Review

Host-Based Diagnostics for Acute Respiratory Infections

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ABSTRACT

Purpose: The inappropriate use of antimicrobials, especially in acute respiratory infections (ARIs), is largely driven by difficulty distinguishing bacterial, viral, and noninfectious etiologies of illness. A new frontier in infectious disease diagnostics looks to the host response for disease classification. This article examines how host response–based diagnostics for ARIs are being used in clinical practice, as well as new developments in the research pipeline.

Methods: A limited search was conducted of the relevant literature, with emphasis placed on literature published in the last 5 years (2014–2019).

Findings: Advances are being made in all areas of host response–based diagnostics for ARIs. Specifically, there has been significant progress made in single protein biomarkers, as well as in various "omics" fields (including proteomics, metabolomics, and transcriptomics) and wearable technologies. There are many potential applications of a host response–based approach; a few key examples include the ability to discriminate bacterial and viral disease, presymptomatic diagnosis of infection, and pathogen-specific host response diagnostics, including modeling disease progression.

Implications: As biomarker measurement technologies continue to improve, host response–based diagnostics will increasingly be translated to clinically available platforms that can generate a holistic characterization of an individual's health. This knowledge, in the hands of both patient and provider, can improve care for the individual patient and help fight rising rates of antibiotic resistance. (*Clin Ther.* 2019;41:1923–1938) © 2019 Published by Elsevier Inc.

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INTRODUCTION

Emerging antibiotic resistance is one of the most pressing medical challenges of our time; recent estimates indicate that in 2010, there were >150,000 deaths attributable to antibiotic-resistant infections in the United States.[1] A large contributing factor to this increasing resistance is the alarmingly high rate of inappropriate antimicrobial use, often due to concerns about missing a bacterial infection. For example, in 2010–2011, there were 506 outpatient antibiotic prescriptions per 1000 people, only 70% of which were considered appropriate.[2] The rate was even more staggering among children: 1287 antibiotic prescriptions per 1000 children aged 0 to 2 years per year. Looking at acute respiratory infections (ARIs), the numbers are even bleaker: among patients presenting to US emergency departments (EDs) with acute respiratory symptoms, 61% of patients received an antibiotic prescription, despite most cases being viral in etiology.[3] Clearly, this trend is a public health issue. However, it is also concerning for individual patients. Complications of antibiotics (eg, allergic reactions, secondary

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infections) represent 16.1% of ED visits for adverse drug events, second only to anticoagulant agents.[4]

These dangers of overusing antibiotics highlight a need for new tests to quickly and accurately discern who needs antibiotic therapy at the point of care, which was echoed in the 2014 Executive Order on Combating Antibiotic-Resistant Bacteria.[5] An exciting new frontier in infectious disease (ID) diagnostics is examining the host response to infection. The current review examines some ways that host response–based diagnostics for ARIs are being used in clinical practice, as well as some exciting new possibilities in the research pipeline.

MATERIALS AND METHODS

A limited search was conducted of the relevant literature, with emphasis placed on literature published in the last 5 years (2014–2019).

PRIMER ON INFECTIOUS DISEASE DIAGNOSTICS

Using the host response as a means for identifying infection is not a new concept. For many centuries, people have looked at syndromic patterns to diagnose infections. The first recorded description of malaria and its characteristic periodic fevers comes from a 2700 BC Chinese medical text.[6] In his collection of works, *Corpus Hippocraticum*, Hippocrates (considered the Father of Modern Medicine) used fever patterns and sputum purulence to characterize and diagnose lower respiratory tract infections ranging from pneumonia to tuberculosis.[7]

As technology advanced, we moved into a pathogen detection era of ID diagnostics. The first successful Gram stain and bacterial cultures were performed in the late 1800s,[8] and the more recent use of antigen detection tests and polymerase chain reaction continued to increase our ability to identify more microbes more quickly than culture. However, these once-landmark technologies have many limitations. They are often lacking in sensitivity or specificity, can take days or weeks to produce results, are limited to culturable or known pathogens, and in some cases require an *a priori* suspicion for a specific pathogen. These are some of the limitations that explain why a pathogen was identified in <40% of patients with community-acquired pneumonia (CAP) enrolled in a high-quality study that used an advanced panel of microbiologic tests.[9] Furthermore, identifying a microbe does not distinguish between infection and colonization, with colonization rates that can exceed 50% in some populations.[10,11]

These shortcomings with traditional pathogen detection approaches create a clear need to look elsewhere for a more comprehensive ID diagnostic strategy. The natural next step is to return to the host response, this time from a molecular rather than a syndromic perspective.

INDIVIDUAL BIOMARKERS

Currently in Clinical Use: Erythrocyte Sedimentation Rate, C-Reactive Protein, and Procalcitonin

A number of individual host response biomarkers have been used in the evaluation of respiratory infections for many years. The erythrocyte sedimentation rate and C-reactive protein (CRP), discovered in 1917 and 1930, respectively, are sensitive but nonspecific markers of inflammation that, when elevated, have traditionally been used to support a diagnosis of bacterial infection.[12,13] Since that time, their use has been widely adopted in both inpatient and outpatient settings, despite their limited specificity.

