Intensive Care Med
https://doi.org/10.1007/s00134-023-07108-6

NARRATIVE REVIEW

Differentiating infection, colonisation, and sterile inflammation in critical illness: the emerging role of host-response profiling

Mark Jeffrey[1,2], Kerina J. Denny[3,4], Jeffrey Lipman[5,6,7] and Andrew Conway Morris[1,2,8*]

Abstract

Infection results when a pathogen produces host tissue damage and elicits an immune response. Critically ill patients experience immune activation secondary to both sterile and infectious insults, with overlapping clinical phenotypes and underlying immunological mechanisms. Patients also undergo a shift in microbiota with the emergence of pathogen-dominant microbiomes. Whilst the combination of inflammation and microbial shift has long challenged intensivists in the identification of true infection, the advent of highly sensitive molecular diagnostics has further confounded the diagnostic dilemma as the number of microbial detections increases. Given the key role of the host immune response in the development and definition of infection, profiling the host response offers the potential to help unravel the conundrum of distinguishing colonisation and sterile inflammation from true infection. This narrative review provides an overview of current approaches to distinguishing colonisation from infection using routinely available techniques and proposes matrices to support decision-making in this setting. In searching for new tools to better discriminate these states, the review turns to the understanding of the underlying pathobiology of the host response to infection. It then reviews the techniques available to assess this response in a clinically applicable context. It will cover techniques including profiling of transcriptome, protein expression, and immune functional assays, detailing the current state of knowledge in diagnostics along with the challenges and opportunities. The ultimate infection diagnostic tool will likely combine an assessment of both host immune response and sensitive pathogen detection to improve patient management and facilitate antimicrobial stewardship.

Keywords: Infection, Colonisation, Host-response, Antimicrobial stewardship, Rapid diagnostics

Introduction

Infection develops when a microorganism enters a space intolerant of that microorganism, overgrows, or releases toxins that damage the host and provoke an inflammatory response, that if severe enough results in organ failure (sepsis). The macroscopic, cellular, and biochemical features of infection overlap with inflammation from sterile tissue damage. The majority of critically ill patients manifest features of systemic inflammation irrespective of their admitting problem [1]. Critically ill patients rapidly develop dysbiosis, with emergence of pathogen-dominant microbiomes in mucosal organs even in the absence of frank infection. Thus, distinguishing colonisation from infection in the critically ill is challenging.

The advent of highly sensitive molecular pathogen detection shows promise in improving antimicrobial prescribing [2, 3]; however, these techniques may exacerbate the problem of unnecessarily treating colonisation, as organisms can be identified in almost all mucosal organ

*Correspondence: ac926@cam.ac.uk
[2] Division of Anaesthesia, Department of Medicine, Level 4, Addenbrooke's Hospital, University of Cambridge, Hills Road, Cambridge CB2 0QQ, UK
Full author information is available at the end of the article

samples [4–6]. Although the use of antimicrobials in infection is lifesaving [7], inappropriate administration is associated with significant harm [8]. Thus, the differentiation of colonisation in the presence of sterile inflammation from infection is crucial to maximising benefits and minimising harm.

Existing approaches to distinguishing colonisation from infection include assessing clinical features of infection [9], determination of pathogen density [10], and use of plasma biomarkers [11]. These measures are imperfect and lack discriminant ability between infection and sterile inflammation.

Identification of the specific host responses to infection offers the prospect of resolving the problems of mistaking colonisation for infection, or sterile inflammation for culture-negative infection, thus reducing the risks of unnecessary antimicrobials [8, 12]. This review summarises current clinical approaches to this issue, which gives an overview of the current understanding of host–pathogen responses, and important sources of heterogeneity. It then describes the various techniques available to profile host responses and how these may become clinically useable tools in the future.

Current approaches to distinguishing colonisation and sterile inflammation from infection

The original conceptualisation of infection, as invasion of previously sterile spaces by pathogens, has evolved with the discovery of natural microbiomes in mucosal organs. Dysbiotic pathogen-dominant 'pathobiomes' can emerge [13], with phenotypic shifts towards virulence amongst microorganisms under metabolic stress [14]. Dysbiosis may occur in a number of disease contexts and does not automatically lead to infection [15], although it does increase the risk [16]. The phenomena of pathogen colonisation in critical illness [17] can, therefore, be reconceptualised as the development of dysbiosis, with colonisation and infection existing on a continuum instead of binary states [18].

Colonisation is most easily distinguished from infection when systemic inflammation is absent; however, given the high prevalence of inflammation in critical illness [1], this situation is rarely clinically encountered. Although scores, such as the clinical pulmonary infection score (CPIS), can reduce subjectivity, such scores have modest diagnostic performance [9].

Plasma biomarkers, such as C-reactive protein (CRP) and procalcitonin (PCT), are commonly used; however, their ability to distinguish infection from sterile inflammation is imperfect. Both markers have better evidence for early discontinuation of antibiotics than initiation and have recently been reviewed elsewhere [11]. A systematic review of other individual soluble markers has also recently been published [19].

Take home message

Infection can be difficult to distinguish from colonisation and sterile inflammation, with only the former requiring antimicrobial therapy. The rise of highly sensitive microbial diagnostics is likely to exacerbate this problem. The key role of the host immune response in defining infection makes it an attractive target to discriminate infection from colonisation, and thereby maximise benefits and minimise harms from antimicrobials. This article describes current approaches to distinguishing colonisation from infection, the underlying immunopathology of infection and summarises the current and future diagnostic tools.

The microbes identified can help to distinguish colonisation from infection. Some organisms are considered 'non-pathogenic' from sites where colonisation is common; for instance, Candida species detected in the lungs, gut, or bladder or Enterococci in the lungs. This is harder to interpret when the patient is immunosuppressed or when skin commensals are found from sterile site cultures (e.g., blood or cerebrospinal fluid).

The true epidemiology of false-positive and -negative culture rates in intensive care unit (ICU) is hard to elucidate, as these are heavily influenced by testing practices and case definitions. Blood cultures are positive in approximately 10% of cases. Reported false-positive rates vary from 0.3% to 5.5%, leading to poor diagnostic performance in low prevalence populations [20, 21]. To give context to the impact of metagenomic next-generation sequencing (mNGS), blood, sputum, and urine cultures in suspected infections were positive in 7.9%, 39%, and 32% of cases, and increased to 41%, 96%, and 100%, respectively, utilising mNGS [4–6].

Quantitation of pathogenic species is commonly used for discrimination, assuming that heavy growth indicates infection. For instance, in the lungs, common cut-offs are 10^4 colony-forming units (CFU)/ml for lavage and 10^5–10^6 CFU/ml for endotracheal aspirate or sputum [22]. These cut-offs have suboptimal sensitivity compared to histological gold standards [22], and do not take into account dilution or growth suppression from intercurrent antimicrobials.

