The host response as a tool for infectious disease diagnosis and management

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REVIEW

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ABSTRACT

Introduction: A century of advances in infectious disease diagnosis and treatment changed the face of medicine. However, challenges continue to develop including multi-drug resistance, globalization that increases pandemic risks, and high mortality from severe infections. These challenges can be mitigated through improved diagnostics, and over the past decade, there has been a particular focus on the host response. Since this article was originally published in 2015, there have been significant developments in the field of host response diagnostics, warranting this updated review.

Areas Covered: This review begins by discussing developments in single biomarkers and pauci-analyte biomarker panels. It then delves into 'omics, an area where there has been truly exciting progress. Specifically, progress has been made in sepsis diagnosis and prognosis; differentiating viral, bacterial, and fungal pathogen classes; pre-symptomatic diagnosis; and understanding disease-specific diagnostic challenges in tuberculosis, Lyme disease, and Ebola.

Expert Commentary: As 'omics have become faster, more precise, and less expensive, the door has been opened for academic, industry, and government efforts to develop host-based infectious disease classifiers. While there are still obstacles to overcome, the chasm separating these scientific advances from the patient's bedside is shrinking.

1 Introduction

Microbial culture has long been the gold standard for the diagnosis of infectious disease. However, the host response has a much longer history in the diagnosis and management of infectious diseases. For many centuries, people have used the most macroscopic observations of host response, syndromic patterns, to diagnose infections. The first recorded description of malaria, the characteristic periodic fevers, comes from a 2700 BC Chinese medical text, and descriptions of other infections, including intestinal worms, dysentery, and abscesses, are found in the Ebers papyri from Egypt *circa* 1550 BC [1,2]. At that time, nothing was known of the microorganism, so the host was the only source of diagnostic information. The seminal experiments of Louis Pasteur, Robert Koch and others, along with the development of improved light microscopy techniques in the late 19th century, led to the germ theory of disease. The paradigm of diagnostics quickly switched to detection and identification of these microbes by culture and microscopy, and later to more advanced techniques such as antigen detection and PCR identification. However, pathogen focused diagnostics often have limited sensitivity and specificity, require a focused clinical suspicion for the pathogen of interest, do not clearly distinguish between infection and colonization, and results may take anywhere from days to weeks depending on the pathogen. Research has returned to the host response to fill these gaps left by pathogen-based diagnostics. This updated review presents a brief summary of current single and pauci-analyte biomarkers followed by a discussion of new developments in 'omics technologies for building host-based infectious disease diagnostics.

2 Single and pauci-analyte biomarkers

2.1 Early biomarkers

Historically, biomarker development has focused on quantifying one or a few easily detectable analytes that serve as surrogates for the host in response to disease (Table 1). This is not a new concept, dating back to the discovery of the erythrocyte sedimentation rate (ESR) in 1917 and C-reactive protein (CRP) in 1930 to diagnose infections such as pneumonia and tuberculosis [3,4]. These are sensitive yet nonspecific measures of inflammation, limiting their use as diagnostic tools. Clinicians commonly use the white blood cell count with differential as a marker of infection. However, white blood cell counts may be low during infection or may be elevated due to physiologic stress or medications. Serology, particularly IgM antibodies, have also been used to diagnose acute illnesses such as viral hepatitis, arbovirus infections, mononucleosis, and HIV for decades. However, the accuracy of serology depends on the timing of presentation and is plagued by false positive results [5].

Table 1. Summary of single-analyte biomarkers for infectious diseases in the clinical and research settings.

Biomarker	Clinical Use Examples	Notable operating characteristics	Clinically available?
Erythrocyte Sedimentation Rate (ESR)	• Rheumatologic conditions • Chronic infections, such as osteomyelitis, prosthetic joint infections, and endocarditis	• Negative predictive value for severe infections • Sensitive for inflammatory diseases • Can also be elevated with increased age, female sex, anemia, and renal disease	Yes
C-reactive protein (CRP)	• Rheumatologic conditions • Sepsis and other severe acute infections	• Specific for sepsis at high levels • More rapid elevation and fall compared to ESR • Measure of response to therapy	Yes
Lactate	• Key component of sepsis management • Indicator of severe disease and tissue damage (infection, heart failure, hypovolemia) • Prognostic marker	• Clearance associated with improved prognosis • Negative predictive value for mortality • Potential for continuous, transdermal monitoring	Yes
Procalcitonin (PCT)	• Empiric antibiotic discontinuation in suspected sepsis • Antibiotic initiation in respiratory tract infection	• Higher elevations for bacterial infections • More specific for infectious processes	Yes
Presepsin sTEM-1 suPAR proADM MMPs	• Candidate protein biomarkers studied in sepsis • Potential use as indicators of sepsis severity and prognosis	• All have been shown to have a lower sensitivity and specificity for sepsis than PCT	No
Cytokines	• Sepsis and other acute infections • States of tuberculosis	• Short serum half-life lowers detection ability	Limited

2.2 Lactate

Elevated serum lactate emerged as a marker of more severe sepsis and is associated with mortality in emergency department (ED) patients [6,7]. As a single measurement, lactate is poorly specific and can be elevated for a variety of reasons [8–11]. It therefore should not be considered a general diagnostic biomarker for infection, but is more useful in identifying severe illness, specifically sepsis. In particular, serial measurements have been shown to have prognostic value [12,13]. As a result of this research, lactate measurement, particularly serial lactate measurement, has become increasingly emphasized in sepsis management. The Surviving Sepsis Campaign updated the International Sepsis Guidelines in 2016 to include lactate measurement as a key component of the three-hour bundle, with repeat measurement included in the six-hour bundle if the initial level was elevated. The guidelines also include using lactate levels to guide fluid resuscitation measures, though this is noted as a weak recommendation with low quality of evidence [14]. Looking forward, there is interest in continuous lactate measurement for critically ill patients. This was shown to be successful utilizing intravascular catheters with a microdialysis function [15,16]. Lactate has also successfully been quantified transdermally [17]. Combining these ideas, transdermal continuous lactate sensors are currently in the prototype stage and may offer a rapid, sensitive way to detect incipient sepsis although specificity for infection may be limited [18].

2.3 Procalcitonin

The discovery that the pro-hormone, procalcitonin (PCT), is induced during inflammation led to great interest in PCT as a biomarker for infection. Interestingly, the rise in PCT is greater for bacterial infection than for viral, fungal, or noninfectious etiologies of the inflammatory response [19–21]. Furthermore, it is technically easy to measure in clinical laboratories, is stable in serum, and has a long half-life making it highly amenable to clinical measurement [22]. PCT was initially FDA-cleared in critically ill patients at the time of admission as a marker for risk of progression to severe sepsis or

shock. Low levels suggest a low likelihood of bacterial infection, spurring many PCT-based antibiotic stewardship algorithms. In a meta-analysis of PCT use in the ICU, PCT-guided antibiotic discontinuation strategies resulted in a significantly shorter antibiotic duration of 1.67 days and lower short-term mortality [23]. Another systematic review looking at PCT in respiratory tract infections showed that PCT guidance reduced antibiotic exposure by 2.4 days, decreased antibiotic-related side effects, and decreased mortality [24]. This evidence prompted the FDA to expand indications for PCT to include antibiotic management for both sepsis and acute respiratory tract infections. It should be noted, however, that high false positive rates for bacterial infection have been observed for patients with major trauma [25], cardiopulmonary bypass surgery [26], liver cirrhosis with ascites [27], chronic kidney disease [28], colonic ischemia [29], acute onset Still's disease [30], heatstroke [31], and burn patients [32], conditions that are frequently encountered in the ICU. Furthermore, while the Surviving Sepsis Guidelines weakly recommended PCT-guided antibiotic discontinuation strategies, they also emphasized that these decisions should never be based solely on the changes of any currently available single biomarker [14].