Studies have shown that in underserved primary care settings, the use of CRP to guide antibiotic therapy in patients with ARIs correlated with a significant decrease in 14-day antibiotic use with no increase in adverse events.[14] In addition, although elevated CRP is traditionally associated with bacterial infection, it has been shown that among patients with viral infections, elevated CRP is strongly associated with an influenza diagnosis over other viral ARIs.[15] These markers can aid in guiding diagnosis and management; however, they are not diagnostic on their own and, as such, have limited utility. This feeling is echoed by primary care physicians, who believe that point-of-care CRP testing can be helpful in supporting decision-making when combined with clinical criteria in ARIs but that care must be taken not to rely too heavily on the test.[16]

Procalcitonin (PCT), the pro-hormone of calcitonin, is an acute inflammatory marker that tends to be higher in bacterial infections compared with viral infections, fungal infections, or noninfectious inflammatory responses.[17–19] It is stable in serum with a long half-life, making it a good candidate for measurement in a clinical setting.[20] Previous studies have shown that, despite being a relatively poor

biomarker for ascertaining bacterial versus viral infection,[21,22] the use of PCT to guide initiation and duration of antibiotic treatment results in lower risks of mortality, reduced antibiotic consumption, and decreased antibiotic-related side effects.[23,24] These findings have been observed across different clinical settings and in patients with both severe and more mild to moderate ARIs,[25] and they supported the clearance by the US Food and Drug Administration to use PCT to guide duration of antibiotic therapy in patients with respiratory infections.[26] This dissonance between the ability of PCT to discriminate bacterial and viral infections and its clinical utility can be partly explained by the high prevalence of viral infections in the general ARI population; even a poorly discriminating biomarker could decrease antibacterial use.[21]

However, the recently published ProACT (Procalcitonin Antibiotic Consensus Trial) study suggests that PCT may not have as much clinical utility as was previously reported.[27] In this study, patients with suspected lower respiratory tract infections in 14 US hospital EDs and hospital medicine departments were randomized to a PCT group or a usual care group (where PCT results remained blinded). Providing PCT results in this setting did not lower antibiotic prescribing rates. Reasons for this deviation from previous conclusions are likely multifactorial. They include increased antibiotic stewardship, leading to lower antibiotic rates in the control arm, as well as high rates of clinician overrule. This high rate of overruling may indicate that providers do not have sufficient confidence in the test to allow it to drive their decision-making or that the test is itself imperfect for differentiating bacterial and viral etiologies and is missing bacterial infections that are clinically identified.

High false-positive rates for PCT have been observed for patients with major trauma, cardiopulmonary bypass surgery, liver cirrhosis with ascites, chronic kidney disease, colonic ischemia, acute-onset Still's disease, and heatstroke, as well as burn patients.[28–35] These limitations further restrict the clinical application of PCT for a diagnosis of ARI, as these are common comorbidities, particularly in the ED and hospital environment, where the product label for PCT indicates it should be used.

Candidate Biomarkers: Cytokines, Metabolites, and Proteins

Numerous biomarkers have been studied but not yet translated into routine clinical practice. Cytokines, which mediate the host inflammatory response, are an obvious choice for candidate biomarkers. For example, a combination of interleukin (IL)-4, IL-5, IL-6, IL-10, and granulocyte-macrophage colony-stimulating factor have shown promise for distinguishing viral and bacterial etiologies in ARIs.[36] In addition, serum IL-6 concentration on admission in children aged <5 years with CAP is independently associated with pneumococcal infection as opposed to other common causes of CAP, such as *Haemophilus influenzae*, *Moraxella catarrhalis*, atypical bacteria, and viruses.[37] However, the diagnostic utility of cytokines is dramatically limited by their short half-lives, sometimes on the order of 1 to 2 hours.[38,39] Moreover, the intersubject variability often exceeds variability due to clinical status, making it difficult to set thresholds that clearly discriminate between diagnoses.

A few metabolites have also been identified as having potential diagnostic utility in ARIs. For example, 2 metabolites measured in exhaled breath condensate, lactic acid and pyroglutamic acid, could detect currently stable patients who would have an acute cystic fibrosis exacerbation in the next 1 to 3 months, and differentiate them with 90% accuracy from patients with stable cystic fibrosis.[40] In addition, a panel of 13 lipid metabolites has been identified that discriminated between CAP and hospitalized patients without CAP (including noninfectious patients, patients with extrapulmonary infection, and patients with non-CAP pulmonary infections) with an AUC > 0.8.[41]

Finally, multiple different proteins (other than cytokines) have been identified that have diagnostic potential. One example is CD64, which has been studied in various populations of patients with ARIs. CD64, a receptor expressed on neutrophils, has been used to correctly differentiate patients with acute respiratory failure who have an underlying infectious process from patients with noninfectious acute respiratory failure within 12 hours of admission.[42] Looking at a slightly different population, one study found that CD64 is a relevant marker for bacterial infection in patients with acute exacerbations of

chronic obstructive pulmonary disease, and CD64-guided antibiotic therapy resulted in reduced length of hospital stays, lower cost, and shortened antibiotic treatment duration than a conventional treatment group.[43] This use of CD64 to guide treatment is similar to the current clinical application for PCT.