Although all currently available measures are imperfect, we propose the matrix in Table 1 as optimal current practice in distinguishing colonisation from infection incorporating a risk-based approach to antimicrobial prescribing as advocated in the Surviving Sepsis Campaign guidelines [23]. A pervading difficulty is the lack of a gold standard for differentiating infection from colonisation with concomitant sterile inflammation. Therefore, the central column of Table 1 encompasses the majority

Table 1 Proposed matrices for distinguishing infection from colonisation with sterile inflammation

Host response / Microbiology	**Elevated specific marker** (PCT >2ng/ml*) *OR* **Frank clinical/ radiological evidence of infection** (e.g. pus, radiological abscess)	**Intermediate specific marker** (PCT 0.5-1.9ng/ml*) *OR* **Elevated non-specific marker** (CRP/WCC/Fever) *OR* **Clinical/radiological suspicion of infection** (e.g. pulmonary infiltrates, peri-pancreatic collections)	**Low clinical suspicion and no markers elevated**
Obligate pathogen at high growth	**Infection** *Organism confirmed* Targeted antimicrobials	**Infection** *Organism confirmed* Targeted antimicrobials	**Colonisation** No antimicrobial indicated (unless eradication considered)
Obligate pathogen at sub-threshold growth *OR* **Non-pathogenic organism**	**Infection** *Organism uncertain* Broad antimicrobials	**Possible infection** High risk** - broad antimicrobials Low risk - watch and wait	**Colonisation** No antimicrobial indicated
Negative microbiology	**Infection** *Organism uncertain* Broad antimicrobials	**Possible infection** High risk** - broad antimicrobials Low risk - watch and wait	**No evidence** of infection or colonisation

PCT: procalcitonin; CRP: C-reactive protein; WCC: white cell count

Approach to differentiating infection from colonisation in ICU with current diagnostics. Simultaneous assessment of microbiology and the host response, adjudicated in ambiguous cases by a clinical risk assessment. Patients are anticipated to move between categories as illness and investigation progress, and should be applied as part of a daily review

*PCT may also be elevated in the setting of recent major surgery, trauma, severe burns, and cardiogenic shock

**High risk indicated by the presence of shock, worsening organ failure (e.g., increase in SOFA score $\geq$ 2), or immunosuppression

of patients, with consequent effects on antimicrobial prescribing.

Host–pathogen interactions

Given the centrality of the host response in defining infection, assessing this response offers the potential to overcome the limitations of the approaches detailed above. To enable this assessment, it is important to understand how the host responds to infectious and non-infectious insults, and sources of heterogeneity in those responses (Fig. 1).

Pathogenic microorganisms cause organ injury via direct and indirect activation of the host immune system. Pathogen-associated molecular patterns (PAMPs) are highly conserved microbial motifs that are recognised by pattern recognition receptors (PRRs) on host cells resulting in immune system activation [24]. The ensuing inflammatory response causes tissue injury and release of damage-associated molecular patterns (DAMPs). DAMPs are host molecules, such as histones, high mobility group box protein 1 (HMGB1), and adenosine [25] that when released into the extracellular environment, act as potent immune system activators and perpetuate the initial inflammatory response [26].

PAMPs and DAMPs both act via PRRs, using similar signal transduction machinery, resulting in an immune response and inflammatory cascade [25]. Tissue injury that results from non-infectious insults (e.g., pancreatitis and trauma) also results in the release of DAMPs [27]. The common pathways result in sterile insults mimicking the immune response to infection, making the distinction of these conditions challenging.

Pathogen-specific responses

Pathogen-specific differences in the host response have been identified, though thus far limited to domain and kingdom taxonomic ranks [28, 29]. The transcriptional responses of patients to pathogens show both common and specific elements, and can distinguish bacterial from fungal [30] and viral [31] infections. There are differences

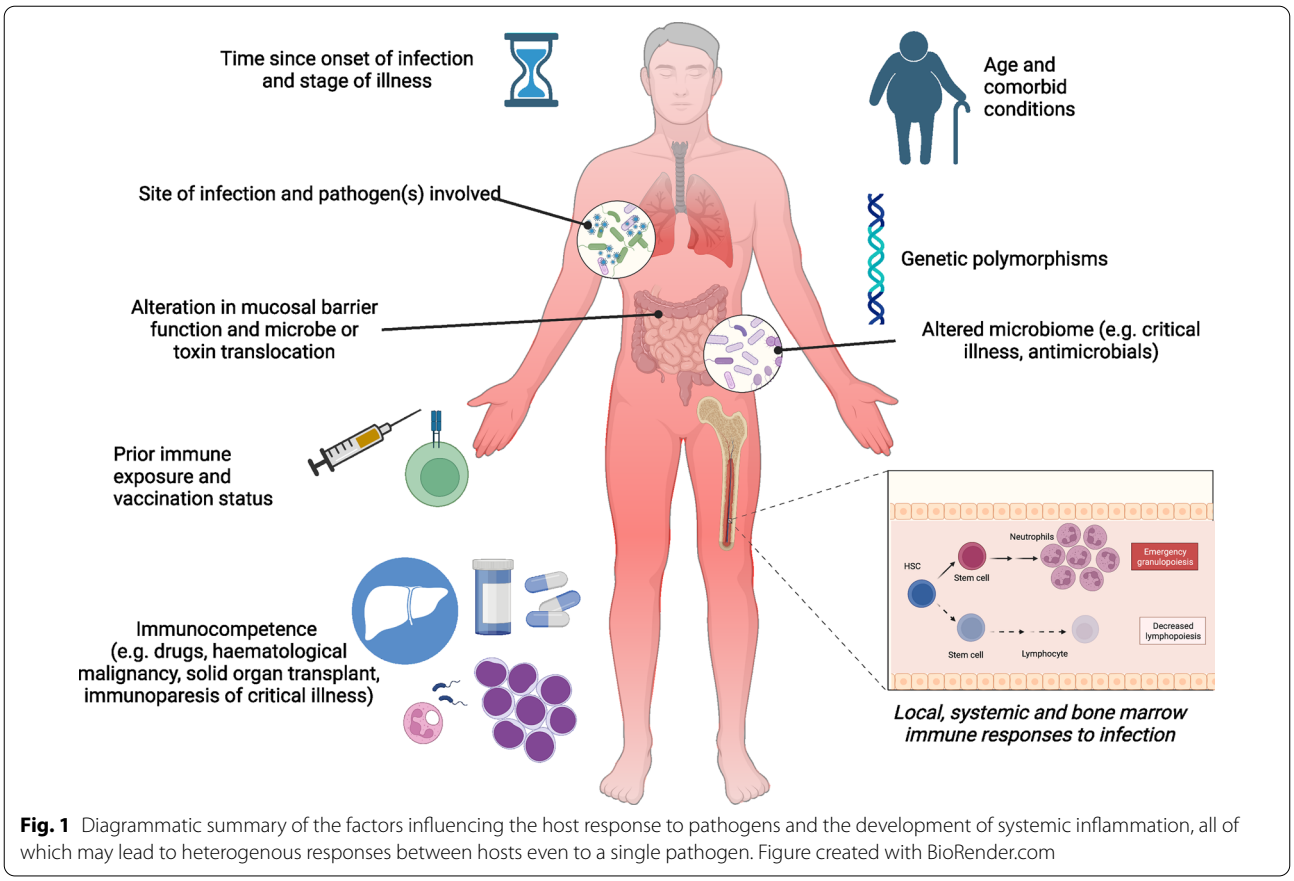

Fig. 1 Diagrammatic summary of the factors influencing the host response to pathogens and the development of systemic inflammation, all of which may lead to heterogenous responses between hosts even to a single pathogen. Figure created with BioRender.com

between the host responses to broad categories of bacteria, such as Gram-positive and negative organisms across microcirculatory alterations [32], cytokine [33], and gene-expression profiles [34]. Heterogeneity in host responses to a particular pathogen may arise from co-infection, differences in the microbiome, genetic background, immunocompetence, and/or the infected organ or body compartment (Fig. 1).

Microbiome

The development of dysbiosis frequently precedes clinically apparent infection. As well as permitting the growth of pathogens, alterations in the microbiome may prime the immune system for a more exuberant response and thus increase the risk of host tissue damage and sepsis [13, 35]. Critical illness also disrupts mucosal barriers and endothelial function, increasing the risk of pathogen translocation [18]. How dysbiosis should be managed remains uncertain. Therapeutic strategies in susceptible populations aimed at reducing the burden of pathogenic microorganisms, such as selective digestive decontamination, have demonstrated promise in reducing morbidity and mortality [36] Other approaches to altering the host microbiome, including probiotics in high-risk patients, have had mixed results [37].

