Omics of endothelial cell dysfunction in sepsis

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Abstract

During sepsis, defined as life-threatening organ dysfunction due to dysregulated host response to infection, systemic inflammation activates endothelial cells and initiates a multifaceted cascade of pro-inflammatory signaling events, resulting in increased permeability and excessive recruitment of leukocytes. Vascular endothelial cells share many common properties but have organ-specific phenotypes with unique structure and function. Thus, therapies directed against endothelial cell phenotypes are needed to address organ-specific endothelial cell dysfunction. Omics allow for the study of expressed genes, proteins and/or metabolites in biological systems and provide insight on temporal and spatial evolution of signals during normal and diseased conditions. Proteomics quantifies protein expression, identifies protein–protein interactions and can reveal mechanistic changes in endothelial cells that would not be possible to study via reductionist methods alone. In this review, we provide an overview of how sepsis pathophysiology impacts omics with a focus on proteomic analysis of mouse endothelial cells during sepsis/inflammation and its relationship with the more clinically relevant omics of human endothelial cells. We discuss how omics has been used to define septic endotype signatures in different populations with a focus on proteomic analysis in organ-specific microvascular endothelial cells during sepsis or septic-like inflammation. We believe that studies defining septic endotypes based on proteomic expression in endothelial cell phenotypes are urgently needed to complement omic profiling of whole blood and better define sepsis subphenotypes. Lastly, we provide a discussion of how in silico modeling can be used to leverage the large volume of omics data to map response pathways in sepsis.

Introduction

Sepsis is a clinical syndrome defined as life-threatening organ dysfunction due to dysregulated host response to infection (1). It is a major health issue with the number of cases ranging from 19 to 50 million per year and is a leading cause of death globally (2). Sepsis can be caused by primary bacterial, fungal or viral infections or secondary infections that can develop following non-infectious insults such as burn or trauma (3). Sepsis is a heterogeneous syndrome...
and diagnosis is complicated due to the broad spectrum of non-specific clinical features (3). In addition, the clinical course is impacted by individual factors relating to infection source, (epi)genetics, comorbidities or demographics (1, 4). Furthermore, there are a multitude of biological signals that play a role in interconnecting pathways, making it difficult to define clinically relevant endpoints besides mortality and to establish a clear understanding of the underlying disease. This wide array of factors determining sepsis onset and response diminishes the likelihood of creating one standard treatment for the heterogeneous cohort of patients. Thus, categorizing sepsis patients into distinct endotype classes should improve the prospects of finding efficacious drugs within each class (5). In sepsis, if organ function is not maintained, organ damage can develop, leading to increased morbidity and mortality (3, 6). Particularly, the microvascular endothelium plays a key role in the development and progression of sepsis (7), but the application of omics, specifically proteomics, towards defining endotypes and unraveling the mechanisms of dysfunction of endothelial cells (ECs) in multiple organs during sepsis is in its infancy. Therapeutic approaches for the treatment of sepsis are supportive, but there are no specific pharmacologic therapies to treat the underlying pathophysiology and maintain endothelial cell function (7).

In the emerging field of omics of sepsis, we believe that this review will provide an initial summary of the literature in the field as a resource and also encourage further studies (8, 9, 10, 11). In particular, as sepsis is a complex process, proteomics provides a quantitative analysis of the protein changes that can help bridge the genotype–phenotype gap (12). Specifically, since proteins are involved in every biological phenomenon, unraveling protein–protein interactions (PPIs) is crucial for identifying pathways contributing to disease (12, 13, 14). In this regard, omics analysis can further our understanding of the subphenotypes of the disease and, in combination with laboratory and clinical variables, suggest future studies with clinical relevance.

In this review, we summarize how omics of various ECs is leveraged to better describe sepsis progression, define sepsis subphenotypes and identify novel therapeutic targets. We not only discuss how genomics has been used to define septic endotype signatures in different populations but also focus on the application of proteomic analysis of organ-specific microvascular ECs during sepsis or septic-like inflammation which has not been reviewed before. Lastly, we provide a brief discussion of how in silico modeling can be used to leverage the large volume of omics data for mapping endothelial response pathways in sepsis.

An overview of sepsis, endothelium and omics

Sepsis and the role of the endothelium

The vascular endothelium is a single layer of cells lining the tunica intima (inner layer) of blood vessels (15). The endothelium regulates several physiological functions including vascular tone, permeability and immune response (15). The endothelium of different organs shows heterogeneity in function and morphology, and organ-specific ECs exhibit distinct barrier properties and interactions with immune cells (16). During sepsis, an intense systemic inflammatory response develops in response to pathogen-associated molecular patterns (PAMPs) (7). This systemic inflammation activates a cascade of pro-inflammatory events that results in leukocyte dysregulation and an altered endothelial phenotype, producing increased barrier permeability, coagulation and neutrophil trafficking into critical organs; this results in host tissue damage and multiple organ dysfunction syndrome (MODS) (3, 7, 17).

Specifically, neutrophils and ECs engage in crosstalk that leads to neutrophil rolling, adhesion and migration across ECs via a multifactorial process controlled by concurrent chemoattractant-dependent signal, hemodynamic shear forces and adhesive events (18). While neutrophils are crucial to host defense, neutrophil dysregulation has a critical role in the early course of death of ECs through the release of proteases and the formation of neutrophil extracellular traps (NETs) (17, 18). Subsequently, ECs dysfunction induces the activation of the complement and coagulation cascades and disseminated intravascular coagulation (DIC) (19).

To date, there are no gold-standard diagnostic measures for sepsis, which complicates hypothesis-driven studies searching for individual biomarkers or therapeutic targets (1). Mechanistic computational modeling based on multi-omic analysis can provide a rational basis for understanding the pathophysiology of sepsis, sepsis phenotypes and design of clinically relevant therapeutics (20).