Other candidate biomarkers include L-lactate, soluble triggering receptor expressed on myeloid cells 1 (sTREM-1), mid-regional pro-adrenomedullin (MR-proADM), and metalloproteinase-9. L-lactate, an isomer of lactate, is a marker for local inflammation. Recent research has found that increased sputum levels of L-lactate are associated with viral lower respiratory tract infections.[44] sTREM-1 is elevated in critically ill patients with infections but does not perform as well as PCT for identifying bacterial infections in ARIs.[45] Similarly, MR-proADM rises in bacterial infections and correlates with increased complications and mortality in patients with CAP.[46,47] However, PCT was again shown to outperform MR-proADM for pneumonia identification among patients presenting to the ED with acute dyspnea.[48] One final candidate is metalloproteinase-9, which is elevated in the acute phase of ventilator-associated pneumonia compared with noninfected ventilated patients.[49] This list of candidate biomarkers is not exhaustive, and many others have and will continue to be evaluated for their ability to diagnose infection and to discriminate between infectious etiologies.

Although all of these individual biomarkers have shown some ability to distinguish between infected and noninfected states, most are inferior to PCT. Although PCT seems to be the best approach available to help manage antibiotic use in ARIs, it has limitations (as reviewed earlier). These limitations may be inherent to the use of a single biomarker approach; perhaps there is simply too little biology captured in a single biomarker. Consequently, integrating multiple complementary biomarkers from different biological pathways into a single classifier offers a new and exciting frontier in ID diagnostics.

OVERVIEW OF "OMICS" TECHNOLOGIES

The ability to integrate multiple biomarkers into a single predictive model is a direct result of technological advances and collaborations between clinical scientists and data scientists, resulting in the introduction of these technologies into clinical practice. Since the completion of the Human Genome Project in 2003, there has been an explosion in the number of "omics," including genomics, transcriptomics, and proteomics, with more recent expansion into metabolomics, lipidomics, phosphoproteomics, and epigenomics (Figure 1).

The first "omics" field to emerge was genomics, which is the study of DNA. As the cost of whole-genome sequencing continues to fall, and public utilization of services such as 23andMe continues to rise, we will have access to ever-expanding databases of genomic information on the population level. In addition, DNA is constantly being modified by methylation, which changes its accessibility and activity. Several studies

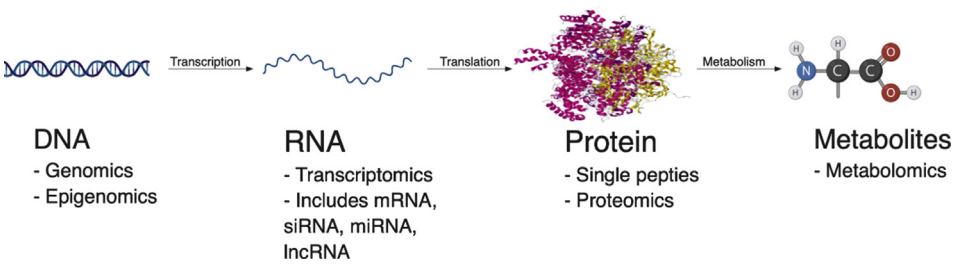

Figure 1. Opportunities to characterize the molecular response to disease. The host's response to disease begins at the level of DNA, helping to determine innate susceptibility or resilience. A more dynamic and acute response to disease can be observed by analyzing the transcriptome, proteome, and metabolome. Each source of information provides another opportunity to understand the host's response and to use that information for diagnostic or prognostic purposes. lncRNA = long noncoding RNA; miRNA = microRNA; siRNA = small interfering RNA.

have suggested that methylation patterns, termed the epigenome, have potential as biomarkers for a diverse set of pathologies, including cancer, neurodegenerative disease, psychiatric disorders, and infectious disease.[50–53]

The transcriptome is the complete set of RNA transcripts in a cell or population of cells at any given time, including mRNA and noncoding RNAs. New RNA sequencing techniques offer a more sensitive, less biased view of the transcriptome than traditional microarray technology. This technology allows for the characterization of rarer transcripts, including splice variants and coding single nucleotide polymorphisms, in addition to other RNA types, such as short noncoding RNA (eg, microRNA, small interfering RNA) and long noncoding RNA, which are critical for posttranscriptional regulation.[54] Limitations of RNA-sequencing include the need for RNA molecules to be copied into complementary DNA and amplified, which introduces bias and increases time to result. Nanopore direct RNA-sequencing is an exciting new technology that allows for real-time sequencing of single molecules of RNA, and it may someday replace RNA-sequencing as the transcriptome analysis technique of choice.[55–57]

Proteomics is the large-scale study of the proteins in a sample. 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 is the current standard. Over the past decade, advances in mass spectrometry techniques and instrumentation combined with the expansion of protein reference libraries have enabled increased sensitivity, allowing for detection of posttranslational protein modifications.[58–60] In addition, platforms already exist that allow for rapid and accurate protein measurements in the clinical setting.