Host genome

Differences in the host genome have been associated with differences in susceptibility to infection [38] and infection-associated end-organ dysfunction. Notably, death from infection is more highly heritable than death from heart disease or malignancy [39], although the absolute size of the impact of genetic factors on infection mortality remains debated [40].

A recent systematic review identified genetic variants associated with a risk of sepsis, including eight variants of PRRs [41]. Similarly, genetic polymorphisms in certain cytokines, such as tumour necrosis factor-alpha (TNF-a) [42] and coagulation factors, may also account for different individual susceptibilities to infection and subsequent sepsis [43].

Host immunocompetence

Differences in host immunocompetence alter the susceptibility to and clinical trajectory of infections, and risks confounding host-response profiling for diagnostic purposes. Immunocompromised patients are at risk of

a broader range of pathogens, with even low virulence organisms causing life-threatening organ dysfunction[31]. Furthermore, the clinical features of infection may be less pronounced, owing to their reduced ability to mount an immune response [44]. The role of host immunocompetence is a complex one and, interestingly, despite an increased susceptibility to infection, patients immunosuppressed following solid-organ transplant have a mortality rate no greater, and in some cases less, than immunocompetent patients even after adjusting for potential confounding factors [44–46].

Immunosuppression is also a complication of sepsis in the previously immunocompetent host. Patients convalescing from a septic insult are at increased risk of recurrent infection [47]. The mechanisms of sepsis-induced immunosuppression are multiple and include inflammation-induced cellular anergy and apoptosis as well as therapy-induced immunosuppression (e.g., glucocorticoid therapy) [48, 49].

Compartmentalisation of infections and host responses

Heterogeneity may also be introduced by infection originating in different organs or compartments. In-hospital mortality differs depending on the origin of infection (e.g., pulmonary, peritoneal, urinary, leptomeningeal, and primary bacteraemia) [50, 51]. The transcriptomic and immune response may also differ depending on what tissue-specific immune cells (e.g., alveolar macrophages) are involved [52].

These factors combine to produce marked heterogeneity in responses to pathogens. The idea that sepsis represents a common, stereotyped host response to infection is giving way to a growing recognition of distinct phenotypes or subclasses [53]. Such subclasses may be characterised using clinical or -omic (i.e., transcriptomic, metabolomic, or proteomic) data. Sepsis subclasses, however, have been difficult to consistently identify. Beyond the heterogeneity outlined above, a further reason for this is timing. Time-series analyses reveal dynamic and rapid transcriptional shifts in immune cells in response to infection over time [54].

Overall, the above host–pathogen interactions are not uniform, but rather show markedly variable pathophysiological features [50, 55]. This heterogeneity complicates attempts to identify a host response that can differentiate between infection and colonisation with sterile inflammation.

Techniques to characterise the host response and distinguish infection from sterile injury

Progress in biological and computational sciences has created tools to characterise specific host immunological responses to the presence of microorganisms. Categorising these tools under transcriptomics, proteomics, and

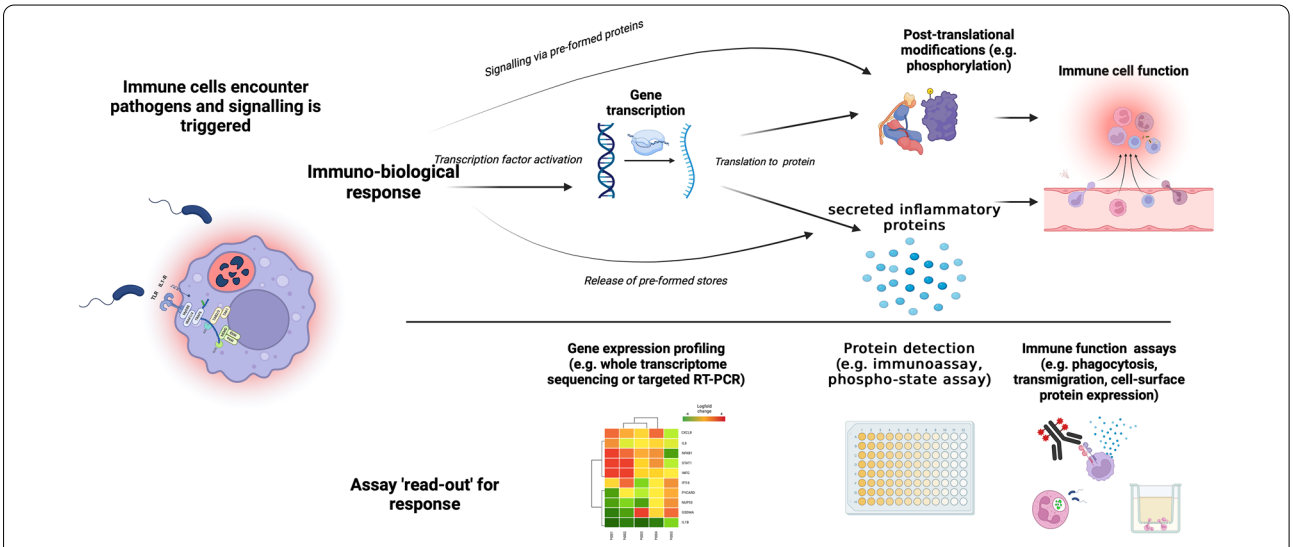

Fig. 2 Diagrammatic summary of how immune cells respond to the stimulus of encountering a pathogen and how these responses can be assessed by laboratory or point-of-care testing. The upper section illustrates some of the immunobiological responses which occur within the patient, including both de-novo protein synthesis following transcription of messenger RNA (mRNA) and activation of existing proteins. Proteins, such as chemokines and cytokines, may be secreted into the extracellular environment and are thus detectable in body fluids. Proteins frequently undergo post-translational modifications, such as the addition or removal of phosphate groups, influencing their function and location within the cell. Protein translation and post-translational modification, combined with the release of soluble signalling molecules, combine to effect immune cell function. Terms in bold are responses which can be assayed, and terms in italics are processes occurring. The lower section illustrates how these various immunobiological responses can be detected and assayed using the tools described in the article. Figure created with BioRender.com

Table 2 Potential influence of a novel host-response marker

Microbiology \ Host response	Sensitive and specific novel host marker(s) of infection positive	Novel host marker negative but clinical/ radiological suspicion of infection	Low clinical suspicion and no markers elevated
Obligate pathogen at high growth	**Infection** *Organism confirmed* Targeted antimicrobials	**Possible infection** High risk* - targeted antimicrobials Low risk - watch and wait	**Colonisation** No antimicrobial indicated (unless eradication considered)
Obligate pathogen at sub-threshold growth *OR* Non-pathogenic organism	**Infection** *Organism uncertain* Broad antimicrobials	**Colonisation** Watch and wait	**Colonisation** No antimicrobial indicated
Negative microbiology	**Infection** *Organism uncertain* Broad antimicrobials	**Unlikely infection or colonisation** Watch and wait High threshold antimicrobials	**No evidence** of infection or colonisation

Rapid advancements in molecular microbiological tests will significantly increase the rate of positive but clinically ambiguous microbe detection. To avoid an increase in inappropriate antimicrobial use, novel host-response markers offer promise in the interpretation of these detections

*High risk indicated by the presence of shock, worsening organ failure (e.g., increase in SOFA score ≥ 2), or immunosuppression

functional immune assays, we review how these phenotyping tools have been used to differentiate infection from non-infectious host responses (Fig. 2). Table 2 illustrates how a hypothetical host-response marker could improve the safety of empiric antimicrobial therapy by reducing the risks of false-negative testing whilst limiting overuse of antimicrobials and should be contrasted with Table 1 showing optimal current practice.