Omics for understanding disease and developing therapeutics

Since the development of the first genome sequencing method, technologies that further allow the quantification and identification of genes, RNA transcripts, proteins and metabolites (Fig. 1) have been instrumental for understanding disease mechanisms and identifying intervention targets for pathological conditions such as cancer (21).
The field of systems biology represents a leap forward from reductionist methods by providing the ability to quantify the entire state of a biological system in the context of the four major classes of biomolecules (DNA, RNA, protein, metabolites) (14). Genomics focuses on whole-genome sequencing, while transcriptomics focuses on RNA-sequencing (RNA-seq) and analyzing differential RNA transcript expression patterns (14). Single-cell RNA-seq (scRNA-seq) is an emerging technology that captures differential transcript expression from individual cells. This technique permits the evaluation of biological events at a greater resolution compared to performing bulk RNA-seq (22). Thus, incorporating scRNA-seq in studying endothelial cell heterogeneity during sepsis would be beneficial in characterizing organ-specific omic expression patterns. Proteomics quantifies differentially expressed proteins (DEPs) in a biological sample, while metabolomics analyzes metabolites within a cell (23). In proteomics and metabolomics, liquid chromatography (LC) methodologies separate complex mixtures based on size, resin affinity or charge; mass spectrometry (MS) ionizes and fragments protein mixtures into peptides and nuclear magnetic spectroscopy (NMR) is used to determine molecular structure (14). Additionally, newer mass spectrometry technologies help capture the heterogeneity of cell response during sepsis by measuring low-abundance proteins in samples, improving detection of peptides and increasing sensitivity over traditional 2-D-LC proteomic assays (24). Once fragmented, protein databases are utilized to determine the targeted protein(s) of interest (23). Omics can help with molecular sub-typing of specific diseases and tailoring of treatment strategies for different patient groups by analyzing large amounts of data to characterize biomolecule expression (25), enabling the development of next-generation therapeutics for complex, poorly characterized diseases such as sepsis. Omics provide tools for the characterization of biomolecule expression in a tempo-spatial manner, thus allowing us to quantify the dynamics of pathway signaling during disease progression (25, 26). Furthermore, omics can generate hypothesis-driven experiments and identify pathways and biomolecules from samples a priori which can then be tested in experimental models to investigate the role of the identified biomolecules in signaling pathways (27).
Omics in septic research and endothelial dysfunction

Omics in sepsis research

Given the dynamic nature of sepsis, omic analysis, combined with clinical input regarding the stage of the disease, can be used to characterize pathologically relevant biomarkers (4). For example, omics can be particularly useful in sepsis research for discovering (a) biomarkers to differentiate between infectious and non-infectious sources, (b) prognostic biomarkers, (c) biomarkers that aid in sepsis therapy and (d) biomarkers to predict individual patient response to therapy (28). Discovering these synergistic combinations of biomarkers is of high interest, given the fact that no single biomarker is sensitive and specific enough to capture the entirety of an individual’s septic condition (29). A recent study found 60 biomarkers that were able to distinguish between sepsis and systemic inflammatory response syndrome (SIRS), but only 7 of these contain sufficient data for further evaluation (30). One of them is PCT, which is the only FDA-approved sepsis biomarker; the other six are presepsin, CRP, IL6, sTREM1, LBP and CD64 (30, 31). PTX-3 is another biomarker that has been studied in septic shock (32). Limitations of these biomarkers include: low diagnostic and prognostic accuracy when used alone, lack of studies directly comparing one over another, variability of concentration during early or late-stage sepsis and lack of standardized diagnostic cut-off values (29, 30). Comprehensive reviews of sepsis biomarkers can be found elsewhere (29, 30).

Several studies have proposed classification systems that stratify sepsis patients into unique endotypes based on genomic data and/or modeling approaches (5, 11, 33, 34, 35). The success in stratifying septic patients into endotypes and in associating these features with clinical outcomes illustrates the clinical relevance of the heterogeneous aspects of sepsis. The papers serve as blueprints for precision medicine to reconsider therapeutic approaches on a patient-by-patient basis depending on individual omic profiling. While research has elucidated clinical signs of these endotypes in septic patients, more investigation on the underlying biomolecules and pathways of disease is needed to establish the physiological basis for these endotypes (25). This is where the integration of systems biology and omics plays a major role. Since sepsis affects multiple cellular compartments and organs in an entropic manner, omics can capture patient-specific biomolecule expression in biological systems and, in combination with computational methods, decipher how underlying biological networks are dysregulated (36). This will then permit sub-typing of patients according to common clinical features (25, 26) and characterize the underlying endotype. Table 1 shows a summary of different genomic and modeling studies that have stratified patients into sepsis endotypes with selected differential gene expression and corresponding outcomes.

As shown in Table 1, a selective number of genes were used to characterize the endotypes of interest in sepsis in several different population/demographic groups. The fact that different genes were identified across studies may in part be based on the type of study (retrospective vs prospective), time of patient recruitment (months vs years), if recruitment occurred before or after the sepsis-3 definition (1), study population (children vs adults), demographics (country of origin, race) and time of assay. Wong et al. performed genome-wide expression profiling using whole-blood-derived RNA from 98 children with septic shock (33). Three subclasses were established via unsupervised hierarchical clustering: subclasses A, B and C. Subclass A had the highest mortality (36%), illness severity and degree of organ failure. Also, subclass A had repressed genes in immunity (44 genes including LAT and TRAIT) and zinc biology which help to maintain homeostasis (181 genes including ZnT), and thus this cohort exhibited lower adaptive immunity and increased mortality than other subclasses (33). Additional pathway hits corresponding with these repressed genes included B-cell and glucocorticoid signaling which further confirms that the subclass A cohort did not have the immune-related genes expressed during sepsis (33). These initial findings support the efforts to stratify patients into various endotypes based on differential omic expression in sepsis. In another study conducted within the Molecular Diagnosis and Risk Stratification of Sepsis (MARS) project (34), a clinical trial investigating sepsis endotypes in ICUs, eight genes were identified which, in specific combinations, could be used to systematically classify patients into the MARS 1, 2, 3 or 4 endotype. The MARS 1 group showed decreased innate and adaptive gene expression, MARS 2 exhibited increased cell motility and cytokine pathway expression, MARS 3 demonstrated increased adaptive immune gene expression and the MARS 4 group had increased IL6, NFKB and interferon gene expression (34). This is of particular importance since having a large, complex omic signature that is sensitive enough to correctly classify different patients into various endotypes is impractical in the clinic and thus having a smaller signature would enable additional studies to evaluate its relevance in sepsis pathophysiology and predict treatment responses on a larger scale (37). The comparison between these studies is further complicated.
Table 1  Examples of genomic and modeling studies to classify septic human patients into various endotypes. Gene definitions can be found in Supplementary Table 1.