2.4 Candidate analytes

In addition to PCT, a number of other single analytes have been associated with heterogeneous infectious syndromes like sepsis. Protein markers correlating with sepsis severity, morbidity, and mortality include the neutrophil activation marker, CD64 [33,34]; soluble fraction of glycoprotein TREM1 (sTREM-1) [35,36]; serum urokinase plasminogen activator (suPAR) [37,38]; proadrenomedullin (proADM) [39,40]; metalloproteinases [41], and presepsin [42,43]. However, when studied in head-to-head comparisons with PCT, these potential analytes all have lower sensitivity and specificity for sepsis [44–47].

2.5 Cytokines

There has also been interest in utilizing patterns of host inflammatory mediators such as cytokines and chemokines to aid in the

diagnosis of infection. One study showed that a combination of IL-4, IL-5, IL-6, IL-10, and GM-CSF showed promise at distinguishing viral and bacterial etiologies in acute respiratory infection [48]. IL-27 was shown to be a potentially useful biomarker for estimating the risk of bacterial infections in the critically ill population [49]. A study of patients with invasive Aspergillosis showed significantly increased levels of cytokines IL-6, IL-8, IL-17A, IL-23, and TNF-alpha in bronchoalveolar lavage fluid; IL-8 showed the best individual performance with a sensitivity of 90% and a specificity of 73% [50]. In the pediatric population, CSF IL-6 levels distinguished between bacterial and aseptic meningitis [51]. These examples highlight the breadth of current research into cytokine-based biomarker strategies. However, the serum half-life of these cytokines is very short; for example, IL-6 and TNF-alpha have been shown to have half-lives on the order of one to two hours [52,53]. This significantly limits diagnostic utility, as levels would vary dramatically based on time of sample collection.

2.6 Pauci-analyte panels

Clearly, the perfect diagnostic test that enables early diagnosis of infection, reliably differentiates between bacterial and non-bacterial etiologies, and provides prognostic information may not be possible from a traditional, single analyte biomarker. Instead, panels of complementary single analyte biomarkers have been tested in the hope of greater diagnostic and prognostic utility. A combination of biomarkers from the same biological pathway are unlikely to demonstrate additive diagnostic utility. Instead, combining biomarkers from different biological pathways is more likely to demonstrate improved performance. For example, measurement of lactate and PCT perform better than either alone for sepsis diagnosis and predicting sepsis progression in ED patients [54,55]. Combining five protein biomarkers, Wong et al. developed a decision tree through the PERSERVERE study to reliably stratify pediatric sepsis risk [56,57]. Further down the diagnostic pipeline, FebriDx® is a diagnostic test manufactured by Rapid Pathogen Screening Diagnostics® that combines CRP (upregulated with bacterial infection) and myxovirus resistance protein A (associated with viral infection) to discriminate between viral and bacterial causes of respiratory illness with 80–87% sensitivity and 83–94% specificity [58]. Notably, this point-of-care, fingerstick-based diagnostic test produces results in under 15 min. Currently, FebriDX is available for commercial use in Canada and Europe, but not in the United States.

Despite the immense amount of research that has been performed studying low-complexity biomarkers and biomarker panels for the diagnosis of infectious processes, these approaches are limited by inadequate sensitivity and specificity. Particularly in the area of infectious disease, where withholding antibiotics due to false negative bacterial results can have catastrophic outcomes, it is absolutely critical to maximize performance. Pauci-analyte panels have shown improvements compared to single analytes, thus it is a natural conclusion that adding analytes will improve diagnostic performance. As our ability to characterize the host with increasingly fine detail has evolved, we have begun to search for even more complex multi-analyte platforms. Specifically, new

technologies in 'omics have offered the promise of identifying such biomarker panels in an unbiased way.

3 Overview of 'omics technology

The completion of the Human Genome Project in 2003 heralded a new era of thinking about biology on a systems level – characterizing, quantifying, and analyzing different types of biological molecules. This has led to the birth the various fields in 'omics, which began as genomics, transcriptomics, and proteomics, but over the last several years has rapidly expanded to include diverse fields as metabolomics, lipidomics, phosphoproteomics, and epigenomics, to name a few. Key to the ability to make use of this explosion of information is the collaboration with biostatisticians and computer scientists to develop advanced computational and statistical tools capable of handling this wealth of data. As these laboratory techniques and computational tools progress, 'omics measurements are seeing rapidly increasing throughput and lower costs, allowing for translation to clinical practice.

3.1 Genomics

The first of the 'omics technologies to emerge was the study of DNA, the initial molecule in the central dogma of molecular biology. The costs for whole genome sequencing have plummeted to less than one thousand dollars, and Illumina, a DNA sequencing giant, recently announced a new machine that is expected to decrease that cost by ten-fold [59]. Biotech companies such as 23andMe and AncestryDNA have taken advantage of low costs and public databases on single nucleotide polymorphisms (SNPs) to put genetic information in the hands of the lay public [60]. While DNA is classically thought of as static genetic information fixed since conception, DNA is constantly being modified by methylation, which changes accessibility and activity. Several studies have suggested that methylation patterns, termed the 'methylome' or 'epigenome,' have potential as biomarkers for a diverse set of pathology including cancer, neurodegenerative disease, psychiatric disorders, and infectious disease [61–64]. The human disease methylation database, DiseaseMeth, will hopefully provide a platform for the expansion of this exciting technology [65,66].

3.2 Transcriptomics

Transcriptomics refers to the complete set of RNA transcripts produced by the genome in a cell or population of cells at any one time, including mRNA and non-coding RNAs. Traditionally, this has focused on global changes in gene expression in response to factors such as development, disease, and environment. At first, microarray technology was heavily utilized due to relatively low cost, short time to results, ease of data generation, and good quantitative accuracy, despite being limited to quantifying only pre-determined sequences. However, microarrays are being quickly outpaced by RNA sequencing techniques, which offer a more sensitive, less biased view of the transcriptome by capturing all sequences [67]. The sensitivity of RNAseq is dependent on the number of

reads obtained per sample; as the number of reads increases, the detection of low abundance transcripts improves. A high read-depth, while more expensive, allows for the characterization of rarer transcripts, including splice variants and specific coding SNPs, in addition to other RNA types, such as short non-coding RNA (miRNA, siRNA) and long non-coding RNA (lncRNA) [68]. Non-coding RNA are critical for post-transcriptional regulation and are starting to be used as biomarkers for various human diseases, both as an adjunct to mRNA and on their own [69]. However, a weakness of both microarrays and RNAseq technology is they require RNA molecules to be copied into cDNA and amplified, which introduces bias and increases time-to-result. Nanopore direct RNA-seq is an exciting new technology that allows for real-time sequencing of single molecules of RNA [70–72]. While this 'third generation' sequencing method is still in its infancy, one day it could replace traditional RNAseq as the transcriptome analysis technology of choice.