The advances seen in proteomics have also applied to metabolomics, a rapidly growing field that uses mass spectrometry technology to characterize metabolites.[61] Application of mass spectrometry techniques to dried blood spot samples could facilitate translation of disease signatures into clinical practice, whereas other metabolomics models, including breath tests and sweat tests, are potential diagnostic tools on the horizon.[62]

Multi-Biomarker Disease Classifiers

Biomarkers for disease classification work by assigning patients to discrete phenotypic groups based on the biomarker measurements. In recent years, great strides have been made to bring "omics"-based disease classifiers to the point of care for ARIs. Much of this research has focused on differentiating between bacterial, viral, and noninfectious illnesses in immunocompetent patients. The tests with the most clinical utility will be those that can differentiate bacterial infections from all other causes of acute respiratory symptoms. The ideal classifier will be able to identify bacterial infections with excellent sensitivity to avoid missing bacterial infections. To avoid antibacterial overuse, however, this test must also have high specificity. As one can imagine, the generation of such a classifier is a challenging task, but great progress has been made in multiple different "omics" fields in recent years.

One challenge for host response biomarker discovery, whether single analyte biomarkers discussed earlier or multi-biomarker classifiers discussed later, is the absence of a gold standard. Indeed, if a gold standard for the diagnosis of infection existed, there would not be as pressing a need for alternative approaches. In the absence of a gold standard, some other reference must be used. Multiple reference standards can be considered such as conventional microbiology, a composite of other biomarkers, clinical adjudication, or some combination thereof. Whichever is chosen, there should be a broad recognition that the reference standard is imperfect, and in some cases, the new biomarker may be better. Another consideration is that a biomarker with 100% accuracy has managed to match an imperfect reference standard perfectly and should be viewed with caution.

PROTEOMIC APPROACHES

One important benefit of a proteomic approach is that the technology needed for rapid, quantitative protein measurements already exists, allowing for more straightforward translation of a protein signature into a diagnostic platform. For example, FebriDx is a fingerstick-based diagnostic test manufactured by RPS Diagnostics (Sarasota, Florida) that is currently approved for commercial use in Canada and Europe.[63] It does this by combining CRP as a marker

of bacterial infection with myxovirus resistance protein A, a marker of viral infection, to discriminate viral and bacterial causes of respiratory illness with 80% to 87% sensitivity and 83% to 94% specificity. Importantly, this test provides results within 15 minutes. Given the high prevalence of viral ARIs in the outpatient setting, these performance characteristics, which are better than PCT, can offer a very useful tool for antibiotic stewardship. The test, however, is limited by its inability to distinguish bacterial/viral co-infection from severe viral infection (as often seen with influenza) because CRP and myxovirus resistance protein A levels would be elevated in both these scenarios.[15] It has also not been extensively evaluated in patients with ARIs caused by noninfectious etiologies.

Another example of a proteomic-derived ARI diagnostic is the 3-protein signature of tumor necrosis factor–related apoptosis-inducing ligand (TRAIL), interferon gamma–induced protein 10 (IP-10), and CRP, discovered in a study by Oved et al[64] of 765 patients presenting to the ED with fever. In this population, those 3 proteins were found to accurately distinguish bacterial ARIs from viral ARIs with an AUC of 0.94, and infected from noninfected patients with an AUC of 0.96. These results have been supported by multiple external validation studies.[65,66] However, in all of these studies, the noninfected group consisted primarily of healthy subjects. This is not a clinically relevant population, as a diagnostic test like this would not be performed on a healthy patient. Moreover, CRP was used as one of the criteria to decide whether patients had a bacterial infection resulting in an incorporation bias, although the magnitude of that bias is unknown. Finally, subjects with indeterminate test results (11%–15% of the published studies) were excluded from the analysis when calculating the test's operating characteristics. If all tested subjects are considered, as is expected when evaluating performance characteristics, sensitivity decreased to 84.5% and specificity decreased to 87.1%. Nevertheless, these results are still better than what has been historically observed with PCT, a finding reported by the test's manufacturer, and which highlights the potential improvements with a multi-marker strategy.[67]

van der Does et al[68] added to these findings regarding TRAIL/IP-10/CRP in a recently published study. In a cohort of 315 febrile patients in the ED, the 3-protein signature identified bacterial infections with an AUC of 0.73. The authors found that adding PCT as a fourth biomarker produced a modest increase in AUC to 0.76. The significant difference in observed AUCs between the 2 studies highlights the importance of including all tested subjects in calculating performance characteristics, which van der Does et al did. Although a test may report an indeterminate result, these results should not be discarded from the denominator when calculating sensitivity and specificity. Despite these limitations, this promising biomarker signature has been developed into the MeMed BV test (MeMed Diagnostics, Haifa, Israel), which produces results in ~1.5 to 2 hours. This long time to results may limit clinical utility, especially in the outpatient setting. The company has therefore been developing a rapid (~15-minute) sample-to-answer platform, although it is not yet commercially available.[69]

