

Microbial Biomarkers and Personalized Medicine in Respiratory Disorders: Bench to Bedside

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Abstract

Respiratory diseases such as asthma, chronic obstructive pulmonary disease (COPD), bronchiectasis, pneumonia, and tuberculosis are a significant health burden to the world because of their heterogeneity, inconsistent progression, and unpredictability to treatment. The development of microbial and host-derived biomarkers promises to aid in improving diagnosis, prognosis, treatment stratification and disease monitoring as a part of personalized medicine. Recent advances in specimen-based techniques including sputum, bronchoalveolar lavage, blood, stool, and exhaled breath, and multi-omics methods, like metagenomics, transcriptomics, proteomics, metabolomics, and breathomics are summarized in this review. Evidence on disease-specific is addressed and microbiome signatures in asthma and COPD, transcriptomic and microbial biomarkers in tuberculosis and the emerging clinical utility of volatile and non-volatile exhaled compounds as non-invasive diagnostics are discussed. The combination of machine learning and artificial intelligence to combine multi-omics data into a clinically actionable model is critically assessed, and several of the major obstacles, including analytical validation, multicenter reproducibility, cost-efficacy, regulatory approval, and equity of access, are considered. This review has emphasized the frameworks such as the FDA-NIH BEST taxonomy, STORMS, STARD-AI, and TRIPOD+AI and has highlighted the need to have rigor in methodology in order to translate clinical findings. When combined, microbial biomarkers, along with host-response and multi-omics approaches, possess high value to change the face of respiratory medicine by focusing on symptoms to precision diagnostics and personalized treatment.

Keywords: Microbial Biomarkers, Personalized Medicine, Respiratory Disorders, Respiratory diseases, Implementation.

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Introduction

Respiratory diseases, including asthma, chronic obstructive pulmonary disease (COPD), bronchiectasis, pneumonia and tuberculosis have a considerable global burden in morbidity and mortality. Activities related to respiratory diseases have been estimated to cause more than 10% of world deaths every year and this testimony to their heterogeneous nature and difficult management (Soriano *et al.*, 2023). Traditional diagnostic and prognostic methods that are based on symptoms, spirometry, and imaging are usually insensitive to underlying biological variability. All of this limits the diagnosis, helps to cause the inappropriate use of therapies, and ultimately results in worse clinical outcomes (Singh *et al.*, 2022). A practice long established within oncology, the development and implementation of personalized medicine in respiratory medicine is becoming more commonplace. Its fundamental philosophy is targeted interventions on patients based on validated biological signature (Hamburg and Collins, 2019). Hence, the emergence of microbial biomarkers as potential tools in this regard. The respiratory tract microbiota - influenced by environmental exposure, immune system engagement and comorbidities - is increasingly recognized as an important determinant of disease pathophysiology and clinical outcome (de Cordon-Martinez *et al.*, 2023). Dysbiosis has been linked with asthma exacerbations (Alexopoulos *et al.*, 2019; Cobo *et al.*, 2020), COPD (Alexopoulos *et al.*, 2017), bronchiectasis (Longcope *et al.*, 2015), pneumonia (Wacker *et al.*, 2016), and tuberculosis (Gallup *et al.*, 2017). Biomarker, as defined in the FDA-NIH best resources document, include diagnostic, prognostic, predictive, monitoring, pharmacodynamic and safety biomarkers (FDA-NIH Biomarker Working Group, 2021). Microbial biomarkers (which could be by direct pathogen detection or host response biomarkers) could be used to fine-tune the clinical practice in each of these groups. Advances in next-generation sequencing (NGS), metagenomic pathogen detection (mNGS), host transcriptomics, proteomics, metabolomics and breathomics have led to an acceleration of biomarker discovery and application. Despite this, there needs to be a logical pathway to translation from bench to bedside, including analytical validation of assays, clinical validation for a variety of people and demonstration of clinical utility (measurement of clinical activity) via improved outcomes and cost-effectiveness (Collins *et al.*, 2024). Reporting standards such as STORMS for microbiome studies (Mirzayi *et al.*, 2021; Bossuyt *et al.*, 2015).

This review summarizes the most recent evidence on microbial biomarkers in respiratory diseases, underlining their diagnostic, prognostic and therapeutic implications. How biological mechanisms are integrated with therapeutic abuse issues helps it offer a translational blueprint to include microbe signatures in personal respiratory medication.

Asthma: Biomarkers for Precision Medicine using Microbes and Hosts

Unmet Needs (Pathobiology)

Asthma is a highly diverse chronic disease of the airways that is marked by fluctuating airflow limitation, inflammation and remodeling. Traditional phenotyping, based on clinical symptoms and spirometry, is often an inadequate measure of the complexity of underlying endotypes. This causes variation in the response to the treatment, especially for inhaled corticosteroids and biologics Jackson *et al.*, (2023). Thus, molecular and microbial biomarkers are important to be able to enable early diagnosis, guide biologic therapy, and to follow the course of the disease.