Transcriptional responses and classifiers

Transcriptomic diagnostics with better performance than PCT for differentiating infection from sterile inflammation are already in development and are likely to be the first of the novel host-response diagnostics discussed here to enter clinical practice [56, 57]. The perceived complexity of sequencing and machine-learning make for excellent marketing, but hide significant challenges. It is therefore important for clinicians to understand the strengths and weaknesses of these diagnostics.

Transcriptomics refers to sequencing messenger ribonucleic acid (mRNA) that bridges the gap between static deoxyribonucleic acid and protein expression, thus identifying patterns of cellular signalling. These patterns develop rapidly, and precede clinically overt infection by days [58, 59]. Sequencing the entire transcriptome is useful to identify diagnostic signatures. However, measuring the expression of a small number of genes with diagnostic value is required to make a clinically useable test. Once developed, turnaround times can be as short as 1 hour [56].

There are trade-offs that clinicians should be aware of. First, prediction models often compromise interpretability (or transparency) for accuracy [60]. To illustrate, we consider several mRNA-based tests, namely *FAIM3:PLAC8* ratio [61], SeptiCyte™ [62], Sepsis Metascore [63], and IMX-BVN-2 neural network classifier [57], which are summarised in Table 3.

At the simplest level, a ratio of two genes with strongly divergent expression (*FAIM3:PLAC8*) can be used to discriminate community-acquired pneumonia (CAP) from non-infectious inflammation with better performance than several plasma biomarkers including PCT [61]. Whilst this ratio was validated in sepsis arising from sources other than CAP [52], its high sensitivity is traded against low specificity which would limit impact on antimicrobial prescribing. SeptiCyte™ is slightly more complex, combining two ratios with an improvement in specificity and no loss of sensitivity compared to *FAIM3:PLAC8* [62]. This is being commercialized as SeptiCyte LAB (6 h test time) and an abbreviated *PLAC8/PLA2G7* ratio as SeptiCyte RAPID (1 h test time) [56].

More complex tests utilise larger numbers of genes, although even these represent a tiny minority of those altered in infection. Sepsis Metascore is an 11-gene signature, validated across multiple datasets to differentiate sepsis from non-infectious inflammation (SIRS, trauma,

Table 3 Transcriptomic host-response diagnostics in development

Diagnostic	Population	Timeframe	Score	Validation performance	Genes
FAIM3:PLAC8 [61]	ICU admission with suspected CAP or sepsis	Within 48 h of ICU admission	Gene expression ratio	CAP: sensitivity 97.1%, specificity 28.6% [61] Sepsis: AUROC 0.69–0.78 [52]	FAIM3 PLAC8
SeptiCyte LAB & RAPID [62]	ICU admission with suspected sepsis	Within 24–48 h of ICU admission	Sum of two logged gene-expression ratios	LAB: sensitivity 99%, specificity 56%, AUROC 0.68–0.89 [52, 62] RAPID: AUROC 0.82–0.85 [56]	CEACAM4 LAMP1 PLAC8 PLA2G7
Sepsis Metascore [63]	ICU admission with suspected sepsis; secondary infection in ICU; neonatal Sepsis	Throughout ICU admission	Infection z-score	Sepsis: AUROC 0.80–0.82 [52] Secondary infection: 0.68–0.84 (using time-matched baseline) [63] Neonatal sepsis: AUROC 0.92 [79]	11 gene sets
IMX-BVN-2 [57]	Emergency department attendances with suspected bacterial or viral infection or sepsis	In the Emergency Department	Neural network output with graded likelihood bands	Bacterial infection: AUROC 0.89 Viral infection: AUROC 0.83 [57]	29 gene sets

and critical illness) [63]. This 'infection z-score' is the difference in geometric means between positive and negative gene expression, which is less intuitive. However, its multiple validation sets [52, 63] and ability to be used at multiple timepoints rather than only at ICU admission are appealing. The most complex of the tests reviewed is IMX-BVN-2, a neural network classifier that utilises 29 genes to identify bacterial and viral infections in patients presenting to the emergency department. Diagnostic performance is good and superiority to PCT is suggested, though this should be interpreted with caution as PCT was available to adjudicating clinicians in the training data. The output is a score between 0 and 1 calculated by the neural network with little transparency. To improve interpretability, cut-offs corresponding to likelihoods of infection are defined [57].

It is important to understand how these gene sets were derived, with the two broad approaches being data- or biology-driven discovery. Data-driven takes a statistical approach, identifying genes best able to discriminate infected patients. Whilst the genes identified may seem plausibly related to mechanisms, pathophysiological relevance cannot be assumed. As an example of a biology-driven approach, Reyes and colleagues identified a new subtype of monocyte (MS1) which is expanded in sepsis compared to uncomplicated infections and non-septic ICU patients, and inducible on LPS stimulation. Using MS1 marker genes to estimate their abundance from RNAseq data proved promising in identification of sepsis, but less effective at differentiating infection from sterile inflammation. They also proposed a hybrid classifier using MS1 marker genes, *PLAC8* and *CLU* (identified with a 'data-driven' approach) which achieved similar performance to *FAIM3:PLAC8* ratio and SeptiCyte™ [64]. Biologically-driven discovery offers the possibility of 'theragnostic testing' which can be both diagnostic and guide disease-modifying therapy.

When assessing machine-learning derived tests, clinicians should be wary of looking at accuracy alone in the way one considers a conventional biomarker. Bias–variance trade-off needs to be considered. Bias is the distance of a model's predictions from truth, and variance how much predictions would change if different training data were used [60]. These qualities form a U-shaped curve, meaning that increasing accuracy often reduces generalizability.

Integrating the host transcriptome response with microbiome sequencing

Integrating host-response diagnostics with microbial detection using mNGS is promising as it may help limit antimicrobials use in the face of drastically increased microbial detection rates [5, 6], though it is further from realisation than the classifiers reviewed above. mNGS, unlike syndromic PCR panel tests, has the potential to detect all microbes present in a sample. This approach has been applied to tracheal aspirates for the diagnosis of lower respiratory tract infection (LRTI) [65] and sepsis in critically ill adults [66].

An important innovation was the application of a rule-based model (RBM) to the mNGS results to differentiate pathogens from commensal organisms by identifying highly abundant organisms weighted by known pathogenicity. This achieved an accuracy of 95.5% in tracheal

aspirates and 78% compared to blood cultures. Identifying polymicrobial growths on culture and respiratory viruses from blood were weaknesses, but it revealed putative pathogens in 62% of clinically adjudicated LRTIs with negative microbiology and 42% of sepsis cases with negative cultures [65, 66].

Host-response profiling from tracheal aspirates in LRTI identified upregulated innate immune responses and downregulation of oxidative stress responses and MHC class II receptor signalling pathways. In viral infections, RSAD2 and OAS3, IFN-inducible anti-viral proteins, were upregulated compared to bacterial infections. Combining the RBM and a 12-gene host-response classifier achieved 100% sensitivity and 87.5% specificity. If used as a clinical rule-out tool, they estimated a 38% reduction in antibiotic days in their no-LRTI group [65].

In sepsis, upregulation of neutrophil degranulation and innate immune signalling was identified. Viral sepsis demonstrated upregulated interferon signalling, and an effective virus-specific classifier was developed. Combining mNGS and host-response classifiers for sepsis achieved a sensitivity of 99% and specificity of 78% [66]. Both integrated models require prospective validation but demonstrate the potential for sensitive and specific identification of infection in critically ill patients (Box 1).