TRANSCRIPTOMIC APPROACHES

Transcriptomic approaches present an exciting opportunity for discovery of disease-classifying biomarkers. Although the technology to bring transcriptomic-based tests to the point of care is still being developed, interest and advances by multiple biotechnology companies are paving the way for gene expression–based tests to be available for clinical practice. One simultaneous strength and challenge of transcriptomic approaches is that gene transcription is a dynamic process that evolves over time, in both composition and amplitude, as the corresponding stimulus changes. For example, Woods et al[70] described how the overall intensity of the transcriptomic response to influenza tracks closely with symptom scores over time, with the observed genomic response significantly preceding changes in clinical scores in symptomatic individuals. Similarly, Dunning et al[71] described how the content of the transcriptomic response is dependent on disease severity and time course. In hospitalized patients with influenza, interferon-inducible genes and type 1 interferons were prominently upregulated early in the disease course while later disease reflected inflammation and neutrophil activation. The best way to overcome the challenge posed by the dynamic nature of the transcriptome is to develop signatures

in populations with variable durations of illness so as to capture the breadth of that biology.

Despite the challenges posed by these temporal changes, the immune response is largely conserved for a given pathogen class (eg, bacterial, viral, fungal). Therefore, pathogen class–specific host response signatures can be generated and provide broadly applicable diagnostic information that is largely independent of the specific pathogen. Perhaps the most relevant distinction is that of bacterial versus viral infection. Here, we discuss only some of the many such classifiers previously published (Table). One example is an 11-gene signature described by Bhattacharya et al[72] that discriminated bacterial and nonbacterial lower respiratory tract infections with 90% sensitivity and 83% specificity. Similarly, Suarez et al[73] described a 10-gene signature that discriminated bacterial and viral causes of lower respiratory tract infection with 95% sensitivity and 92% specificity.

One limitation of these classifiers, and others like them, is that they were discovered in populations consisting only of patients with confirmed infection, and their control groups consisted of healthy patients. A more relevant discovery population would be one that more closely mimics the complete population a test of this type would be used in. This population would contain not only patients with infection but also patients with noninfectious causes of respiratory symptoms. For example, allergic rhinitis, asthma exacerbation, chronic obstructive pulmonary disease exacerbation, and postinfectious cough, as well as other cardiopulmonary diseases can mimic ARIs. Therefore, a more clinically useful test would account for these conditions when developing the signature. Recognizing this consideration, Tsalik et al[74] developed their signature in a cohort of patients presenting to the ED with bacterial ARI, viral ARI, and noninfectious illness. From this cohort, 3 separate classifiers were developed for bacterial (71 genes), viral (33 genes), and noninfectious (26 genes) etiologies that classified subjects with 87% overall accuracy. The 3-classifier system has many advantages, including the ability to diagnose coinfection because there are independent probabilities assigned to the likelihood of bacterial and viral infection. However, it is a larger signature and therefore more challenging to translate. Nevertheless, the group has recently found that a much smaller set of transcripts remains highly accurate in discriminating bacterial, viral, and noninfectious illness.[75]

Liu et al[76] found that including gene expression data from the baseline healthy state for each subject further improved the accuracy of these tests despite using fewer genes. This study looked at 151 subjects in various phases of viral ARI and found that including a reference sample improved predictive accuracy by 6% to 14%, while using 31% to 39% fewer genes per signature. This finding is of great interest scientifically but currently is not feasible because a patient's baseline gene expression data are not available. However, high-risk populations such as those with structural lung disease may benefit from having an established baseline so that deviations from baseline are more easily identified.

IMMUNOCOMPROMISED HOSTS

The immunocompromised population represents a unique diagnostic challenge. Their immunocompromised state predisposes them to developing serious infections. For example, infections are the leading cause of mortality in lung transplant patients between 30 days' and 1 year posttransplant, acute respiratory failure due to pneumonia is the leading cause for intensive care unit admission in patients with HIV/AIDS, and febrile neutropenia remains the main dose-limiting toxicity of chemotherapy.[77–79] Furthermore, the blunted immune response means that clinicians cannot rely as strongly on traditional clinical signs of the host response to aid in their diagnostic decisions.

Due to this population's higher risk of developing serious infections, the threshold to treat empirically with antibiotics is lower, which further drives the development or acquisition of antibiotic-resistant infections, creating a vicious circle.[80,81] Clearly, this population group could benefit significantly from improved "omics"-based diagnostic platforms. However, the altered immune function in these patients may in principle make host response–based diagnostics inherently inaccurate.

Unfortunately, very little research has been done on this topic. To address this oversight, an examination of the host response in immunocompromised patients is essential. Relevant clinical phenotypes to study include those with solid organ transplant, bone marrow transplant, HIV with or without AIDS, and

Table. Overview of various host response–based approaches for disease classifier creation. Although representative examples for each approach are included, this is not an exhaustive list.