3.3 Proteomics and metabolomics

Proteomics, or the large-scale study of the proteins in a sample, provides a different level of understanding of the host response compared to genomics or transcriptomics. While standard teaching states that genetic information flows simply from DNA to RNA to protein, several studies have shown that RNA content only weakly correlates with protein content [73,74]. Many factors, including translation efficiency, mRNA and protein half-life, and numerous post-translational modifications could explain this discordance [75]. Early methods of studying proteins, namely antibody-based assays and protein microarrays, required a priori knowledge of the protein(s) of interest. For unbiased quantitation of the proteome, mass spectrometry (MS) is the current standard. Over the past decade, advances in MS techniques and instrumentation combined with the expansion of protein reference libraries have enabled picogram per milliliter detection of proteins [76]. The increased sensitivity also allows for detection of post-translational modifications, leading to fields like glycoproteomics and phosphoproteomics. This diversity of information is crucial in understanding the dynamics of protein networks and cell signaling, and at the same time, it provides more material for the development of protein-based disease signatures [77,78]. These advances have also applied to metabolomics, a rapidly growing field that characterizes proteins and other small molecules related to metabolism, as it similarly utilizes MS technology [79]. Furthermore, recent application of MS techniques to dried blood spot samples could facilitate translation of disease signatures into clinical practice [80].

4 Development of disease classifiers

4.1 Clinical precedent

Disease classifiers are patterns of biomarker changes that classify patients into discrete phenotypic groups. Many transcriptomic-based classifiers are already commercially available for a wide variety of disease processes. In addition to improving and individualizing treatment, these classifiers have reduced overtreatment and led to significant cost reductions for health care. For example, the breast cancer classifiers BluePrint, MammaPrint, and Oncotype Dx Breast Cancer measure the expression of 80, 70, and 21 genes, respectively, to prognosticate and guide therapeutics [81,82]. DecisionDx Melanoma, MyPRS, Prolaris, and Afirma guide clinical decision-making for melanoma, multiple myeloma, prostate cancer, and thyroid cancer, respectively [83–87]. Beyond the field of oncology, Corus CAD CardioDx is a 23 gene assay that detects the presence of obstructive coronary artery disease, and AlloMap is a 20 gene classifier that identifies heart transplant patients with acute rejection [88,89]. Notably, all of these tests require the clinical samples to be sent to an off-site laboratory, and results take days to weeks to become available. Nevertheless, these success stories pave the way for biological classifiers in infectious disease to allow personalized treatment of infections and solve long-standing diagnostic dilemmas.

4.2 General approach

Any 'omic dataset can be used to develop a disease signature (Figure 1). The first critical step is selecting clinically relevant populations on which to develop the classifier. While comparison to healthy patients is invaluable to understanding host biology, these groups are not representative of the population likely to undergo testing in the field of infectious disease; healthy individuals do not typically require a test to differentiate them from ill patients. For example, respiratory classifiers are most clinically helpful if able to differentiate viral, bacterial, and noninfectious causes of respiratory symptoms. As such, they should be derived from a population that includes all such possibilities. Likewise, signatures of sepsis will be most useful if they have the power to differentiate between infectious and noninfectious causes of the systemic inflammatory response syndrome (SIRS). Many classifiers in infectious disease thus far have been derived relative to healthy controls. While scientifically useful, these signatures will need to be refined and tested in broader, more relevant patient populations to be clinically deployable [90].

Next, biological samples from the populations of interest undergo laboratory analysis to generate genomic, transcriptomic, proteomic, or other 'omic data. This complex, high dimensional dataset is then reduced to a list of useful biomarkers using well-studied statistical methods, including but not limited to sparse factor modeling [91], Bayesian constructions of the elastic net [92], sparse principal component analysis [93], and the molecular distance to health [94]. Finally, the classifier is ideally validated in a separate population from which it was derived in order to determine the performance characteristics and diagnostic accuracy.

Multiple, equivalently performing signatures would be expected for any defined clinical question. The reason these multiple signatures often demonstrate little overlap is because the process of defining them is highly susceptible to experimental and analytical variability. This variability can derive from the composition of the discovery cohort, study design, how the analytes were measured (e.g. microarray type for mRNA), or statistical methods. Consequently, one should not expect to
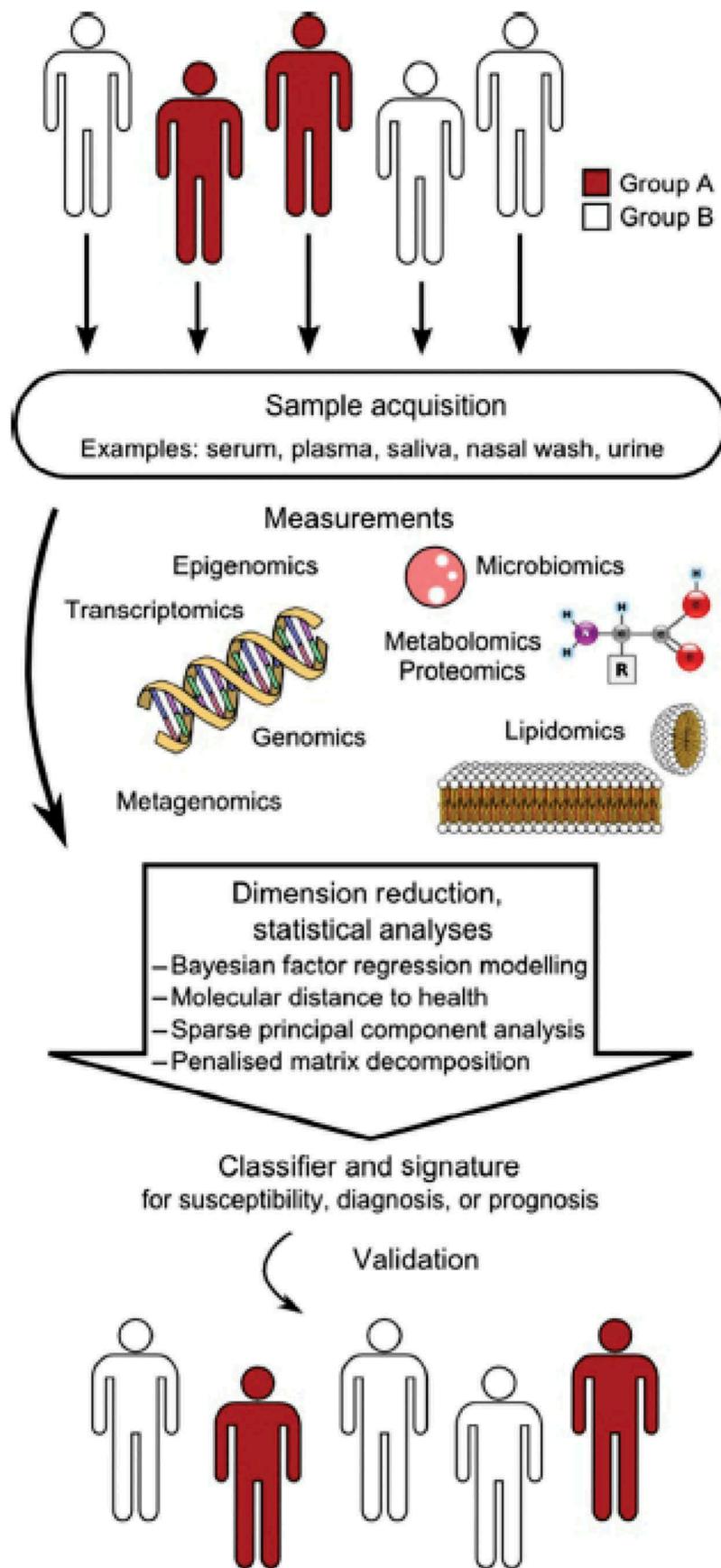
Figure 1. (Reprinted from Methods in Microbiology, Vol 42, William E. Yang, Christopher W. Woods, Ephraim L. Tsalik, Host-Based Diagnostics for Detection and Prognosis of Infectious Diseases, 465–500, Copyright (2015), with permission from Elsevier). Overview of the development process for a host diagnostic biomarker. Beginning with a population that is dichotomised by susceptibility, diagnosis, or prognosis, biological samples are acquired. 'Omic measurements are run on these samples, which generates large quantities of data. Dimension reduction and statistical analyses generate a classifier or signature that distinguishes the desired characteristic from the original population. The classifier is then validated against a different population to test its generalisability.