Pre-symptomatic identification of infection

Infection specific host-response signatures can be identified before clinical presentation. Tsitsiklis and colleagues [58] examined the host response before development of ventilator-associated pneumonia (VAP) complicating coronavirus disease 2019 (COVID-19). They demonstrated changes in the host response consistent with bacterial infection (neutrophil degranulation, antigen presentation, Toll-Like Receptor (TLR), and cytokine signalling) 2 days before clinical recognition of secondary pneumonia.

The same pathways were suppressed immediately after intubation and as much as 2 weeks before the development of VAP compared to non-VAP patients. These findings are consistent with previous data which demonstrate impaired host responses identifying patients at risk of VAP during their admission, as well as identifying the development of VAP before clinical recognition in COVID-19 [58]. These findings highlight the importance of compartment-specific host-response signatures, and of dynamic pre-disposing and infection-specific changes affecting the same pathways, in different directions, at different time points.

A pre-symptomatic host-response signature of infection has also been identified in elective surgery patients 3 days before clinical recognition. Gene signatures from blood were able to discriminate infection from non-infectious systemic inflammation, and sepsis from uncomplicated infection [59].

Proteins and proteomics

CRP and PCT are the archetypal biomarkers of an inflammatory host response but are unable to differentiate infection from sterile inflammation [11]. Neutrophil surface CD64 expression is the only novel marker to have shown consistently better performance than these in larger trials [19] and is discussed under functional assays. Alveolar cytokines have also shown promise in distinguishing pneumonia from non-infectious inflammation with notable compartmentalisation of cytokine responses [67]. However, implementation of this test in clinical practice did not alter antibiotic prescribing [68]. This failure to impact clinical decision-making highlights a challenge for novel technologies with a lack of familiarity amongst clinicians.

A newer approach is the analysis of the whole proteome. Yuxin and colleagues [69] made significant advances scaling proteomic analysis with high-throughput tandem mass spectrometry. They examined thousands of proteins in septic patients admitted to ICU compared to healthy volunteers, elective surgery, and uninfected patients. Fourteen differentially abundant proteins were consistently identified, though diagnostic performance has yet to be explored. Whole proteome analysis is an emerging area with greater similarity to transcriptomics than traditional biomarkers. Integration with the transcriptome and microbiome, a multi-omics approach [70], offers novel methods to identify host inflammatory responses that are specific to infection.

Signalling within cells is frequently effected through post-translational modification (PTM) of proteins, with the addition or removal of chemical groups, such as acetyl, phosphoryl, or methyl on amino acids. These side-chain alterations change protein function, solubility, trafficking, or location. Exposure to pathogens induces rapid and extensive changes in protein side chains, such as the changes in phosphorylation seen in neutrophils on exposure to *Staphylococcus aureus* [71]. Whilst many of these responses to pathogens are common to multiple stimuli, identifying specific signalling responses offers the potential for accurate identification of infection. The potential for detecting PTM as a diagnostic tool has been noted in oncology [72], although, in infection, this remains an underexplored area.

Relationships between multiomic datasets

Although mRNA encodes proteins, there are consistent discrepancies between the abundance of proteins and their encoding mRNA. Although technical difficulties during measurement should not be disregarded, Wang and colleagues [73] hypothesise that information flows between the genome, transcriptome, and proteome analogously to memory in a computer from the hard disc to RAM to cached memory, resulting in related but functionally different records.

As an example of this complexity consider PCT. PCT is encoded by *CALC-I* gene and cleaved to form N-procalcitonin, calcitonin carboxypeptide-1 (CCP-I), and calcitonin (CT). In sepsis, PCT production increases due to widespread expression of *CALC-I* in tissues. Measuring blood *CALC-I* gene expression may not reflect this, and it is notably absent from the host-response classifiers reviewed. PCT and N-procalcitonin remain significantly elevated in serum, but this does not translate to elevated CT levels [74]. These examples show the challenge of understanding complex, dynamic systems from snapshots of -omic data. These datasets are non-synonymous and have counterintuitive interactions, and intellectual humility regarding our understanding is vital.

Functional immune assays

Functional Immune Assays (FIAs) are a diverse group of tests. We discuss three with potential in identifying infection-specific host responses that can differentiate infection from sterile inflammation; neutrophil CD64 expression, neutrophil transmigration, and pulmonary intravital microscopy.

The expression of CD64, an IgG receptor, on neutrophils is increased following stimulation with a variety of inflammatory mediators. Increased expression has shown diagnostic utility in infection vs sterile inflammation, with best performance at ICU admission [19] although more modest performance in later ICU-acquired infections. Measurement requires flow cytometry, and although point-of-care flow cytometers are increasingly available, this test is not yet commonly offered.

Cell transmigration is an important functional component of the host immune response. Using microscopy and timelapse photography, cell migration can be quantified. Neutrophil migration patterns revealed a unique phenotype of spontaneous migration which was specific for burns patients with sepsis and rarely observed in burns patients without sepsis. The pattern often developed 1–2 days before clinical recognition of sepsis. It achieved sensitivity of 80% and specificity of 77% for the identification of sepsis in patients with major burns [75].

Miniaturisation of microscopes has led to the development of clinically useable in-vivo endoscopic microscopes. Akram et al. combined these with administration of fluorescent probes, visualizing bacteria in alveoli [76]. Limitations include invasiveness and location specificity.

Challenges and limitations of host-response diagnostics

Despite their promise, host-response diagnostics face challenges (Box 2). Significant inter-individual variation in the host response to infection exists (Fig. 1). The response to infection is also demonstrably different in primary versus secondary infections and following distinct insults (e.g., trauma, surgery, etc.) [63]. The impact of co-infection also remains to be characterised. Identifying the impact of these heterogenous conditions on diagnostic performance is crucial, and individual baseline comparisons may be required.

The use of machine-learning models should also be considered. The biological implications and limitations of CRP and PCT are well known and incorporated into clinical decision-making. Scores from complex models have no such biological basis from which to reason. Bias in machine-learning is also well recognised. To avoid harm, developers and clinicians must be clear what a model is classifying, use diverse training data, inspect decision-making through interpretability and comparison to prior knowledge, and monitor post-deployment performance [77].

Finally, results must be translated into clinical benefit. This may be a significant challenge as the potential for rapid proliferation of transcriptomic host-response diagnostics without clearly defined clinical roles and, often, only marginal gains over current practice, may undermine any benefits. Conversely, they might also prove too inaccessible. Opaque, time-consuming and expensive tests are unlikely to see widespread adoption.

To realise the potential of host-response diagnostics, developers and clinicians must prioritise the most robust, interpretable, and accessible. A focus on challenging specific clinical problems would also be welcomed, such as the identification of secondary infections in critical illness and sterile inflammation, or treatment-altering conditions such as non-bacterial sepsis. Clinicians should familiarise themselves with concepts introduced by -omics and machine-learning, and work with developers to ensure robust validation and effective implementation, for example by embedding them in adaptive clinical trials, engaging in post-deployment monitoring and diagnostic stewardship [78].

Box 1: Simultaneous assessment of the host transcriptome and microbiome may

- Identify causative organisms in those with negative cultures [65, 66].
- Identify organisms likely to be commensal or colonising [65, 66].
- Identify a specific host response to infection despite negative microbiology [65, 66].
- Indicate whether the immune response is targeting bacteria or viruses [31].
- Increase the proportion of patients in which infection can be accurately excluded [65, 66].
- Identify evidence of infection before clinical signs appear [58, 59].