Diagnostic Category	Example	Advantages	Disadvantages	Clinically Available in the United States
Single biomarker	CRP	Established biomarker with long history of use	Nonspecific	Yes
	PCT	FDA cleared to guide antibiotic usage	High false-positive and false-negative rates	Yes
Protein panel	TRAIL + IP-10 + CRP[64]	Outperforms PCT Platform under development may offer point-of-care capability	Utility limited by indeterminate result in up to 15% of subjects Unable to identify co-infection Not validated in patients with noninfectious illness Current platform requires ~2 h to produce results	No
	CRP + MxA[63]	Fingerstick test Results in 15 min Outperforms PCT	Inability to distinguish bacterial/viral co-infection from severe viral infection Not validated in ill patients with noninfectious illness	No
Gene expression	10-Gene signature[72]	High sensitivity and specificity	Not available as a clinical test Not validated in ill patients with noninfectious illness Unable to identify co-infection	No
	130-Gene signature[74]	High sensitivity and specificity Detects co-infection Discriminates patients with noninfectious illness	Not available as a clinical test Large signature more challenging to translate	No
Wearable technology	ADAMM-RSM	Noninvasive Continuous monitoring	Data collected but not yet developed into a diagnostic strategy	Yes
	Fitbit	Noninvasive Easily accessible to patients Continuous monitoring	Diagnostic applications not developed	Yes

ADAMM-RSM = Automated Device for Asthma Monitoring and Management; CRP = C-reactive protein; FDA = US Food and Drug Administration; IP-10 = interferon gamma–induced protein 10; MxA = myxovirus resistance protein A; PCT = procalcitonin; POC = point-of-care; TRAIL = tumor necrosis factor–related apoptosis-inducing ligand.

patients on high doses of prednisone, disease-modifying antirheumatic drugs, biologics, or chemotherapy.

PRESYMPTOMATIC DIAGNOSIS

Symptoms in ARIs are largely mediated by the immune response to the pathogen. It is therefore understandable that host response measures, particularly gene expression, can identify patients exposed to a pathogen but not yet symptomatic (Figure 2). Much of the discoveries made in diagnostic presymptomatic disease derive from human challenge studies, in which healthy volunteers are inoculated with a pathogen and serial samples are drawn from preinoculation through resolution of illness. In influenza, a 6-gene signature predicted H3N2 infection within 48 hours of inoculation, whereas another signature predicted both H1N1 and H3N2 infection within 29 hours of inoculation.[70,82] In both cases, this was 24 to 48 hours before symptom onset. The identification of exposed but asymptomatic subjects also enabled a characterization of the biological response in symptomatic and asymptomatic individuals despite the same exposure.[83] Indeed, some biological pathways and genes were identified that may confer innate susceptibility or resilience to infection.[84]

Although these signatures are also relevant to the patient presenting with acute symptoms, the ability to identify an exposed but presymptomatic patient offers unique opportunities. For example, the motivation behind these human challenge studies was a Defense Advanced Research Projects Agency program called Predicting Health and Disease. In that program, the goal was to develop a test that could clear soldiers or key personnel before deployment. Someone exposed but presymptomatic who deploys into a far-forward environment or a submarine, for example, risks spreading that infection to others in an environment with limited access to medical care. A similar approach could be taken for any individual in a high-risk work environment. As another example, nursing homes

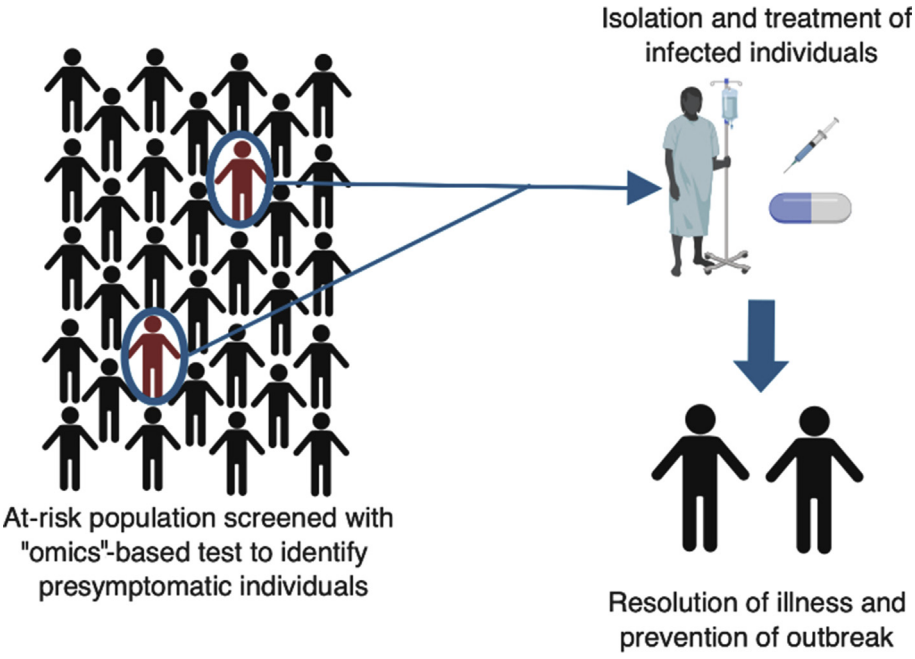

Figure 2. "Omics"-based tests can identify presymptomatic individuals, improving patient care and preventing outbreaks. A screening test that diagnoses presymptomatic individuals, when deployed uniformly across an at-risk population, can identify infected individuals before they can spread the disease to others, thus helping slow or prevent outbreaks.

could screen presymptomatic residents in the setting of an influenza outbreak to direct who should receive oseltamivir prophylaxis. This very scenario was modeled by McClain et al,[85] who showed that presymptomatic antiviral treatment initiated at the time of transcriptomic divergence resulted in reduced length of illness, milder symptoms, and lower expression of inflammatory cytokines.

