	1.1 (0.9–3.2)	0.046
Ornithine	59.1 (44.8–72.7)	104.3 (68.3–116.6)	0.010
Phenyalainine	73.7 (64.9–81.2)	86.9 (77.0–96.8)	0.009
Phosphatidylcholine acyl-alkyl C30:1	0.03 (0.02–0.07)	0.06 (0.04–0.12)	0.042
Phosphatidylcholine diacyl C42:5	0.20 (0.17–0.25)	0.16 (0.12–0.18)	0.042
Trans-OH-proline	6.5 (5.3–9.1)	10.8 (8.4–18.3)	0.016
Proteins			
Apolipoprotein A-IV	41.1 (31.0–54.5)	60.3 (46.1–109.7)	0.046
Apolipoprotein B-100	694 (445–830)	354 (231–523)	0.003
Coagulation factor V	7.8 (6.0–8.5)	4.4 (3.7–7.0)	0.002
Complement component C7	38.0 (27.8–52.9)	62.1 (52.0–79.3)	0.004
IgGFc-binding protein	10.0 (8.3–12.4)	27.7 (11.0–42.7)	0.019
Serotransferrin	784 (618–1196)	1293 (878–1611)	0.025
Thyroxine-binding globulin	3.1 (2.5–4.0)	2.5 (2.0–2.8)	0.026

proteins, lipids and metabolites compared with controls (Genga KR 2018). We evaluated PCSK9 gene variants and found significant differences in proteins, lipids and metabolites between PCSK9 loss-of-function and wild-type patients (Table 1; supplement text).

In summary, focus on early sepsis, harnessing the power of multi-'omics and inverting the drug discovery sequence could enhance drug discovery in sepsis.

Electronic supplementary material
The online version of this article (https://doi.org/10.1007/s00134-018-5122-z) contains supplementary material, which is available to authorized users.

Author details
[1] Division of Critical Care Medicine, Centre for Heart Lung Innovation, St. Paul's Hospital, 1081 Burrard Street, Vancouver, BC V6Z 1Y6, Canada. [2] Department of ICU, Gelre Hospitals Apeldoorn, Apeldoorn, The Netherlands.

Received: 9 February 2018 Accepted: 1 March 2018
Published online: 15 March 2018

References

1. Singer M, Deutschman CS, Seymour CW et al (2016) The third international consensus definitions for sepsis and septic shock (sepsis-3). JAMA 315:801–810
2. Opal SM, Laterre PF, Francois B et al (2013) Effect of eritoran, an antagonist of MD2-TLR4, on mortality in patients with severe sepsis: the ACCESS randomized trial. JAMA 309:1154–1162
3. Seymour CW, Gesten F, Prescott HC et al (2017) Time to treatment and mortality during mandated emergency care for sepsis. N Engl J Med 376:2235–2244
4. Rhodes A, Evans LE, Alhazzani W et al (2017) Surviving sepsis campaign: international guidelines for management of sepsis and septic shock: 2016. Crit Care Med 45:486–552
5. Kumar A (2010) Early antimicrobial therapy in severe sepsis and septic shock. Curr Infect Dis Rep 12:336–344
6. Angus DC, van der Poll T (2013) Severe sepsis and septic shock. N Engl J Med 369:840–851
7. Sorensen TI, Nielsen GG, Andersen PK, Teasdale TW (1988) Genetic and environmental influences on premature death in adult adoptees. N Engl J Med 318:727–732
8. Petersen L, Sorensen TI, Andersen PK (2010) A shared frailty model for case-cohort samples: parent and offspring relations in an adoption study. Stat Med 29:924–931
9. Walley KR, Thain KR, Russell JA et al (2014) PCSK9 is a critical regulator of the innate immune response and septic shock outcome. Sci Transl Med 6:258
10. Roth EM, McKenney JM, Hanotin C, Asset G, Stein EA (2012) Atorvastatin with or without an antibody to PCSK9 in primary hypercholesterolemia. N Engl J Med 367:1891–1900
11. Seidah NG, Abifadel M, Prost S, Boileau C, Prat A (2017) The proprotein convertases in hypercholesterolemia and cardiovascular diseases: emphasis on proprotein convertase subtilisin/kexin 9. Pharmacol Rev 69:33–52
12. Stein EA, Mellis S, Yancopoulos GD et al (2012) Effect of a monoclonal antibody to PCSK9 on LDL cholesterol. N Engl J Med 366:1108–1118
13. Abifadel M, Rabes JP, Devillers M et al (2009) Mutations and polymorphisms in the proprotein convertase subtilisin kexin 9 (PCSK9) gene in cholesterol metabolism and disease. Hum Mutat 30:520–529
14. Stower H (2011) Human genetics: pleiotropic mutations. Nat Rev Genet 13:5