Box 2: Barriers to the implementation of transcriptomic, proteomic and functional immune assays in clinical practice

1. Elucidating relevant mechanisms, disease phenotypes, and treatment targets.
2. Reducing the financial, time and computational resource cost associated with the analysis and interpretation of results.
3. Standardisation of assays to allow comparison between patients, labs and batches.
4. Robust quality control, measurement error identification and reporting.
5. Minimising the impact of cellular stress during sampling and processing.
6. Defining 'normal values' in a dynamic, heterogeneous group of patients.
7. Enabling suitable technological platforms are widely available to allow routine assessment of these parameters in clinical practice.

Conclusions

Diagnosing infection in the systemically inflamed, critically ill patient is challenging. The advent of molecular pathogen tests offers the prospect of greater sensitivity in the detection of microbes but may provide further challenges in distinguishing infection from sterile inflammation and colonisation. The host response to invasive microorganisms is critical to defining, and thus diagnosing, infection, and sepsis. These responses include aspects which are microorganism specific and those that overlap with host tissue damage arising from sterile insults. Host responses are modulated by numerous factors, including underlying genotype, immunocompetence, intercurrent medications, and the patient's endogenous microbial flora. Thus, the identification of specific host markers of infection is challenging.

The host response to infection can be interrogated via a range of modalities, including transcriptional, proteomic, and functional profiling, taking samples from the blood or the site of the infection. Although we are increasingly able to obtain unbiased profiles of all available transcripts or proteins present, and indeed can resolve these down to the single cell level, clinically useable tools will need to focus on the most informative markers, return reproducible and reliable results in a short time without the need for complex and skilled analytics. Although there are several promising techniques available, most clinically applicable assays have not yet made it past the development stage and their impact on clinical decision-making remains uncertain.

Author details
[1] John V Farman Intensive Care Unit, Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK. [2] Division of Anaesthesia, Department of Medicine, Level 4, Addenbrooke's Hospital, University of Cambridge, Hills Road, Cambridge CB2 0QQ, UK. [3] Department of Intensive Care, Gold Coast University Hospital, Southport, QLD, Australia. [4] School of Medicine, University of Queensland, Herston, Brisbane, Australia. [5] University of Queensland Centre for Clinical Research, The University of Queensland, Brisbane, Australia. [6] Jamieson Trauma Institute and Intensive Care Services, Royal Brisbane and Women's Hospital, Brisbane, Australia. [7] Nimes University Hospital, University of Montpellier, Nimes, France. [8] Division of Immunology, Department of Pathology, University of Cambridge, Cambridge, UK.

Author contributions
MJ reviewed and appraised the literature, drafted and revised the manuscript, and approved the final version. KD reviewed and appraised the literature, drafted and revised the manuscript, and approved the final version. JL conceived the article, reviewed and appraised the literature, revised the manuscript, and approved the final version. ACM conceived the article, reviewed and appraised the literature, drafted and revised the manuscript, and approved the final version.

Funding
ACM is supported by a Clinician Scientist Fellowship from the Medical Research Council (MR/V006118/1). MJ is supported by a Clinical Research Fellowship from Addenbrookes Charitable Trust.

Declarations

Conflicts of interest
JL has received honoraria from Pfizer and MSD. ACM has received speaking fees from Boston Scientific, Biomerieux and Fischer and Paykel, and ACM is a member of the scientific advisory board of Cambridge Infection Diagnostics. All other authors declare that they have no relevant conflicts of interest.

Publisher's Note

Received: 7 March 2023 Accepted: 22 May 2023
Published online: 21 June 2023

References

1. Griffith DM, Lewis S, Rossi AG et al (2016) Systemic inflammation after critical illness: relationship with physical recovery and exploration of potential mechanisms. Thorax 71:820–829. https://doi.org/10.1136/thoraxjnl-2015-208114
2. Hayakawa K, Mezaki K, Kobayakawa M et al (2017) Impact of rapid identification of positive blood cultures using the Verigene system on antibiotic prescriptions: a prospective study of community-onset bacteremia in a tertiary hospital in Japan. PLoS ONE 12:e0181548. https://doi.org/10.1371/JOURNAL.PONE.0181548
3. Echavarría M, Marcone DN, Querci M et al (2018) Clinical impact of rapid molecular detection of respiratory pathogens in patients with acute respiratory infection. J Clin Virol 108:90–95. https://doi.org/10.1016/J.JCV.2018.09.009
4. Geng S, Mei Q, Zhu C et al (2021) Metagenomic next-generation sequencing technology for detection of pathogens in blood of critically ill patients. Int J Infect Dis 103:81–87. https://doi.org/10.1016/J.IJID.2020.11.166
5. Zhou JJ, Ding WC, Liu YC et al (2022) Diagnostic value of metagenomic next-generation sequencing for pulmonary infection in intensive care unit and non-intensive care unit patients. Front Cell Infect Microbiol 12:936. https://doi.org/10.3389/FCIMB.2022.929856