see the same analytes appearing in independently derived signatures [90]. However, one should expect to see the same biological pathways represented, which is indeed the case.

Another related observation is that analytes from different 'omic approaches do not always correlate. For example, IL-6, CRP, and PCT are upregulated in sepsis. In contrast, the mRNAs encoding these proteins are not prominently featured in sepsis gene expression signatures. However, other studies have shown considerable overlap between different omics measurements that are particularly congruent at the biological pathway level [95]. This again indicates that individual analytes may not necessarily correlate with their upstream (i.e. mRNA) or downstream (i.e. protein or metabolite) target for biological or technical reasons. Instead, one should expect to see consistency in the pathways across multiple 'omic measurements.

4.3 Translation to a clinical diagnostic platform

The final step in the development of a clinically useful disease classifier is translation onto a diagnostic device capable of performing in a clinical environment. In fields like oncology and rheumatology where the allowable time-to-result can be longer, the technical demands on such a platform are less stringent making it possible for specialized referral laboratories to run the tests. However, infectious diseases require fast and accurate data to facilitate rapid decisions about antibiotic use. Therefore, an effective diagnostic must be located on site and return accurate results on the scale of minutes to hours, which is a difficult technical challenge. No 'omic-derived point-of-need diagnostics are currently available in the clinical setting, but there is exciting progress in the area over the past several years. In transcriptomics, for example, BioFire Diagnostics™ has developed a patented FilmArray® technology that incorporates sample preparation, reverse transcription, PCR, and detection within one pouch, with only two minutes of hands-on time and results available in 45 min. Products commercially available on this platform, such as the blood culture identification panel and respiratory panel, are based on pathogen detection. However, BioFire™ and researchers from Duke University have developed a host response-based assay on the FilmArray® platform that identifies patients with viral or bacterial infection [96]. Duke has also collaborated with Qvella™, currently a pre-market company, to apply their patented FAST™ technology to the host response [97]. In the field of proteomics and metabolomics, 908 Devices' ZipChip™ combines microfluidics with mass spectrometry to produce results in under 3 min, with minimal sample preparation [98]. In addition to industry, the federal government also has an interest in this area. In 2016, the U.S. Department of Health and Health Services announced a 20 million-dollar prize for the delivery of point-of-need diagnostics to identify bacterial infections. The Defense Threat Reduction Agency, an organization within the Department of Defense, also recently announced funding opportunities for biomarker research, with point-of-need medical devices noted as specific areas of interest. With growing interest and funding availability, it is only a matter of time until a rapid, robust, on-site testing platform for the host response becomes available.

The use of 'omics-derived classifiers has the ability to revolutionize the recognition and treatment of infection. One can imagine a healthcare system where host-based diagnostics can diagnose infection early, differentiate between infectious states, and help identify pathogens within a few hours of presentation. Not only this, but because of the focus on the host response, one has the unique opportunity to tailor and personalize treatment plans based on an individual's genetic make-up. Next, we will go into several case examples in specific areas of infectious disease where these ideas are taking hold.

5 Defining and diagnosing sepsis: a modern approach

Infections can lead to sepsis, a condition where the body's inflammatory response to the infection causes injury to its own tissues and organs. Sepsis is life-threatening and carries a mortality of 20–35% [99–101]. Despite many advances in critical care, the management of sepsis remains challenging due to its heterogeneous nature. Pathogen-specific factors (e.g. bacterial versus fungal) and patient-specific factors (e.g. co-morbidities, differences in immune response) combine to produce a wide spectrum of disease severity and outcomes. Sepsis is clearly not a single disease, yet sepsis treatment is largely uniform and far from personalized. Fortunately, 'omics has the potential to change the sepsis landscape. As the pathogenesis of sepsis is directly related to an overwhelming immune response, technologies based on the host response are an ideal avenue for sepsis research. Over the past several years, new studies utilizing 'omic technologies have refined how we diagnose, classify, and prognosticate sepsis; these developments are reviewed below.

5.1 Sepsis vs. SIRS

Early diagnosis and initiation of therapy is critical to lowering mortality rates. An important decision point in clinical management of a patient with systemic inflammatory response syndrome (SIRS) is determining whether or not an infection is present. In the past few years, there has been exciting progress in sepsis versus SIRS host-response classifiers. In 2015, Immunexpress described a four-transcript classifier (CEACAM4, LAMP1, PLA2G7, and PLAC8) from 105 patients hospitalized with either sepsis or infection-negative SIRS [102]. After validation in both adult and pediatric cohorts, SeptiCyte Lab™ received 510(k) clearance from the FDA in 2017 [102,103]. To date, SeptiCyte™ is the first and only infectious disease host gene expression diagnostic cleared by the FDA. Unfortunately, the time-to-result of 4–6.5 h and the assay complexity will limit routine clinical use for the time being although efforts are underway to generate a faster test. However, filings with the FDA revealed poor specificity in non-White racial/ethnic groups [104]. For example, healthy White subjects had an elevated SeptiSCORE of 3 or 4 (on a 1–4 scale where higher scores correlate with sepsis) in only 17% of cases (e.g. false positives). However, 28% of healthy Hispanic subjects had elevated scores; 44% of healthy Asian patients had scores of 3 or 4; and 78% of healthy Black subjects had SeptiSCOREs indicative of sepsis. These results highlight the importance of generating and

validating signatures in a broadly heterogeneous population. Furthermore, a recently published study evaluating SeptiCyte in 467 patients with acute respiratory failure showed that it had limited utility, failing to adequately identify patients with sepsis or ruling out patients without sepsis [105]. Performance was equivalent to CRP alone.