In addition to predicting who will fall ill after exposure, the ability to predict which individuals will become contagious after exposure is equally valuable. In 2017, the Defense Advanced Research Projects Agency sponsored the Prometheus program, which aims to answer that question. The goal of Prometheus is to discover biomarkers that predict future contagiousness when measured within 24 hours of pathogen exposure.[86]

PATHOGEN-SPECIFIC EXAMPLES

Although much of the progress using "omics" technologies in ARIs has been to classify pathogen class, there are also important strides being made in pathogen-specific diagnostics. Two clinically important pathogens being explored include tuberculosis (TB) and respiratory syncytial virus (RSV).

TUBERCULOSIS

The diagnosis of TB remains a challenge. Cultures are insensitive and slow. Tests such as a purified protein derivative (PPD) or interferon-ɣ release assays cannot distinguish between active, latent, or treated TB. They also provide false-positive results in those with bacille Calmette-Guérin (BCG) vaccination. A diagnostic platform that could accurately differentiate between these various states, and predict active infection, would be of significant clinical utility.

One important aspect of TB diagnostics is the ability to accurately identify which individuals are infected and if that infection is active or latent. With respect to host response, Singhania et al[87] built on previous transcriptomic work to develop a 20-gene signature that differentiated active TB and latent TB, as well as between active TB and other bacterial and viral illnesses. This signature does not contain any mediators from the interferon-γ pathway, which is the primary moderator of the immune response in viral infections and was highly represented in previous published signatures. Excluding transcripts from this pathway helped mitigate the high false-positive rate previously observed in patients with viral infection. In addition, a recently described 7-gene signature differentiated active TB from latent TB in subjects with concurrent HIV infection, regardless of HIV treatment status.[88]

Importantly, these signatures are generalizable across diverse populations; one classifier that distinguished active TB from pneumonia had an AUC of 0.96 in an American cohort and 0.91 in a Sub-Saharan African cohort.[89] Given the widespread global disease burden of TB, the generalizability of these signatures is vital.

A similar but slightly different question is how to predict which exposed individuals will go on to develop infection and which infected individuals are likely to progress from latent to active infection. Roe et al[90] recently described a 3-gene signature that predicted infection within 90 days of TB exposure with a 50% positive predictive value and 99% negative predictive value. In patients already known to have latent disease, Zak et al[91] published a 16-gene signature that predicted progression to active disease with 66.1% sensitivity and 80.6% specificity.

RESPIRATORY SYNCYTIAL VIRUS

RSV causes severe disease in infants and toddlers, occasionally leading to prolonged hospitalizations for respiratory distress. Fortunately, tests that detect RSV with high sensitivity and specificity are abundant. However, detecting RSV is only part of the picture. Identifying and predicting which children will become severely ill is not currently possible but is amenable to a host response approach. To that end, a 5-gene signature from nasopharyngeal aspirates described by van der Kieboom et al[92] differentiated mild and severe RSV infection in a cohort of children aged <5 years. Similarly, Mejias et al[93] created a genomic "molecular distance to health" score that correlated with disease severity in infants with RSV. Together, these show that transcriptomic changes are correlated with clinical severity, and they open the door for prognostic signatures to be used clinically in the coming years. The ability to predict an individual patient's clinical courses could fundamentally alter how these infections are treated, enabling a preventive as opposed to reactionary approach.

Although most signatures have been discovered based on gene expression in peripheral blood, transcriptomic signatures in nasal epithelial samples

can also help identify the presence of viral infection. For example, recent work by Yu et al[94] found that transcriptomic analysis of nasal epithelial cells was comparable to whole-blood transcript signatures to distinguish viral infection from various control groups with either no detectable virus or with asymptomatic rhinovirus shedding. A potential benefit of using nasal epithelial cell–based tests is that this approach provides an opportunity to combine host response and pathogen detection tests using the same clinical sample. To date, studies of nasal epithelial host response have only been described for viral infections.[94–96] Unless the host response test can also identify the presence or absence of bacterial infection, the clinical utility of such tests will be limited. Although a nasal host response to bacterial infection has not yet been described, the simultaneous measurement of host and viral pathogen would be an improvement over current methods.

WEARABLE TECHNOLOGIES

One additional space in which progress is being made in ARI diagnostics is the field of wearable technologies. Here, the biomarkers are not necessarily molecular in nature, such as proteins, metabolites, or RNA transcripts; instead, the host response is measured noninvasively by analyzing high-density data and detecting an individual's deviations from baseline (Figure 3). Abundant access to smart, wearable, continuous vital sign–monitoring devices enables the collection of massive amounts of data that could be used for ARI monitoring.