6. Duan W, Yang Y, Zhao J et al (2022) Application of metagenomic next-generation sequencing in the diagnosis and treatment of recurrent urinary tract infection in kidney transplant recipients. Front Public Heal 10:2517. https://doi.org/10.3389/FPUBH.2022.901549
7. Vincent JL, Sakr Y, Singer M et al (2020) Prevalence and outcomes of infection among patients in intensive care units in 2017. JAMA 323:1478–1487. https://doi.org/10.1001/JAMA.2020.2717
8. Arulkumaran N, Routledge M, Schlebusch S et al (2020) Antimicrobial-associated harm in critical care: a narrative review. Intensive Care Med 46:225–235. https://doi.org/10.1007/s00134-020-05929-3
9. Zilberberg MD, Shorr AF (2010) Ventilator-associated pneumonia: the clinical pulmonary infection score as a surrogate for diagnostics and outcome. Clin Infect Dis 51:S131–S135. https://doi.org/10.1086/653062
10. Conway Morris A, Kefala K, Simpson AJ et al (2009) Evaluation of the effect of diagnostic methodology on the reported incidence of ventilator-associated pneumonia. Thorax 64:516–522. https://doi.org/10.1136/THX.2008.110239
11. Póvoa P, Coelho L, Dal-Pizzol F et al (2023) How to use biomarkers of infection or sepsis at the bedside: guide to clinicians. Intensive Care Med 49:142. https://doi.org/10.1007/S00134-022-06956-Y
12. Denny KJ, Cotta MO, Parker SL et al (2016) The use and risks of antibiotics in critically ill patients. Expert Opin Drug Saf 15:667–678. https://doi.org/10.1517/14740338.2016.1164690
13. Miniet AA, Grunwell JR, Coopersmith CM (2021) The microbiome and the immune system in critical illness. Curr Opin Crit Care 27:157. https://doi.org/10.1097/MCC.0000000000000800
14. Wu LR, Zaborina O, Zaborin A et al (2005) Surgical injury and metabolic stress enhance the virulence of the human opportunistic pathogen *Pseudomonas aeruginosa*. Surg Infect (Larchmt) 6:185–195. https://doi.org/10.1089/SUR.2005.6.185
15. Maes M, Higginson E, Pereira-Dias J et al (2021) Ventilator-associated pneumonia in critically ill patients with COVID-19. Crit Care. https://doi.org/10.1186/S13054-021-03460-5
16. Betzold RD, Dennis BM, Bellister SA et al (2020) Antibiotic exposure Is not associated with clearance of bronchoalveolar growth: results From a prospective ventilator-associated pneumonia study. Am Surg 87:1347–1351. https://doi.org/10.1177/0003134820966281
17. Ewig S, Torres A, El-Ebiary M et al (2012) Bacterial colonization patterns in mechanically ventilated patients with traumatic and medical head injury. Am J Respir Crit Care Med 159:188–198. https://doi.org/10.1164/AJRCCM.159.1.9803097
18. Roquilly A, Torres A, Villadangos JA et al (2019) Pathophysiological role of respiratory dysbiosis in hospital-acquired pneumonia. Lancet Respir Med 7:710–720. https://doi.org/10.1016/S2213-2600(19)30140-7
19. Pierrakos C, Velissaris D, Bisdorff M et al (2020) Biomarkers of sepsis: time for a reappraisal. Crit Care 24:1–15. https://doi.org/10.1186/S13054-020-02993-5
20. Kseibi E, Naesah N, Hajja A, Kseibi F (2022) Utilization and diagnostic yield of blood cultures in a surgical intensive care unit. Crit Care Med 50:339–339. https://doi.org/10.1097/01.CCM.0000809088.07896.4D
21. Zanella MC, De Lorenzi-Tognon M, Fischer A et al (2022) Bacteremia detection in second or subsequent blood cultures among hospitalized patients in a tertiary care hospital. JAMA Netw Open 5:e228065–e228065. https://doi.org/10.1001/JAMANETWORKOPEN.2022.8065
22. Chastre J, Fagon JY (2012) Ventilator-associated Pneumonia. Am J Respir Crit Care Med 165:867–903. https://doi.org/10.1164/AJRCCM.165.7.2105078
23. Evans L, Rhodes A, Alhazzani W et al (2021) Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. Crit Care Med 49:E1063–E1143. https://doi.org/10.1097/CCM.0000000000005337
24. Mogensen TH (2009) Pathogen recognition and inflammatory signaling in innate immune defenses. Clin Microbiol Rev 22:240–273. https://doi.org/10.1128/CMR.00046-08
25. Denning NL, Aziz M, Gurien SD, Wang P (2019) Damps and nets in sepsis. Front Immunol. https://doi.org/10.3389/FIMMU.2019.02536
26. Schaefer L (2014) Complexity of danger: the diverse nature of damage-associated molecular patterns. J Biol Chem 289:35237–35245. https://doi.org/10.1074/jbc.R114.619304
27. Vourc'h M, Roquilly A, Asehnoune K (2018) Trauma-induced damage-associated molecular patterns-mediated remote organ injury and immunosuppression in the acutely Ill patient. Front Immunol 9:1. https://doi.org/10.3389/FIMMU.2018.01330
28. Goh C, Knight JC (2017) Enhanced understanding of the host–pathogen interaction in sepsis: new opportunities for omic approaches. Lancet Respir Med 5:212–223. https://doi.org/10.1016/S2213-2600(17)30045-0
29. Li Y, Oosting M, Deelen P et al (2016) Inter-individual variability and genetic influences on cytokine responses against bacterial and fungal pathogens. Nat Med 22:952. https://doi.org/10.1038/NM.4139
30. Cheng SC, Scicluna BP, Arts RJW et al (2016) Broad defects in the energy metabolism of leukocytes underlie immunoparalysis in sepsis. Nat Immunol 174(17):406–413. https://doi.org/10.1038/ni.3398
31. Sweeney TE, Wong HR, Khatri P (2016) Robust classification of bacterial and viral infections via integrated host gene expression diagnostics HHS Public Access. Sci Transl Med 8:346–391. https://doi.org/10.1126/scitranslmed.aaf7165
32. Legrand M, Klijn E, Payen D, Ince C (2010) The response of the host microcirculation to bacterial sepsis: does the pathogen matter? J Mol Med (Berl) 88:127. https://doi.org/10.1007/S00109-009-0585-6
33. Feezor RJ, Oberholzer C, Baker HV et al (2003) Molecular characterization of the acute inflammatory response to infections with gram-negative versus gram-positive bacteria. Infect Immun 71:5803–5813. https://doi.org/10.1128/IAI.71.10.5803
34. Wang Q, Li X, Tang W et al (2022) Differential gene sets profiling in gram-negative and gram-positive sepsis. Front Cell Infect Microbiol 12:38. https://doi.org/10.3389/fcimb.2022.801232
35. Adelman MW, Woodworth MH, Langelier C et al (2020) The gut microbiome's role in the development, maintenance, and outcomes of sepsis. Crit Care. https://doi.org/10.1186/S13054-020-02989-1
36. Hammond NE, Myburgh J, Seppelt I et al (2022) Association between selective decontamination of the digestive tract and in-hospital mortality in intensive care unit patients receiving mechanical ventilation: a systematic review and meta-analysis. JAMA. https://doi.org/10.1001/JAMA.2022.19709
37. Sulaiman Alsuwaylihi A, McCullough F (2023) The safety and efficacy of probiotic supplementation for critically ill adult patients: a systematic review and meta-analysis. Nutrition. https://doi.org/10.1093/nutrit/nuac059
38. Rogne T, Damås JK, Solligård E, Dewan AT (2020) Genome-wide linkage analysis of the risk of contracting a bloodstream infection in 47 pedigrees followed for 23 years assembled from a population-based cohort (the HUNT Study). Crit Care Med 48:1580–1586. https://doi.org/10.1097/CCM.0000000000004520
39. Sørensen TIA, Nielsen GG, Andersen PK, Teasdale TW (2010) Genetic and environmental influences on premature death in adult adoptees. N Engl J Med 318:727–732. https://doi.org/10.1056/NEJM198803243181202
40. Obel N, Christensen K, Petersen I et al (2010) Genetic and environmental influences on risk of death due to infections assessed in Danish Twins, 1943–2001. Am J Epidemiol 171:1007. https://doi.org/10.1093/AJE/KWQ037
41. Lu H, Wen D, Wang X et al (2019) Host genetic variants in sepsis risk: a field synopsis and meta-analysis. Crit Care 23:1–13. https://doi.org/10.1186/S13054-019-2313-0
42. Georgescu AM, Banescu C, Azamfirei R et al (2020) Evaluation of TNF-α genetic polymorphisms as predictors for sepsis susceptibility and progression. BMC Infect Dis. https://doi.org/10.1186/S12879-020-4910-6
43. Arcaroli J, Fessler MB, Abraham E (2005) Genetic polymorphisms and sepsis. Shock 24:300–312. https://doi.org/10.1097/01.SHK.0000180621.52058.E1
44. McCreery RJ, Florescu DF, Kalil AC (2020) Sepsis in immunocompromised patients without human immunodeficiency virus. J Infect Dis 222:S156–S165. https://doi.org/10.1093/INFDIS/JIAA320
45. Malinis MF, Mawhorter SD, Jain A et al (2012) Staphylococcus aureus bacteremia in solid organ transplant recipients: evidence for improved survival when compared with nontransplant patients. Transplantation 93:1045–1050. https://doi.org/10.1097/TP.0B013E31824BF219
46. Donnelly JP, Locke JE, MacLennan PA et al (2016) Inpatient mortality among solid organ transplant recipients hospitalized for sepsis and severe sepsis. Clin Infect Dis An Off Publ Infect Dis Soc Am 63:186. https://doi.org/10.1093/CID/CIW295