<table>
<thead>
<tr>
<th>Endotype classification</th>
<th>Endotype outcome</th>
<th>Genes</th>
<th>Study population</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, B, C</td>
<td></td>
<td></td>
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<td>(33)</td>
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</tbody>
</table>

Subclass A group  
- Increased organ failure, highest mortality  
- 44 key adaptive immune genes (i.e. T/B-cell related such as KAT2B, SOS1, JAK2, G6, TAF1, PTPRC, MA3K7 etc.) in subclass A compared to B and C.  
- 181 key zinc biology-related genes (i.e. ZnT/SLC, etc) downregulated in subclass A compared to B and C.  

Subclasses B and C groups SRS 1, 2  
- Decreased mortality  
- SRS1 group  
  - Higher mortality and T-cell exhaustion  
  - IRAK3, TOLLIP, CBL, PAG1, HIF1A, EPAS1, IL18RAP, CCR1, LDHA, GAPDH  
  - LAT, CD247, HLA family, CIITA, RFX5, CCR3, MTOR, SIRT1, CD247  
- SRS2 group  
  - Increased cell response to infection, low mortality  
  - HLA family class II, T-cell and B-cell complexes  

MARS 1–4  
- MARS 1 group  
  - Highest 28-day mortality, decreased immune gene expression  
  - BPGM, TAP2  
- MARS 2 group  
  - Increased cytokine pathway expression  
  - GADD45A, PCGF5  
- MARS 3 group  
  - Increased adaptive immunity expression, lowest 28-day mortality  
  - AHNAK nucleoprotein, PDCD10  

(Continued)
The fact that these patterns characterize differences in gene expression at different times. For example, in many studies outlined in Table 1, data are collected and profiled within the first 24–48 h of hospital admission; however, another study indicated that 50% of patients can change from one endotype to another within the first 5 days of hospital admission (4). Thus, tracking of omic expression in a time-dependent manner is important.

Although each study in Table 1 utilizes unique methods to categorize sepsis patients into their own endotype groups, there are commonalities across different endotypes groups which can be utilized to promote future therapeutic research. Many studies have performed genomic profiling of leukocytes or mononuclear cells (33, 38, 39, 40, 41, 42, 43); however, only a few studies focus on grouping patients into different endotypes. Focusing on endotype-dependent studies is critical for developing appropriate therapeutic intervention, since one needs to identify which pathway is critical to target in a particular patient, a goal of precision medicine. Among endotypes, low mortality groups (SRS 2 (11), MARS 3 (34), adaptive (35), α (5)) shared the common characterization of increased adaptive immune signaling, but high mortality groups (subclass A (33), SRS 1 (11), MARS 1 (34), Inflammopathic (35), δ (5)) were not as uniformly characterized by immune status and had repressed immune function. Certain groups were characterized by hyperinflammation (inflammopathic (35), δ (5)), and others were linked to immunosuppression (inflammopathic (35), δ (5)), and others were linked to immunosuppression (inflammopathic (35), δ (5)), and others were linked to immunosuppression (inflammopathic (35), δ (5)), and others were linked to immunosuppression (inflammopathic (35), δ (5)), and others were linked to immunosuppression (inflammopathic (35), δ (5)), and others were linked to immunosuppression (inflammopathic (35), δ (5)), and others were linked to immunosuppression (inflammopathic (35), δ (5)), and others were linked to immunosuppression (inflammopathic (35), δ (5)), and others were linked to immunosuppression (inflammopathic (35), δ (5)). Furthermore, emerging studies are beginning to evaluate different endotype signatures across populations (e.g. evaluating SRS endotype signatures in pediatric patients) to investigate their performance with respect to mortality (44). Additional knowledge from the combination of endotypes can verify common biological targets between populations leading to an endotype, as described in other diseases such as acute respiratory distress

### Table 1

<table>
<thead>
<tr>
<th>Endotype classification</th>
<th>Endotype outcome</th>
<th>Genes</th>
<th>Study population</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MARS 4 group</td>
<td>Increased interferon gene expression</td>
<td>IFIT5, GLTSCR2/NOP53/NOL5A</td>
<td>Retrospective study; Total of 23 bacterial sepsis/inflammation datasets (12 in children, 11 in adults) were analyzed; Majority of patients in the cohorts were males from first-world nations</td>
<td>(35)</td>
</tr>
<tr>
<td>Inflammopathic, adaptive and coagulopathic group</td>
<td>Highest mortality and innate immunity expression</td>
<td>ARG1, LCN2, LTF, OLFM4</td>
<td>HLA-DMB</td>
<td></td>
</tr>
<tr>
<td>Adaptive group</td>
<td>Lowest mortality and increased adaptive immunity expression</td>
<td>YKT6, PDE4B, TWISTNB/POLR1F, BTN2A2</td>
<td>GADD45A, CD24, S100A12, STX1A</td>
<td></td>
</tr>
<tr>
<td>Coagulopathic group</td>
<td>High mortality and coagulopathy</td>
<td>KCNMB4, CRISP2, HTRA1, PPL</td>
<td>RHBDL2, ZCCHC4, YKT6, DDX6</td>
<td></td>
</tr>
<tr>
<td>Alpha, beta, gamma, delta group</td>
<td>Less organ dysfunction, normal blood tests and lowest mortality</td>
<td>IL10</td>
<td>v-dimer, IL6, IL8, TNFa, Procalcitonin, C-reactive protein</td>
<td>(5)</td>
</tr>
<tr>
<td>α group</td>
<td>Chronic illness and renal dysfunction</td>
<td>IGFBP7, COL4, TIMP2</td>
<td>IL10, IL66, procalcitonin, SELE, PAI1</td>
<td></td>
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<tr>
<td>β group</td>
<td>Increased inflammation and fever</td>
<td>IL6, KIM1/HAVCR1, procalcitonin, PAI1, ICAM1, SELE</td>
<td>SELE, PAI1</td>
<td></td>
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<tr>
<td>γ group</td>
<td>High coagulation and hypotension and the highest mortality</td>
<td>IL10, IL6, IL8, procalcitonin, TNFa, COL4, v-dimer, PAI1, VCAM1, TAT complex</td>
<td>SELE, PAI1</td>
<td></td>
</tr>
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</table>

MARS, Molecular Diagnosis and Risk Stratification of Sepsis; SRS, sepsis response signature.
syndrome (ARDS) (45). However, there is currently a lack of standards by which common endotypes can be identified in different studies.