Several other transcriptomic-based classifiers have been recently published. Sweeney et al. developed an 11-gene classifier from publically available gene expression data comparing sepsis to SIRS. This 'Sepsis MetaScore' had an AUC of 0.87 and 0.83 in discovery and validation cohorts, respectively. Scicluna et al. compared community acquired pneumonia (CAP) versus non-CAP admissions to the ICU to produce a 78-gene signature for CAP-related sepsis [106]. They narrowed this to a two-gene signature (FAIM3 and PLAC8) whose ratio had an overall accuracy of 82.8% in distinguishing CAP from non-CAP. This outperformed PCT, IL-8, and IL-6 highlighting the opportunity for improvement over existing inflammatory biomarkers.

As gene expression studies are published, that data is typically made publically available in the Gene Expression Omnibus (National Center for Biotechnology Information) or ArrayExpress (European Molecular Biology Laboratory-European Bioinformatics Institute). This ever-growing wealth of data has fostered new techniques that combine disparate datasets in an effort to define disease-related signatures. Sweeney et. al demonstrated this approach by combining multiple publically available databases containing 2,604 patients, more than could reasonably be obtained in any single study. Applying their methods to the question of sepsis vs. SIRS, they developed their own 'Sepsis Metascore,' which they compared to the previously described FAIM3:PLAC8 ratio and SeptiCyte Lab. Performance was similar: 0.82 (range 0.73–0.89), 0.78 (range 0.49–0.96), and 0.73 (0.44–0.90), respectively [107]. This highlights that there are multiple signatures for any given clinical question. Provided the signatures were designed to answer the same clinical question, sepsis vs. SIRS in this case, then many different signatures exist and will perform similarly when evaluated head to head [90].

5.2 Subtyping sepsis

Sepsis can be classified in multiple ways: the first and most obvious is by causative pathogen. While bacterial infections are the most common cause of sepsis, fungal organisms, most commonly Candida species, are another important cause particularly in the immunocompromised. Empiric antibiotics for presumed sepsis typically cover a broad spectrum of bacteria, but anti-fungal agents are usually neglected until cultures detect a fungal etiology or the patient fails to respond to antibacterials. Dix et al. examined patients with sepsis caused by bacterial pathogens (*Staphylococcus aureus* and *Escherichia coli*) and fungal pathogens (*Candida albicans* and *Aspergillus fumigatus*) to develop a transcriptomic classifier. This 38-gene classifier had 96% accuracy in the discovery cohort, and 92% accuracy in a validation cohort of *Crypotoccus neoformans* infections [108].

Sepsis is not a homogeneous disease but rather a syndrome encompassing many heterogeneous pathophysiologies. Patient factors including genetics predispose to poor outcomes, though current clinical characterizations fail to identify those at greatest risk of progression and mortality. This heterogeneity is also the reason why countless sepsis therapeutic trials have all failed: because available treatments are not being targeted to patients most likely to benefit. To address this knowledge gap, discovery oriented computational methods centered on self-organizing maps offer an opportunity to define sepsis subtypes as defined by function or biology. Davenport et al. conducted global gene expression analysis on 265 patients admitted to the ICU with sepsis secondary to CAP and discovered two distinct sepsis response signatures, SRS1 and SRS2. SRS1, which represented 41% of the cohort, was a relatively immunosuppressed phenotype with downregulated HLA class II, T-cell exhaustion, and endotoxin tolerance, when compared to SRS2 [109]. In a similar study, Sweeney et al. employed clustering analysis on publically available sepsis datasets to separate patients into 3 different groups: Inflammopathic, Adaptive, and Coagulopathic. The Inflammopathic cluster was exemplified by innate immunity expression (IL-1 receptor, complement activation), the Adaptive cluster showed adaptive immunity expression and interferon signaling, and the Coagulopathic cluster was notable for increased expression of genes related to clotting (platelet degranulation and coagulation cascade). Furthermore, a 33-gene classifier was generated to classify patients into these three phenotypes, with 83% accuracy [110]. There is also evidence that sepsis subtypes can change as illness progresses. Wong et al. had previously discovered two pediatric sepsis subtypes, called Endotype A and Endotype B, which differed in corticosteroid responses and outcomes [111]. Recently, changes in endotype during the initial three days of illness were studied; 35% of patients transitioned between endotypes, which correlated with outcomes [112]. These studies provide a framework for understanding the biology of sepsis and may open the door for individualized treatment regimens based on the underlying pathophysiology.

5.3 Sepsis prognosis

Sepsis is associated with a high mortality rate. However, the majority (56%) of patients who succumb to sepsis in the hospital do not have severe disease on initial presentation [113]. Early and aggressive therapy is only effective if provided early and aggressively. Unfortunately, the inability to identify subjects who will progress limits the effectiveness of our clinical interventions. In order to better identify subjects at risk of sepsis progression and of poor outcomes, several studies have looked to the host response as a prognostic and risk stratification tool. Tsalik et al. compared 78 sepsis survivors and 28 sepsis non-survivors and found 1,238 differentially expressed genes, many of which were related to immune function [114]. Continuing this initial transcriptomic work, Sage Bionetworks supported a federated analysis of publically available data [115]. In that publication, multiple analytical approaches were leveraged to analyze publically available discovery and validation data. Four models were generated. Despite there being little to no overlap in their gene lists, they all performed similarly. AUCs to predict 30-day mortality based on gene expression obtained at initial sepsis diagnosis was 0.77–0.89. In the field of proteomics, Wong et al. identified five
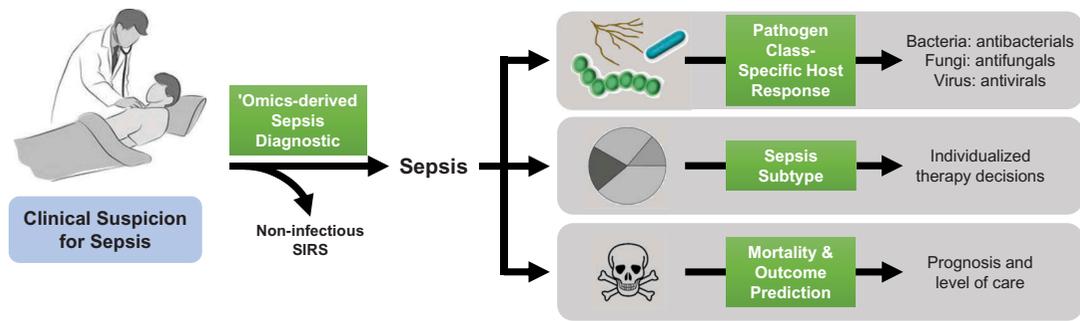
Figure 2. Host-based diagnostics to improve sepsis management. A patient meeting SIRS criteria would undergo an 'omics-based rapid diagnostic test to distinguish between sepsis and sterile SIRS. Once sepsis has been confirmed, further host-based testing could help narrow down the causative pathogen, determine the sepsis subtype, and stratify mortality risk. Information from such tests would allow for proper therapy, individualized care decisions, and earlier escalation of care.