47. Yao RQ, Ren C, Zheng LY et al (2022) Advances in immune monitoring approaches for sepsis-induced immunosuppression. Front Immunol. https://doi.org/10.3389/fimmu.2022.891024
48. Heming N, Sivanandamoorthy S, Meng P et al (2018) Immune effects of corticosteroids in sepsis. Front Immunol 9:1736. https://doi.org/10.3389/FIMMU.2018.01736
49. Torres LK, Pickkers P, Van Der Poll T (2022) Sepsis-Induced Immunosuppression. Annu Rev Physiol 84:157–181. https://doi.org/10.1146/ANNUREV-PHYSIOL-061121-040214
50. Pieroni M, Olier I, Ortega-Martorell S et al (2022) In-hospital mortality of sepsis differs depending on the origin of infection: an investigation of predisposing factors. Front Med 9:915224. https://doi.org/10.3389/FMED.2022.915224
51. Conway Morris A, Rynne J, Shankar-Hari M (2022) Compartmentalisation of immune responses in critical illness: does it matter? Intensive Care Med 48:1617–1620. https://doi.org/10.1007/S00134-022-06871-2
52. Maslove DM, Shapira T, Tyryshkin K et al (2019) Validation of diagnostic gene sets to identify critically ill patients with sepsis. J Crit Care 49:92–98. https://doi.org/10.1016/J.JCRC.2018.10.028
53. Maslove DM, Tang B, Shankar-Hari M et al (2022) Redefining critical illness. Nat Med 28:1141–1148. https://doi.org/10.1038/s41591-022-01843-x
54. Maslove DM, Wong HR (2014) Gene expression profiling in sepsis: timing, tissue, and translational considerations. Trends Mol Med 20:204. https://doi.org/10.1016/J.MOLMED.2014.01.006
55. Prescott HC, Calfee CS, Taylor Thompson B et al (2016) Toward smarter lumping and smarter splitting: rethinking strategies for sepsis and acute respiratory distress syndrome clinical trial design. Am J Respir Crit Care Med 194:147–155. https://doi.org/10.1164/RCCM.201512-2544CP
56. Balk R, Esper AM, Martin GS et al (2023) Validation of SeptiCyte RAPID to discriminate sepsis from non-infectious systemic inflammation. medRxiv. https://doi.org/10.1101/2022.07.20.22277648
57. Bauer W, Kappert K, Galtung N et al (2021) A Novel 29-messenger RNA host-response assay from whole blood accurately identifies bacterial and viral infections in patients presenting to the emergency department with suspected infections: a prospective observational study. Crit Care Med 49:1664. https://doi.org/10.1097/CCM.0000000000005119
58. Tsitsiklis A, Zha BS, Byrne A et al (2021) Impaired immune signaling and changes in the lung microbiome precede secondary bacterial pneumonia in COVID-19. Res Sq. https://doi.org/10.21203/RS.3.RS-380803/V1
59. Lukaszewski RA, Jones HE, Gersuk VH et al (2022) Presymptomatic diagnosis of postoperative infection and sepsis using gene expression signatures. Intensive Care Med 48:1133–1143. https://doi.org/10.1007/s00134-022-06769-z
60. James G, Witten D, Hastie T, Tibshirani R (2021) An introduction to statistical learning with applications in R, 2nd edn. Springer, New York
61. Scicluna BP, Klein Klouwenberg PMC, Van Vught LA et al (2015) A molecular biomarker to diagnose community-acquired pneumonia on intensive care unit admission. Am J Respir Crit Care Med 192:826–835. https://doi.org/10.1164/RCCM.201502-0355OC
62. McHugh L, Seldon TA, Brandon RA et al (2015) A molecular host response assay to discriminate between sepsis and infection-negative systemic inflammation in critically ill patients: discovery and validation in independent cohorts. PLoS Med. https://doi.org/10.1371/JOURNAL.PMED.1001916
63. Sweeney TE, Shidham A, Wong HR, Khatri P (2015) A comprehensive time-course–based multicohort analysis of sepsis and sterile inflammation reveals a robust diagnostic gene set. Sci Transl Med 7:287ra71. https://doi.org/10.1126/SCITRANSLMED.AAA5993
64. Reyes M, Filbin MR, Bhattacharyya RP et al (2020) An immune-cell signature of bacterial sepsis. Nat Med 26:333–340. https://doi.org/10.1038/s41591-020-0752-4
65. Langelier C, Kalantar KL, Moazed F et al (2018) Integrating host response and unbiased microbe detection for lower respiratory tract infection diagnosis in critically ill adults. Proc Natl Acad Sci USA 115:E12353–E12362. https://doi.org/10.1073/pnas.1809700115
66. Kalantar KL, Neyton L, Abdelghany M et al (2022) Integrated host-microbe plasma metagenomics for sepsis diagnosis in a prospective cohort of critically ill adults. Nat Microbiol 711(7):1805–1816. https://doi.org/10.1038/s41564-022-01237-2
67. Hellyer TP, Morris AC, Mcauley DF et al (2015) Diagnostic accuracy of pulmonary host inflammatory mediators in the exclusion of ventilator-acquired pneumonia. Thorax 70:41–47. https://doi.org/10.1136/thoraxjnl-2014-206280
68. Hellyer TP, McAuley DF, Walsh TS et al (2020) Biomarker-guided antibiotic stewardship in suspected ventilator-associated pneumonia (VAPrapid2): a randomised controlled trial and process evaluation. Lancet Respir Med 8:182–191. https://doi.org/10.1016/S2213-2600(19)30367-4
69. Mi Y, Burnham KL, Charles PD et al (2022) High-throughput mass spectrometry maps the sepsis plasma proteome and differences in response. medRxiv. https://doi.org/10.1101/2022.08.07.22278495
70. Rajczewski AT, Jagtap PD, Griffin TJ (2022) An overview of technologies for MS-based proteomics-centric multi-omics. Expert Rev Proteomics 19:165–181. https://doi.org/10.1080/14789450.2022.2070476
71. Wood AJT, Vassallo AM, Ruchaud-Sparagano MH et al (2020) C5a impairs phagosomal maturation in the neutrophil through phosphoproteomic remodeling. JCI Insight. https://doi.org/10.1172/JCI.INSIGHT.137029
72. Ghosh R, Ahmed R, Ahmed H, Chatterjee BP (2022) Phosphorylated proteins from serum: a promising potential diagnostic biomarker of cancer. Int J Mol Sci 23:12359. https://doi.org/10.3390/IJMS232012359
73. Wang D (2008) Discrepancy between mRNA and protein abundance: Insight from information retrieval process in computers. Comput Biol Chem 32:462–468. https://doi.org/10.1016/j.compbiolchem.2008.07.014
74. Becker KL, Nylén ES, White JC et al (2004) Procalcitonin and the calcitonin gene family of peptides in inflammation, infection, and sepsis: a journey from calcitonin back to its precursors. J Clin Endocrinol Metab 89:1512–1525. https://doi.org/10.1210/JC.2002-021444
75. Jones CN, Moore M, Dimisko L et al (2014) Spontaneous neutrophil migration patterns during sepsis after major burns. PLoS ONE 9:e114509. https://doi.org/10.1371/JOURNAL.PONE.0114509
76. Akram AR, Chankeshwara SV, Scholefield E et al (2018) In situ identification of Gram-negative bacteria in human lungs using a topical fluorescent peptide targeting lipid A. Sci Transl Med. https://doi.org/10.1126/scitranslmed.aal0033
77. Vokinger KN, Feuerriegel S, Kesselheim AS (2021) Mitigating bias in machine learning for medicine. Commun Med 11(1):1–3. https://doi.org/10.1038/s43856-021-00028-w
78. Morgan DJ, Malani PN, Diekema DJ (2023) Diagnostic stewardship to prevent diagnostic error. JAMA. https://doi.org/10.1001/JAMA.2023.1678
79. Sweeney TE, Wynn JL, Cernada M et al (2018) Validation of the sepsis metascore for diagnosis of neonatal sepsis. J Pediatric Infect Dis Soc 7:129. https://doi.org/10.1093/JPIDS/PIX021