Though biomarkers can provide valuable insight to guide therapeutic decisions and enhance patient management by preventing, for example, unnecessary antibiotic therapy (29) and commonalities between omic studies can be further validated experimentally, it is highly improbable that a universal endotype signature for sepsis can be developed due to the heterogeneity of the disease (37). It is therefore important to understand different endotypes in the disease to allow the development of tailored therapeutics. It is important to note that the overall goal of endotyping is to unravel molecular subtypes of a disease, and it should not be used as a definitive prognostic tool (44). An international effort to form a standardized consensus on omic profiling procedures would be beneficial (37). Additionally, it would be useful to further validate the omic expression changes in each endotype across populations in time-lapse, multi-institutional prospective studies and plan endotype studies that employ the current definition of sepsis (1), since many omic studies in Table 1 were conducted using the former consensus definition (33, 46, 47, 48).

Omics of microvascular endothelium in sepsis/inflammation

Microvascular ECs play a central role in neutrophil-endothelial crosstalk, and excessive neutrophil migration leads to edema, shock and MODS (7, 17). Since sepsis progresses rapidly, and there are no standard diagnostic procedures to determine a patient's clinical condition between admission and first course of ‘treatment’ (1), omics would be beneficial in determining how ECs of vital organs are impacted in the early phase of sepsis (7, 17, 27). Understanding organ-specific omics of ECs should be of high importance due not only to its role in maintaining homeostasis and immunity but also for how its dysfunction can lead to organ failure (7, 17, 28). Furthermore, characterizing differential omic expression patterns of ECs phenotypes will help us better understand how each vascular bed responds to inflammatory insults and what gene ontologies (GO) and signaling pathways are unique to each bed or common across beds. A summary of the genomic, sepsis/inflammatory studies performed in mice is presented in Table 2. Though there have been concerns about whether results from mice can translate to human trials, data from mouse models are still needed to help understand the pathology of sepsis (49). Mouse models are also critical for establishing the response of ECs phenotypes to inflammatory stimuli for the evaluation of genetic (e.g. knock-in or knock-out) or pharmacological effects in a living system, since these studies cannot be done in patients. In this section, we discuss genomic studies, followed by proteomic studies of ECs in ‘Proteomics of ECs and in silico modeling of omics’. To our knowledge, there are no published metabolomic or epigenomic studies of mouse microvasculature ECs challenged with an inflammatory insult.

Organ-specific ECs have been stimulated with exogenous substances (e.g. LPS, bacteria) to induce septic-like conditions over different time points (e.g. 6 or 24 h) (50, 51, 52, 53) to identify unique genes, pathways or GO differentially expressed in various organs using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and GO database. Overall, most of the KEGG signaling pathways, gene families (e.g. Cxcl, Tnfα, Sele, Selp) and GO overexpressed in the ECs beds correlate with the activation of the innate immune system (e.g. TLR signaling), leukocytes (e.g. leukocyte migration) and coagulation (54). These findings are consistent with our understanding that sepsis causes dysregulated host response to infection, leading to activation of ECs and immunity pathways (7, 17).

Additional organ-specific pathways based on endothelial-specific gene expression have also been reported (54). For example, upregulation of adipose tissue-specific ECs genes (e.g. Car3, Csf2rb) drives osteoclast differentiation, kidney-specific ECs genes (e.g. Dram1, Dkk2) aid in endocytosis, cardiac-specific ECs genes (e.g. Kcna5, Myadn) drive axon guidance and brain-specific ECs genes (e.g. Edn3, Foxj2) help maintain ErbB signaling (54). To date, most omic endothelial-based sepsis/inflammatory studies have focused on the lung, liver and brain showing that a number of unique as well as common pathways and genes are associated with these different ECs (50, 51, 52, 53, 54). Even though common pathways are expressed among all ECs, differential gene expression still occurs. For example, Wnt signaling is a common pathway among ECs, but brain ECs show higher expression of Nkd1 or Fzd6 while liver ECs express Apc or Ep300 (54). Though this initial study (54) was not done under inflammatory stimuli, these findings can provide an organ-specific understanding of the signaling mechanisms to examine during sepsis. Additionally, pathway changes in adipose tissue, mammary or adrenal glands or skeletal muscle ECs during normal or disease conditions have not been systematically studied and warrant further investigation. Furthermore, omics studies investigating intra-organ endothelial heterogeneity...
<table>
<thead>
<tr>
<th>Reference</th>
<th>Methodology</th>
<th>Region</th>
<th>KEGG pathway hits</th>
<th>GO pathway hits</th>
<th>Genes</th>
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<td>(S4)</td>
<td>Embryonic stem cells were differentiated into organ-specific ECs</td>
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<tr>
<td>AT</td>
<td>Osteoclast differentiation, MAPK signaling, metabolism</td>
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<tr>
<td>Brain</td>
<td>ErbB signaling, RPAR signaling, MAPK signaling</td>
<td></td>
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<tr>
<td>Diaphragm</td>
<td>Toxoplasmosis, RIG-I-like signaling, apoptosis</td>
<td></td>
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<tr>
<td>Heart</td>
<td>Focal adhesion, axon guidance signaling, ECM-receptor interaction</td>
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<td>Kidney</td>
<td>Endocytosis, hematopoietic cell lineage, calcium signaling</td>
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<td>Liver</td>
<td>TGF-B signaling, complement and coagulation, hematopoietic cell lineage</td>
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<tr>
<td>Lung</td>
<td>Neuroactive ligand-receptor interaction, Wnt signaling, metabolism</td>
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<tr>
<td>MG</td>
<td>JAK-STAT, NOD-like receptor signaling, MAPK signaling</td>
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<td></td>
<td></td>
<td></td>
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<td>Upregulated</td>
<td>Downregulated</td>
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<tr>
<td>Pancreas</td>
<td></td>
<td></td>
<td>Adherens junction, focal adhesion, MAPK signaling</td>
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<tr>
<td>SM</td>
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<td></td>
<td>JAK-STAT, TLR signaling, metabolism</td>
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<tr>
<td>Trachea</td>
<td></td>
<td></td>
<td>Gap junction, NOD-like receptor signaling, TLR signaling</td>
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Cultured brain, lung and heart ECs were stimulated with LPS for 6 and 24 h