This massive amount of data produced by continuous monitoring simultaneously presents opportunity and challenge; for a wearable technology to be diagnostic, it must sift through all of the data to identify signals amidst the noise. This will require machine-learning algorithms to establish what is

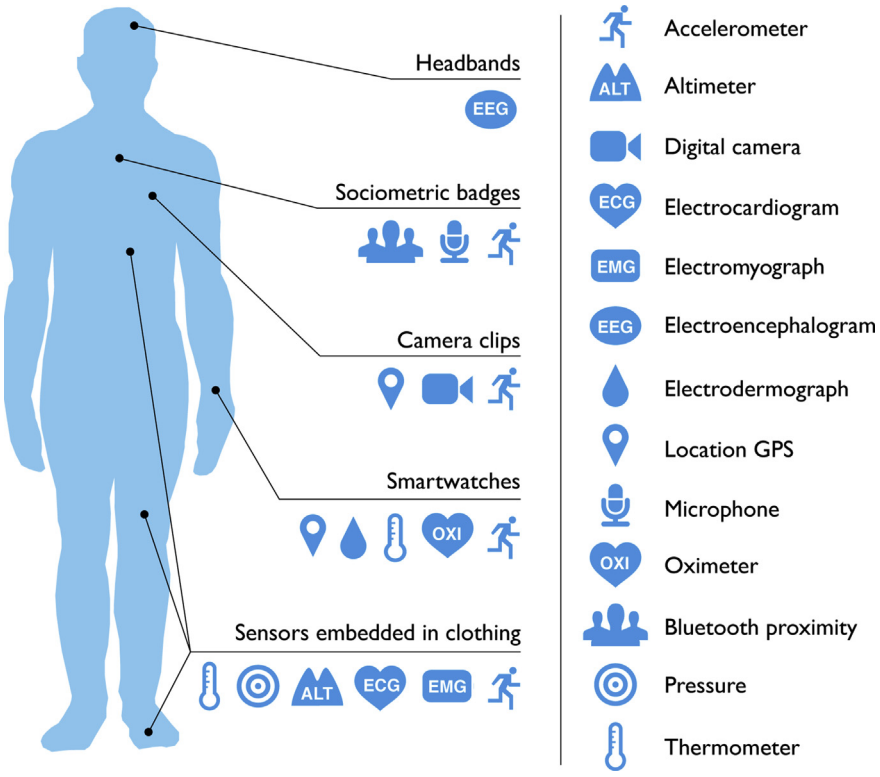

Figure 3. Overview of potential applications of wearable technologies. Continuous noninvasive measurement of various nonmolecular biomarkers via the methods illustrated here provides an opportunity for individuals to detect variations from their personal baselines, opening the door for improved early detection of illness. GPS = Global Positioning System. Reprinted from Piwek et al.[102]

normal for each individual, facilitating the detection of deviations from one's baseline. Rather than defining "normal" for a population, machine learning on continuously collected data enables us to define "normal" for that user.

For example, changes in heart rate variability, easily assessed by current commercial technologies, predict infection 3 to 4 days before symptom onset, and the degree of these changes predicts infection severity.[97–99] Furthermore, the next generation of wearable technology is not limited to vital sign monitoring: mechanical, physiological, and biochemical monitoring options are increasingly available. These functions include glucose, alcohol, electrolyte, pH, oxygenation/gas, and humidity sensing, all of which dramatically increases their potential applications.[100] Individualizing the interpretation of these various monitoring methods will provide additional diagnostic utility.

Much of the wearable technology that currently exists is not yet diagnostic in and of itself but has the ability to aid in diagnosis. For example, Health Care Originals, Inc (Rochester, New York) developed a wearable respiratory monitoring device, the Automated Device for Asthma Monitoring and Management (ADAMM-RSM), that is being marketed for asthma surveillance. It has the ability to detect abnormal breath sounds in real time, from the wheezes typically associated with asthma to the focal crackles heard in pneumonia.[101] Although this technology does not distinguish bacterial from viral etiologies, it can forewarn an impending illness, allowing for early detection and treatment that could subsequently avert severe disease requiring hospitalization.

In addition, several research groups have developed mechanically flexible and fully integrated wearable sweat sensors that can be worn in various locations such as wristbands, temporary tattoos, eyeglasses, and mouth guards. These sensors allow for analysis of metabolites and electrolytes that could bring continuous molecular measurements into routine clinical practice.[100]

CONCLUSIONS

A renewed focus on the host response has become possible in recent years due to advances in biomarker discovery tools and the technologies that would measure them in clinical practice. From single biomarkers to "omics"-based classifiers to wearable technologies, progress is being made to directly affect patient care. As technology continues to advance, it will be possible to integrate these tools so they may generate a holistic characterization of an individual's health. This knowledge, in the hands of both patient and provider, can help fight global antibiotic resistance and improve care for the individual patient.

DISCLOSURES

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