protein biomarkers that when combined with age, admission lactate level, and chronic disease burden had 94% sensitivity and 56% specificity for mortality [116]. Metabolomic data has also been a robust tool to stratify patients with sepsis. Langley et al. presented a comprehensive analysis of patients with sepsis, focusing on survivors and non-survivors [95]. An algorithm derived from clinical features together with five metabolites predicted patient survival more effectively than initial sepsis severity or clinical sepsis scores such as APACHE-II. In sum, these studies show the potential for future diagnostics to more clearly define patients at risk of sepsis progression to severe sepsis and shock, allowing for escalation of care earlier, when it can make the most difference.

The 'omics-based research presented above will hopefully pave the way for a more complete understanding of all aspects of sepsis. In the future, point-of-need, host response-based diagnostic tests administered on admission may have the capability to diagnose sepsis, classify sepsis subtype, guide initial therapies, and recommended escalation or de-escalation of care (Figure 2). This type of precision medicine could truly revolutionize the care of ICU patients, reducing the economic burden of sepsis and, more importantly, decreasing patient morbidity and mortality.

6 Antibiotic stewardship: bacterial vs. viral infection

A second area where host response-based disease classifiers could have a profound impact is in the area of antibiotic stewardship, specifically by differentiating viral from bacterial infection. Numerous studies have shown that antibiotics are prescribed inappropriately at an alarmingly high rate. The annual outpatient antibiotic prescription rate was 506 per 1000 patients, but only 353 (70%) were considered appropriate [117]. Among patients presenting to US EDs with acute respiratory infection, 61% resulted in an antibiotic prescription, despite most of them being viral in etiology [118]. Driven in part by this prescribing practice, multidrug resistant pathogens are rapidly emerging. In 2016, the first case of a totally drug resistant organism, a Klebsiella species, claimed the life of a woman in the United States [119]. Furthermore, antibiotics are not benign medications; complications of antibiotics (e.g. allergic reactions, secondary infections) represented 16.1% of ED visits for adverse drug events, second only to anticoagulants [120]. Given the consequences of current

antibiotic prescribing practices, there is a significant need for a rapid, reliable bacterial-versus-viral diagnostic test to aid clinical decision-making.

6.1 Transcriptomics approaches

Many bacterial-versus-viral transcriptomic signatures have been published in the past decade. To highlight three recent studies: Herberg et. al. discovered a two-transcript signature (ILI44L and FAM89A) with 100% sensitivity and 96.4% specificity for bacterial infections in febrile children; Suarez et al. identified a 10-gene classifier for adults with acute respiratory infection (ARI) with 95% sensitivity and 92% specificity, far superior to PCT; and Bhattacharya et al. described an 11-gene signature with 90% sensitivity and 83% specificity [121–123]. While their performance is impressive, it is important to note that these signatures were developed on patients with only bacterial or viral etiologies. A more relevant testing population would also include patients with non-infectious causes of illness. Mahajan et al. published a bacterial versus non-bacterial transcriptomic signature in children, where the non-bacterial training group contained a heterogeneous mixture of patients with confirmed viral infections and no identified infection [124]. Taking it one step further, Tsalik et al. developed separate classifiers for bacterial ARI (71 genes), viral ARI (33 genes), and non-infectious causes of illness (26 genes), with an overall accuracy of 87%. Notably, this approach has the potential to also diagnose co-infections, given that each patient receives an individual probability for bacterial, viral, or non-infectious etiologies [125]. In contrast, classifiers developed in a purely binary manner (i.e. bacterial vs. viral) are unable to identify co-infection since the model is forced to choose one or the other diagnosis.

6.2 Proteomics approaches

Whereas the majority of research in this area has been in transcriptomics, significant progress has also been made with proteomic approaches. One advantage of proteomics over transcriptomics is that methodologies are already in place that allow for rapid, quantitative protein measurements, so diagnostic development can progress more quickly. For example, Oved et al. identified a three-protein signature that discriminated bacterial and viral causes of respiratory illness [126]. This study

screened 600 proteins in 765 patients presenting to the emergency room with fever. The protein levels of TRAIL1, IP10, and CRP were found to accurately distinguish bacterial from viral (AUC 0.94) and infectious (bacterial and viral) from noninfectious (AUC 0.96) patients in validation sets. However, most of the non-infectious group consisted of healthy subjects, which are not of clinical relevance and make the classification task easier. Moreover, these performance characteristics were calculated based only on results that gave a clear bacterial or viral call. Subjects with indeterminate test results (11% of the cohort) were excluded from the denominator highlighting an excluded data bias. There was also an incorporation bias as CRP was both part of the test and also part of the adjudicated reference standard. Despite these limitations, this promising biomarker signature has been developed into the ImmunoXpert™ test, providing results in approximately 1.5–2 h. Subsequent evaluations, particularly in pediatric cohorts demonstrated a potential for bacterial/viral discrimination (> 90% sensitivity) with notable improvements over PCT [127,128]. If all tested subjects are included such that indeterminate test results are counted as a missed diagnosis, sensitivity decreased to 84.5% and specificity was 87.1%, highlighting the challenges with this classification task. As with PCT, false positive results can also be seen for TRAIL1, IP10, and CRP such as in patients with cancer [129,130] and immunosuppression [131]. It will therefore be important to evaluate this biomarker panel in a more heterogeneous cohort, inclusive of non-infectious illness, and where all evaluable subjects are included.

Substantial progress has been made in signature development to distinguish between bacterial and viral causes of illness. Proteomic tests have already been developed, and transcriptomic tests are in the pipeline. However, several obstacles need to be addressed prior to widespread adoption of bacterial versus viral point of care testing, notably a robust method of diagnosing co-infection, and validation in complex populations of patients with chronic infections, immunosuppression, and inflammatory conditions.

7 Pre-symptomatic diagnosis

An additional area where host-based diagnostics could have a profound impact is in early diagnosis of pre-symptomatic states. This century has witnessed two pandemics with SARS-CoV in 2003 and Swine flu (H1N1/05) in 2009, in addition to regional epidemics such as Avian flu (H5N1) in 2004, MERS-CoV in 2012, and most recently, Ebola hemorrhagic fever in 2013. For all of these infections, there is a variable latency between exposure and clinical infection. This latent period offers an opportunity for the pre-symptomatic host to travel and introduce the disease to new populations. Identifying patients in this pre-symptomatic window would be monumental from a public health perspective, in addition to allowing early diagnosis and early treatment, if available.