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<thead>
<tr>
<th>Region</th>
<th>Methodology</th>
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<th>KEGG pathway hits</th>
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<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td></td>
<td>6 h</td>
<td>Leukocyte migration, response to LPS</td>
<td></td>
<td>Ccl11, Timp1, Tnfa, Il1a, Il1b, Sele, Selp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24 h</td>
<td>Response to chemokine, cell chemotaxis, leukocyte/neutrophil migration</td>
<td></td>
<td>Ccl3, Timp1, Ccl11, Selp, Sele</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td>6 h</td>
<td>Cell chemotaxis, leukocyte migration</td>
<td></td>
<td>Ccl3, Sele, Selp, Cxcl5, Cxcl11, Il6, Cxcl3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24 h</td>
<td>Leukocyte migration, neutrophil/leukocyte chemotaxis, response to chemokine</td>
<td></td>
<td>Cxcl5, Cxcl3, Sele, Selp, Acod1</td>
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<tr>
<td>Lung</td>
<td></td>
<td>6 h</td>
<td>Acute inflammatory response, cell chemotaxis</td>
<td></td>
<td>Cxcl1, Cxcl9, Ilr2, Casp6, Il10, Ly96</td>
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<tr>
<td></td>
<td></td>
<td>24 h</td>
<td>Cell chemotaxis, leukocyte/neutrophil chemotaxis, leukocyte migration</td>
<td></td>
<td>Mmp8, Il10, Acod1, Cxcl9</td>
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<tr>
<th>Reference</th>
<th>Methodology</th>
<th>Region</th>
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<th>GO pathway hits</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>(51)</td>
<td>Mice were injected with LPS for 4 h prior to isolation of heart, brain liver and lung ECs</td>
<td>Kidney, brain, liver, lung, heart</td>
<td></td>
<td>Leukocyte migration, response to lipopolysaccharide, response to bacterium</td>
<td>\begin{itemize} \item Sele, Selp, Cdh5, Ctnna1, Cldn5, Thb, Vwf, Jam2 \end{itemize}</td>
</tr>
<tr>
<td>(52)</td>
<td>Mice were injected with LPS for 3 h prior to isolation of adrenal ECs</td>
<td>Brain</td>
<td></td>
<td></td>
<td>\begin{itemize} \item Sele, Selp, Cdh5, Ctnna1, Cldn5, Thb, Vwf, Jam2 \end{itemize}</td>
</tr>
<tr>
<td>(53)</td>
<td>Mice were injected with influenza infection for 6 h prior to isolation of lung ECs</td>
<td>Heart</td>
<td></td>
<td></td>
<td>\begin{itemize} \item Sele, Selp, Cdh5, Ctnna1, Cldn5, Thb, Vwf, Jam2 \end{itemize}</td>
</tr>
<tr>
<td>(54)</td>
<td>Mice were injected with influenza infection for 6 h prior to isolation of lung ECs</td>
<td>Liver</td>
<td></td>
<td></td>
<td>\begin{itemize} \item Sele, Selp, Cdh5, Ctnna1, Cldn5, Thb, Vwf, Jam2 \end{itemize}</td>
</tr>
<tr>
<td>(55)</td>
<td>Cultured mouse brain ECs were stimulated with avian E. coli for 1–6 h</td>
<td>Lung</td>
<td></td>
<td>Blood vessel development, positive regulation of cell motility, sprouting angiogenesis</td>
<td>\begin{itemize} \item Gpihbp1, Ifi47, Pivpa, Sox17, Aft3, Nr11, Nusap1, Birc5, Cd1k, Top2a, Hki1, Kdr, Aft3, Cd34 \end{itemize}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brain</td>
<td>Ribosome, legionellosis, TNF signaling, HIF-1 signaling</td>
<td></td>
<td>Nuclear part, intracellular part, intracellular organelle, cellular macromolecule metabolic process</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Biosynthesis of amino acids, glycolysis</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
are needed to understand how inflammatory stimuli impact different ECs of the same organ. These urgently needed studies will help in generating hypotheses for the validation of experiments in experimental models to enhance our understanding of how sepsis impacts ECs in various organs and to identify druggable targets. Most studies in Table 2 report genes that are upregulated, but all use either KEGG or GO to find the biological processes or signaling pathways these genes play a role in. However, only two report KEGG and/or GO hits that are downregulated (52, 55). More studies reporting what processes and pathways are downregulated are necessary to obtain a comprehensive understanding of how the functionality and structural properties of organ-specific ECs are altered in response to sepsis.

Proteomics of ECs and in-silico modeling of omics

Proteomics of mouse ECs

A summary of proteomic organ-specific ECs studies in mice under septic/inflammatory conditions is outlined in Table 3. Most studies report up- and downregulated proteins and use either KEGG or GO to find the biological processes or signaling pathways in which these proteins play a role. If KEGG or GO hits were not reported, protein lists were submitted to these databases, and the hits are reported in Table 3. None of these studies report KEGG pathways and/or GO hits that are downregulated. Consistent with genomic studies, proteins expressed correlate with the upregulation of KEGG pathways and GO hits related to coagulation, cell adhesion and immune response (56, 57, 58, 59, 60, 61, 62). Thus, proteins correspond with gene expression to determine final cellular pathways and biological processes overexpressed in septic-like conditions. Interestingly, studies have also shown that COVID-19, which has been described as a form of viral sepsis, significantly affects the endothelium (63). For example, the proposed KEGG COVID-19 pathway is shown to play a role in endothelial dysfunction (56), thus implicating the endothelium as a potential target for COVID-19 therapeutics. Though there have been emerging studies on the omics of COVID pathogenesis and progression (64), there are currently no omic studies on endothelial dysfunction in COVID-19.

Many studies discussed in Tables 2 and 3 do point to the molecular players and pathways already known in sepsis that produce a cellular phenotype. Nevertheless, differential omic analysis can provide insights for the design of future studies based on shared and unique proteins across organ-specific ECs. While certain pathways are highly upregulated among ECs (e.g. Wnt signaling), there are also organ-specific upregulated pathways such as axon guidance in cardiac ECs or endocytosis in kidney ECs (54). Additionally, organ-specific ECs express cell surface proteins, and thus the variety of genes and proteins shown in Tables 2 and 3 could be classified as potential therapeutic targets to preserve the vasculature of the tissue and prevent downstream damage such as edema and MODS, which are hallmarks of sepsis damage to the endothelium (54). However, it should be noted that all of these studies were performed in mouse ECs, and further validation of these findings must be complemented with experimental models such as microphysiological systems (MPS) using human cells that recapitulate the 3-D geometry and physiologically relevant flow conditions of the microvasculature (18, 65).

Other than causing differential regulation of coagulation, cell adhesion and immune response proteins in ECs, sepsis has been shown to affect the glycocalyx (a gel-like layer composed of proteoglycans coating ECs) and vascular smooth muscle cells (VSMCs) (7, 58). The synthesis of new peptides in response to sepsis related to lipid transport (e.g. Apo family), immunity or oxidative stress (C7) is all downregulated in the glycocalyx (58). Thus, designing studies investigating potential proteins that shed from the glycocalyx during sepsis would be beneficial. While proteomic studies in mouse microvascular ECs provide a better understanding of the basic mechanisms of sepsis, in vitro proteomic studies using human ECs, specifically with ECs exposed to physiological and abnormal shear flow conditions (66), have been done providing potential relevance to clinical studies.

Proteomics of human ECs

ECs under shear stress convert mechanical stimuli into intracellular signals that affect cellular functions under both normal and diseased conditions. However, traditionally, proteomic expression patterns of ECs have been studied under static conditions, mostly in human umbilical vein ECs (HUVECs) (67, 68). For example, IL1B and IL13 are two cytokine proteins that are released from ECs during inflammation, thus inflammatory and cell adhesion proteins (e.g. RIPK22, SERPINB2, VCAM1) were upregulated in HUVECs (67, 68). Most proteins involved in molecular functions in ECs such as enzyme regulation and metabolic regulation of the cytoskeleton (e.g. cystatin-SN and profilin-1) are upregulated in inflammation (69). While HUVECs are well-established and easy to use in vitro models for studying ECs function, for the most part, they are
Table 3  Summary of proteomic studies in mouse microvascular endothelial cells investigating differential protein expression after inflammatory/septic-like stimulation. Protein definitions can be found in Supplementary Table 3.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Methodology</th>
<th>Region</th>
<th>KEGG pathway hits</th>
<th>GO pathway hits</th>
<th>Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Upregulated</td>
<td>Downregulated</td>
<td></td>
</tr>
<tr>
<td>(56)</td>
<td>Mice were</td>
<td>Lung</td>
<td></td>
<td></td>
<td>SAA1, VCAM1, C4BP, C4,</td>
</tr>
<tr>
<td></td>
<td>injected</td>
<td></td>
<td></td>
<td></td>
<td>COL5A1, GNAI1, CFB, MLST8,</td>
</tr>
<tr>
<td></td>
<td>with LPS</td>
<td></td>
<td></td>
<td></td>
<td>ATP8, POSTN, TH, ST3GAL1,</td>
</tr>
<tr>
<td></td>
<td>over 48 h</td>
<td></td>
<td></td>
<td></td>
<td>POLR2M</td>
</tr>
</tbody>
</table>

Mice were injected with oleic acid for 6 h before lung ECs isolation

Liver

<table>
<thead>
<tr>
<th>Region</th>
<th>Methodology</th>
<th>KEGG pathway hits</th>
<th>GO pathway hits</th>
<th>Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA was</td>
<td></td>
<td>Cell adhesion</td>
<td>Leukocyte</td>
<td>SAA1</td>
</tr>
<tr>
<td>injected</td>
<td></td>
<td>molecules,</td>
<td>proliferation,</td>
<td>VCAM1</td>
</tr>
<tr>
<td>in mice</td>
<td></td>
<td>Staphylococcus</td>
<td>cell–cell</td>
<td>CXCL9</td>
</tr>
<tr>
<td>for 24 h</td>
<td></td>
<td>aureus</td>
<td>adhesion,</td>
<td>SAA1</td>
</tr>
<tr>
<td>before</td>
<td></td>
<td>infection</td>
<td>positive</td>
<td>HPGD</td>
</tr>
<tr>
<td>kidney, liver, heart, brain and white adipose tissue ECs isolation</td>
<td></td>
<td>regulation of cell death</td>
<td>APOE, MUP3</td>
<td></td>
</tr>
</tbody>
</table>