Human viral challenge experiments provide a unique opportunity to study the human response to viral pathogens in a controlled and comprehensive manner. In these studies, healthy volunteers are exposed to viruses (influenza, respiratory syncytial virus, rhinovirus, etc) and provide samples at predetermined time points. Several recent transcriptomic challenge studies have attempted to predict which exposed individuals would eventually become symptomatic. In one challenge study using influenza-H3N2, Davenport et al. discovered a six-gene signature that successfully predicted infection 48 h post inoculation [132]. In another, investigators exposed volunteers to H1N1 and H3N2 influenza strains and identified transcriptomic signatures capable of predicting infection as early as 29 h post inoculation [133]. These various challenge studies were aggregated and used to support a DREAM Challenge, sponsored by the Defense Advanced Research Projects Agency (DARPA) and administered by Sage Bionetworks. In this community challenge, participants were tasked with identifying a gene expression signature present at baseline (pre-inoculation) or early (within 24 h) that could discriminate people who were naturally resilient or susceptible to respiratory viral infection. The results of that challenge are not yet published although many teams participated and offered successful solutions.

Knowing whether a patient is going to become sick before symptoms have manifested may be of significant academic interest but does it have real-world relevance? In another human challenge experiment using influenza, McClain et al. showed that administration of antiviral medications at the time of transcriptomic divergence before symptoms successfully mitigated symptoms, length of illness, and expression of inflammatory cytokines [134]. This study provided evidence that transcriptomic signatures can detect pre-symptomatic infection and that interventions at these early time points could significantly improve clinical outcomes. Looking forward, DARPA recently funded a program called Prometheus to answer a slightly different question: who will become contagious after exposure to a pathogen? Some infected individuals exhibit obvious symptoms, while others may just be carriers, yet still capable of spread to others. The goal of Prometheus is to develop a set of host biomarkers that will predict contagion within 24 h of exposure.

Following these discoveries, the ultimate goal is to develop of a rapid, minimally invasive assay that could be deployed in affected settings. Large numbers of individuals would need to be screened for such a public health approach to be effective, and blood sampling may not be feasible. Nasal sampling, for example, could be more acceptable to the public. In a study recently published by Burke et al., unbiased proteomic analysis of nasopharyngeal lavage samples successfully distinguished between viral infected and uninfected individuals, with an AUC of 0.86 in validation cohorts [135]. Another even less invasive option could be breath-based diagnosis [136]. Eventually, one could envision a screening program for passengers flying from epidemic affected locations, or for troops prior to deployment (Figure 3).

8 Pathogen-specific cases

Finally, host-based diagnostics have the potential to revolutionize management of more perplexing diagnostic dilemmas. Here, we highlight three case examples: tuberculosis, Lyme disease, and Ebola, each of which have specific clinical challenges associated with them.
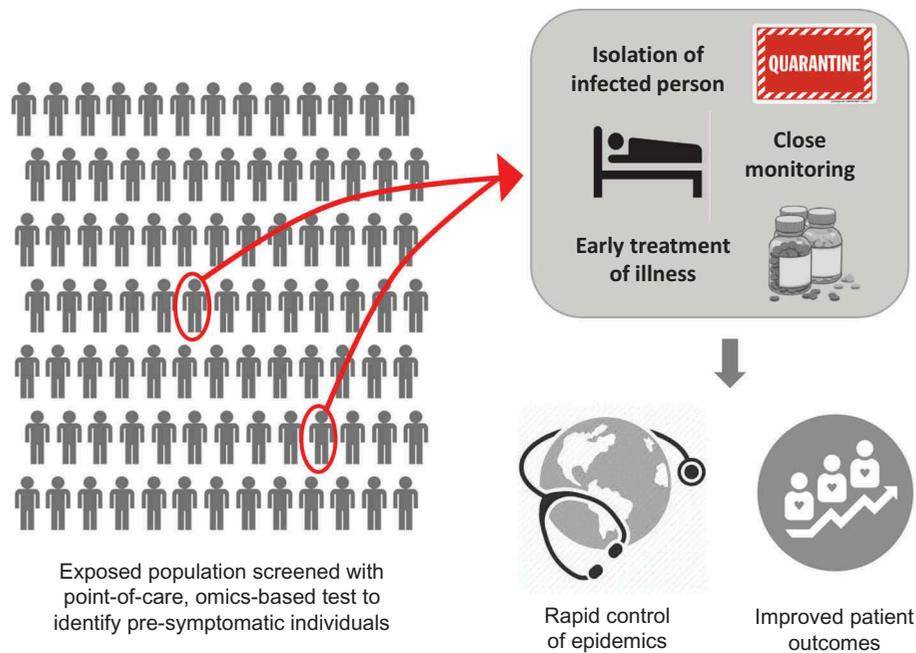
Figure 3. 'Omics-based screening tests for pre-symptomatic patients. An at-risk, exposed population would undergo rapid, minimally invasive, host-based screening for the pathogen of interest. Infected individuals would then be isolated, closely monitored in a hospital setting, and provided early treatment, if available. The goal would be improvement in individual patient outcomes and faster containment of emerging epidemics and pandemics.

8.1 Tuberculosis

Mycobacterium tuberculosis (TB) is a relatively common infection worldwide, with 9 million new cases per year and 1.5 million deaths worldwide [137]. Two disease states are important to differentiate: latent and active disease. Additionally, active disease can exist as a primary pulmonary infection, an extra-pulmonary infection, or as disseminated disease. The bacterium is slow growing and culture results are insensitive, taking weeks to return. Furthermore, screening PPD tests will be positive if the patient has ever been exposed to TB, regardless of infection status, thus prompting unnecessary diagnostic workup and use of potentially harmful anti-tuberculosis drugs. Diagnostics that could readily distinguish between the multiple states of TB or predict progression to active TB would radically improve clinical care. The field of metabolomics has discovered a host of biomarkers in varied sample types, including blood, sputum, urine, and even breath [138]. One promising study elucidated a 6-marker serum signature for active TB with 90% sensitivity and 80% specificity [139]. In transcriptomics, Zak et al. compared two groups with latent TB: those that eventually progressed to active TB, and those that remained healthy. Using RNAseq on whole blood, they developed a 16-gene signature that had 66.1% sensitivity and 80.6% specificity for predicting TB progression [140]. MicroRNAs have also been utilized; Zhou et al. found a signature of 8 microRNAs that showed 95.8% sensitivity and 100% specificity in diagnosing active TB in childhood [141]. These studies are just several examples of different 'omics platforms that have shown promise in areas where traditional pathogen detection techniques have failed.