Brain

Kidney

Heart

WAT

(Continued)
Table 3  Continued.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Methodology</th>
<th>Region</th>
<th>KEGG pathway hits</th>
<th>GO pathway hits</th>
<th>Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelial secretome</td>
<td>Metabolic pathways, endocytosis, biosynthesis of antibiotics, complement and coagulation, viral carcinogenesis, cell adhesion</td>
<td>Metabolic pathways, endocytosis, remodeling of epithelial adherens junction</td>
<td>Inflammatory response, cell assembly and organization, DNA repair/replication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycocalyx</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular smooth muscle</td>
<td>Metabolic pathways, endocytosis, pathways in cancer, PI3k-Akt signaling, cGas/STING-pathway</td>
<td>Metabolic pathways, endocytosis, pathways in cancer, PI3k-Akt signaling, cGas/STING-pathway</td>
<td>Metabolic pathways, endocytosis, pathways in cancer, PI3k-Akt signaling, cGas/STING-pathway</td>
<td></td>
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<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
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</table>

(59) Mice received a dose of cardiac radiation at 8 or 16 Gy before isolation of heart ECs

8 Gy        | EIF2 signaling, remodeling of epithelial adherens junction | Inflammatory response, cell assembly and organization, DNA repair/replication |

|            | SAA1, HX and HPX | CLU, AZGP1, C6, CFD, TLN1, GSN, F10 |
| Heart      | WVF, ICAM1, LAMB, DLAT, NCL, VCP, FH1, HIST1HE, LMNB2 |

(60) Mice were irradiated with a dose of 10 Gy at the thorax prior to the isolation of lung ECs

16 Gy        | EIF2 signaling, actin cytoskeleton signaling | Energy production, cell–cell signaling, cell movement |

|            | CDH13, GDI2, LC25A4, DYN1C1H1, CLTC, GBP2, ISG15, H2D1, SERPINB2, B2M, CASP7 |
| Heart      | ACADM, ACTB, CALD1, DES, EC11, MSN, PRKCDBP, TPM1, FADS1, GBE1, DNH1, MLYCD, FAM120C, ALDOC |

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unsuitable models for sepsis research, since they come from large vessels of the umbilical cord which are not considered early targets of sepsis. Also, HUVECs do not recapitulate the 3-D, morphological and functional microenvironment of organ-specific microvascular ECs (70). A study investigating the effect of a bacterial strain on human brain microvascular ECs was performed (71). Exposure to bacterial strains can disrupt the blood–brain barrier (BBB) and increase the likelihood of toxins to enter the brain resulting in sepsis-associated encephalopathy (SAE) (6, 72). Studies such as these, given the significant level of heterogeneity in ECs from different organs, and additional studies of omic analysis of phenotypes of ECs under flow conditions directly affected by inflammation are urgently needed (70).

Pioneering shear flow-based studies by McCormick in HUVECs and Chen in human aortic ECs in the early 2000s highlighted gene expression changes under flow conditions in a time-dependent manner during 6 or 24 h of flow (73, 74). Endothelial survival genes involved in angiogenesis or matrix remodeling (e.g. TIE2, FLK1) were upregulated during long-term shear exposure which help maintain an anti-inflammatory phenotype, while genes which switch an endothelial phenotype from anti- to pro-inflammatory such as MYD88 and CD30 were downregulated during flow (73, 74). Following these initial studies, a number of other investigators have reported similar gene expression trends under laminar or abnormal shear flow (75, 76, 77).

Particularly, proteomic studies of ECs under shear flow, again in HUVECs, have identified proteins corresponding with the underlying genes. Proteomic analysis of the secretome, defined as proteins secreted from cells, following exposure of ECs to shear stress, could determine if plasma proteins are altered in flow-dependent vascular diseases (66). Over 100 proteins were identified to be secreted under control, laminar or abnormal shear stress conditions (66). Those identified under laminar (e.g. PTHR and LTBP4) or abnormal shear flow (e.g. endothelin-1 and insulin-like growth factor II) conditions are again proteins involved in conferring an anti- or pro-inflammatory ECs phenotype and thus correspond to endothelial genes identified under similar conditions (66). Thus, anti-inflammatory genes and proteins are upregulated by exposure to laminar flow, while pro-inflammatory proteins contributing to vascular inflammation (e.g. DKK1 and Endothelin-1) are expressed by exposure to abnormal shear flow (78, 79). Specifically, during sepsis, the endothelium becomes dysfunctional due to abnormal shear stress, resulting in decreased oxidation in ECs and increased coagulation, among other outcomes (80). Pathogen-associated molecular patterns (PAMPS) and pro-inflammatory cytokines initiate
sepsis leading to the breakdown of ECs–ECs contact, ECs exhibiting a procoagulant phenotype and the shedding of the glyocalyx; these events could lead to hemorheological defects (increased red blood cell aggregation and viscosity) and subsequent reduced arterial pressure, hypotension and abnormal shear stress (80). One of the first proteins found to be impacted by shear stress in vascular inflammation was forkhead box P (a gatekeeper of vascular inflammation) (81) which is downregulated by Kruppel-like factor 2 (a protein involved in adipogenesis, inflammation and T-cell viability) and, in turn, is suppressed by abnormal shear stress (82); this same mechanism applies to sepsis (83). Low shear or no shear stress decreases the activation of Prospero homeobox 1 (a protein-regulating cell fate) and forkhead box C2 (a protein involved in mesenchymal tissue development) and thus decreases thrombomodulin (a glycoprotein that controls coagulation) and endothelial protein C receptor (another protein that regulates coagulation) expression, causing pro-inflammation and leukocyte adhesion/migration (80). Overall, in sepsis, abnormal shear stress significantly impacts endothelial function and glyocalyx shedding and contributes to MODS and death. Thus, additional studies investigating the detrimental effects of differential shear stress on ECs proteomic expression are needed to provide further insight on how sepsis progresses and causes tissue damage.

**In silico modeling of omics**

The large volume of data obtained in omic studies is inherently complex and requires special computational tools for mapping endothelial response pathways, understanding the evolution of inflammatory signaling during sepsis, identifying druggable targets and predicting how different therapeutics may impact the progression of inflammatory signaling. Thus, in addition to experimental approaches, *in silico* modeling can generate testable hypotheses, and simulations can provide new, non-intuitive knowledge on complex systems (20). These models can accelerate the process of discovering novel therapeutic candidates (20) and have been used to investigate how ECs interact with a pathological microenvironment or respond to stimuli. For example, *in silico* modeling has been used to examine how ECs interact with the tumor microenvironment in angiogenesis (84). Other *in silico* models study ECs interacting with other cell types such as hepatocytes (85) or responding to shear stress (86). More recent studies have used organ-specific ECs in pathway models to predict therapeutic targets for specific pathologies, such as diseases in the brain (87). Despite these models providing further insight on how ECs are regulated under various conditions, they have not been applied to investigate the dysfunction of ECs in sepsis which is urgently needed.

In systems biology, several different methods of *in silico* modeling are implemented including agent models, equation models and network models (20). Specifically, network models, based on Boolean logic, are constructed through the integration of the interactome (e.g. protein–protein interaction data) and omics data and do not require a priori quantitative knowledge of biological reactions, which is difficult to achieve (36, 88). Network models are initially constructed by submitting a gene/protein list to a database that maps the entities onto a global PPI model to illustrate their physical interaction or functional association with other entities based on statistical parameters (e.g. confidence scores) (36). Figure 2 is a general workflow of biological network construction and the application of network algorithms.

PPIs are constructed from large-scale experiments or computational predictions and maintained in databases such as BioGRID or STRING (36). Additionally, there are tissue-specific PPI (such as GIANT and TISPIN), and since sepsis affects multiple tissues, the incorporation of omics in multiple tissue-specific *in silico* models for the comparison of differential disease-associated signaling would be beneficial (36). Once an *in silico* network model is generated, graph theory analyses are performed to characterize topological features of the network such as network diameter or node degree (36). Pathway databases such as KEGG can be used in the early stages of modeling to examine signaling pathways in omics data to evaluate the relationships between the data and disease pathways (36). There are numerous strategies to construct and validate *in silico* network models that are discussed elsewhere (88). Overall, *in silico* models have the potential to model drug–protein or protein–protein interactions for a specific pathology, and it is critical to develop such models to decipher omic changes in ECs during sepsis to determine how endothelial inflammatory signaling evolves within and between tissues affected in sepsis.

**Conclusions**

In this review, we discuss the importance of omics, specifically proteomics, profiling of microvascular endothelial cells and leukocytes under septic and/or inflammatory conditions in humans and mice, respectively. In sepsis, microvascular ECs are key targets leading to edema, capillary leak syndrome and MODS...
Since endothelial cells are early targets of sepsis, research focused on creating a systems biology, mechanistic understanding of microvascular endothelial cell dysfunction in sepsis, especially across organs damaged early on such as the lungs, liver and kidneys, is critical. A key area where future research should be directed is profiling the proteome of microvascular endothelial cells in microphysiological systems using cultured organ-specific human microvascular endothelial cells subjected to shear flow to identify differentially expressed proteins (DEPs) and protein–protein interactions that contribute to a disease subphenotype or endotype. Thus, incorporating physiologically relevant flow conditions and organ-specific primary endothelial cells would provide more realistic microenvironments to help identify how organ-specific endothelial proteomes are altered in response to shear and aid in the discovery of therapeutics targeting endothelial cells. Omics can not only characterize the underlying molecular mechanisms of diseases by identifying and quantifying all the biomolecular interactions in a biological system but also categorize patients into endotypes based on their omic expression patterns. Using bioinformatic tools to identify differentially expressed cellular pathways or GO in ECs could answer questions such as where genes or proteins are expressed in a system during different pathologies and their corresponding molecular functions. While genomics can identify causative variants that contribute to disease, complementary experimental and validation studies are often required and needed to identify and characterize the functionality of the variant(s) in disease progression within a heterogenous population. Proteomics, in particular, can yield novel insight since proteins are involved in every biological phenomenon and thus unraveling the complex protein–protein interactions (PPIs) in a cell can identify DEPs between disease and control groups. In addition, one needs to consider that proteins in vivo are subjected to post-translational modifications during and/or after synthesis, and thus future research should focus on identifying these modifications, their potential role in disease and how these modifications can be targeted for therapy.

Another critical area of research is the application of in silico network modeling incorporating proteomics data to screen and test therapeutics for a disease in a realistic model prior to in vitro and in vivo experimentation. Evaluating the effect of a therapeutic on protein(s) or protein complex(es) in silico, especially if the protein complex plays a role in multiple pathways or processes leading to disease, will generate novel hypotheses that (a) would be tested and validated experimentally, (b) complement and refine experimental testing and protocol and (c) potentially reduce the number of animal models needed for experimental studies. In silico models that enable pharmacological intervention of a target (e.g. in silico simulation of knock-out or pharmacological alteration of a biological pathway) would be beneficial.
(91). Additionally, models that can simulate therapeutic responses over time in multiple biological compartments will provide an even more physiologically relevant tool for drug screening and evaluation. Using in silico modeling to repurpose existing therapeutics for treating other diseases (92) is an emerging area of research interest, and its potential for screening therapeutics to target endothelial cells for treating sepsis should be further investigated. Further application of the emerging field of omics for treating sepsis is needed in studies comparing biomarkers used alone or in combination in a time-dependent manner to evaluate their impact on disease progression. A major hurdle for implementing omics in medical practice is a lack of consensus on standardization of methodologies in the scientific community (4). Validation in multicenter, diverse cohorts is urgently needed to effectively test these omic models in clinical trials before translation, and further investigation can assess their utility and cost effectiveness (4). Furthermore, since sepsis progresses rapidly, the quick turn-around time from omic testing to results in healthcare, which is necessary for effective, tailored therapy, has not been achieved yet. Addressing these issues will allow for the translation of omics from the bench to the bedside and, coupled with advances in in silico modeling, establishment of scientific and clinical standards to utilize the potential of omic analyses for clinical treatment of sepsis. Overall, this will significantly advance the goal of precision medicine for delivering the right therapeutic to the right patient at the right time.

**Supplementary materials**

This is linked to the online version of the paper at https://10.1530/VAB-22-0003.

**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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**Author contribution statement**

J C L, M T R, W O, E P and Q Y prepared the manuscript. M F K, L E K and B P developed the ideas and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Leveraging omics to understand endothelium

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