8.2 Lyme disease

Lyme disease, a tick-borne bacterial illness found most commonly in the Northern Hemisphere, has the nickname 'the great imitator,' and can be difficult to diagnose. Lyme disease progresses from early localized disease (e.g. erythema migrans) to early disseminated disease (e.g. neurological or cardiac manifestations) and eventually to late disease (e.g. arthritis and other neurological manifestations). However, these characteristic symptoms do not present in most individuals, and many do not recall a tick bite. Furthermore, the bacterium is difficult to culture in the laboratory, so diagnosis is primarily based on clinical findings. Serological testing can be used to confirm the clinical diagnosis, but is plagued by false negatives and false positives [142]. More accurate diagnostics in the acute phase of Lyme disease could ensure proper treatment of infected patients, which could limit disease progression. A metabolomic approach found a 44-marker signature that distinguished early Lyme disease from healthy controls, with a sensitivity of 88% and specificity of 95% [143]. Soloski et al. discovered a signature of cytokines and chemokines that were strongly associated with acute Lyme disease compared to healthy controls [144]. These studies compared Lyme disease to healthy controls, which is not clinically useful. However, another study of 29 patients with acute Lyme disease identified a transcriptomic signature that persisted for at least three weeks and was unique compared to other infections, including tularemia, anaplasmosis, and candidemia [145]. This longitudinal study also attempted to distinguish between patients with post-treatment Lyme disease syndrome and those with resolved illness, another important diagnostic challenge in Lyme disease. Unfortunately, they did not observe any differential gene expression.

8.3 Ebola

Ebola, a deadly viral hemorrhagic fever, is now a household name since the recent West African outbreak from 2013–2016 caused

>11,000 deaths [146]. Given that the virus has a long incubation period of up to 21 days and has an alarmingly high fatality rate, two important diagnostic questions are raised: 1) can we rapidly identify those early in infection (even presymptomatic), and 2) can we predict which individuals will have poorer outcomes? Fortunately, pathogen detection-based diagnostics have provided the answer to the first question. The ReEBOV® Antigen Rapid test, developed by Corgenix, is a point-of-care device that produces results in 15 min. After showing 100% sensitivity and 92% specificity in both field testing and laboratory settings, the device was approved for use by the CDC [147]. Such diagnostic tests allow for earlier treatment of patients, in addition to quicker quarantine to prevent further dissemination of the virus. For the second question, 'omics technologies may provide the answer to predicting fatal and non-fatal outcomes of Ebola virus. Liu et al. utilized a transcriptomic approach to identify a signature of 10 genes that was predictive of fatal outcomes [148]. Additionally, a multi-omics approach with metabolomics, proteomics, lipidomics, and transcriptomics successfully identified multiple biomarkers associated with disease outcome [149]. These promising studies could pave the way for early identification of higher risk patients, leading to personalized treatment decisions and earlier escalation or de-escalation of care. Hopefully, this could improve outcomes in such a devastating disease.

9 Conclusion

In summary, investigations of the host response have come to the forefront of infectious disease diagnostics in the past decade, with significant progress made in the past several years. There have been developments in single biomarkers, particularly lactate and PCT, and pauci-analyte biomarker panels. However, the truly exciting progress has been in the field of 'omics. As 'omic technologies have become faster, more precise, and less expensive, the door has been opened for academic, industry, and government efforts to develop infectious disease classifiers and to take steps toward their implementation into patient care. In particular, progress has been made in sepsis diagnosis and prognosis; differentiating viral, bacterial, and fungal pathogen classes; pre-symptomatic diagnosis; and understanding disease-specific diagnostic challenges in tuberculosis, Lyme disease, and Ebola.

10 Expert commentary

While progress in this area has indeed been exciting, there are several crucial steps that need to be overcome for 'omics-based classifiers to transition from bench to bedside. One is expansion of the discovery and validation cohorts. Investigators are starting to select the proper patient populations, for example, with the inclusion of non-infectious populations in several of the recently developed classifiers. However, this needs to be further expanded to encompass all types of patients that may encounter this diagnostic test in the future. This includes complex patients with multiple co-morbidities, chronic inflammatory conditions, immunocompromised individuals, and patients with chronic infections, all of which will likely impact classifier performance

due to their intrinsic involvement of the immune response. Age is another consideration – it is critical to understand how the signatures perform at the extremes of age, especially given that the elderly comprise a large percentage of patients. Another area that needs improvement is co-infection, i.e. patients with concurrent infections from multiple pathogen classes. Few studies have looked at co-infection, and those that have unfortunately did not find a robust method for dealing with this population. Proven success of classifiers in these often-excluded patients would be a step in the right direction, particularly in the eyes of regulatory agencies.

That being said, the chasm separating these scientific advances in 'omics from reaching the patient and provider has grown smaller and smaller over the past several years. Collaborations between academia and biotech companies, in addition to growing interest from government agencies like the Department of Defense and the U.S. Department of Health and Human Services, have quickened the development of point-of-need diagnostic devices and increased available funding for 'omics-based research. 2017 saw the first 'omics-derived infectious disease classifier, SeptiCyte™, receive FDA clearance. Though this test quantifying only four transcripts is not yet commercially available, this regulatory approval still represents an important step. Looking forward, more complex disease signatures that encompass tens to hundreds of targets will require advanced algorithms to interpret results, rather than simple reporting of concentrations. Diagnostic algorithms are somewhat of a novel concept with respect to regulation and will require engagement with relevant agencies to find the best path forward.

11 Five-year view

With the incredible progress in infectious disease classifiers over the past few years, it is only a matter of time before these developments translate to the clinical care setting. Progress is being made toward the development of rapid, point-of-need technologies along with a regulatory approval pathway. If the last five years have been any precedent, these developments will continue to race along in the next five.

What remains to be seen, however, is exactly how these novel diagnostics will impact clinical care. Clinicians face enormously difficult decisions every day, as they balance the potentially dire consequences of withholding treatment with importance antibiotic stewardship. Immediate patient care usually supersedes, and as a result, empiric antibiotics are used for extended periods of time, even when evidence suggests another course of action. Host-based diagnostics will need to be incredibly sensitive and specific to inspire confidence in clinicians and patients alike. Even then, the results from the host response may need to be combined with single-analyte biomarkers and pathogen-based diagnostics, providing a mass of information that could leave even the brightest clinicians at a loss. Steps will need to be made for effectively combining all sources of information into specific clinical recommendations, or better yet, incorporating these recommendations in the electronic medical record. Even then, assay results will at best inform but never replace clinical judgement.

Key issues

- Single and pauci-analyte biomarkers, lactate and PCT in particular, are helpful to determine severity of illness and prognosis.
- Systems biology research using 'omics technologies has developed classifiers that demonstrate improved sensitivity and specificity over traditional single or pauci-analyte biomarkers.
- 'Omics technologies are rapidly becoming higher throughput and lower cost, allowing for translation into clinical practice.
- Host response classifiers have shown promise in personalizing sepsis care, particularly in early discrimination between sepsis and sterile SIRS, classification of disease pathophysiology, and prognostication.
- Transcriptomic and metabolomics classifiers that discriminate between bacterial and viral pathogens have the potential to greatly reduce unnecessary antibiotic prescriptions, especially in acute respiratory illnesses.
- Human viral challenge experiments provide a controlled way to understand the host response to viral infection, leading to accurate classifiers that can identify infected but pre-symptomatic individuals.
- Thanks to collaborations between academia, industry, and regulatory bodies, there has been incredible progress in development of point-of-need, host response-based diagnostics.

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Declaration of Interest

Reviewer Disclosures

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