Microbial Biomarkers

The airway microbiome of the asthmatic individual is quite different or distinct from that of a healthy individual. Enrichment of *Moraxella*, *Haemophilus*, and *Streptococcus* species has been linked to severe and corticosteroid-resistant asthma, and decreases in microbial diversity have been linked to increase in risk of exacerbation (Durack *et al.*, 2020). gut microbiome signatures are also important, with depletion of short-chain fatty acid-producing bacteria associated with early-life asthma susceptibility He *et al.*, (2022). These results point to the existence of a gut-lung axis in asthma pathogenesis.

Host Biomarkers

Host derived biomarkers are a part of asthma management. Fractional exhaled nitric oxide (FeNO) is a commonly performed non-invasive test as a biomarker of type-2 (T2) inflammation to direct treatment with corticosteroids (Agache *et al.*, 2022). Blood eosinophil counts and serum periostin concentrations have also been confirmed as predictive biomarkers of responses to anti-IL-5, anti-IL-13 biologics (Hanania *et al.*, 2022). More recently, gene expression signatures called transcriptomic profiling have also been found in airway epithelial cells, differentiating between T2-high vs. T2-low asthma endotypes (Thorsen *et al.*, 2023).

Multi Omics and New Technology

Multi-omics integration is going beyond single biomarkers. Some metabolomic studies on exhaled breath condensate and serum have reported the potential of volatile organic compounds (VOCs) and lipid mediators for predicting the risk of exacerbation (de Vries *et al.*, 2022). Breathomics platforms, like electronic nose (eNose) technologies, are being tested for the accuracy of their diagnoses with promising outcomes in classification of asthma with respect to chestnoughtsPT with respect to their control CP(COPD) and control (healthy) (Kuo *et al.*, 2024). Combining microbial, host transcriptomic, and breathomics data using AI-driven models is expected to provide precision diagnostic models.

Clinical Translational and Utilitarian

Several biomarkers have already entered clinical practice, e.g. FeNO and blood eosinophils. However, most of the candidates based on microbes and multi-omics are still in the discovery or validation stages. barriers are variability in sampling methods, lack of standardization in pipelines and a lack of multicenter validation. Prospective studies with biomarker-based interventions integrated within respiratory care for asthma need to be undertaken to establish the clinical utility of biomarker and cost effectiveness (Reddel *et al.*, 2023).

Chronic Obstructive Pulmonary Disease (COPD): Biomarkers: Microbiological & Host

Pathobiology and Issues for Clinical Practices

Chronic obstructive pulmonary disease (COPD) is a progressive, heterogeneous disorder with symptoms of airflow obstruction, chronic inflammation and frequent exacerbations. Despite the standardization of diagnosis through spirometry, clinical outcome is highly variable across these patients and the response to treatment is unpredictable (Singh *et al.*, 2022). Current classification systems - such as GOLD - group patients according to their symptoms and a history of exacerbations, but don't do a good job of capturing underlying molecular or microbial endotypes. This reflects the need for biomarkers to better risk stratification, treatment response and disease progression.

Microbial Biomarkers

Alterations in the lung microbiome have been increasingly linked with the pathogenesis of asthma during the course of the disease and of allergic asthma. Overt or reduced microbial diversity (Overt- or reduced microbiota associated with exacerbations and accelerated decline in lung function) *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae* are over-represented. Previously, airway dysbiosis has been linked to the exacerbation rate and systemic inflammation (Molyneaux *et al.*, 2022). Gut microbiome changes have also been implicated in this, providing evidence for a gut--lung axis in the pathogenesis of COPD. Butyrate deficiency hinders the development of a state of systemic inflammation and regulates immune responses (Li *et al.*, 2023).

Host Biomarkers

Blood eosinophil count is the most validated biomarker in the field of subject reported in CTC in Data * given limited studies are generally not reproducible. It predicts response to ICS and has now been incorporated in GOLD guidelines for treatment decisions (Agusti *et al.*, 2023). Other systemic markers

such as C-reactive protein (CRP), fibrinogen and interleukin 6 (IL-6) have been explored as predictors of exacerbations, but with some inconsistent reproducibility (Faner *et al.*, 2021). Recent transcriptomic and proteomic analyses have found molecular signatures relating to the development of rapid lung function decline and frequent exacerbators phenotypes (Singhania *et al.*, 2022).

Multi-Omics and new Approaches

Multi-Omics Approach Is Changing the Course of Benchmark XP of Biomarkers for Chronic Obstructive Pulmonary Disease Metabolomics studies show a specific lipid and amino acid profile which separates frequent exacerbators from the stable patients (Wang *et al.*, 2021). Proteomic profiling of plasma has shown potential candidate markers of emphysema progression, including surfactant protein D (SP-D) and club cell protein 16 (CC-16). Breathomics is also under active evaluation and volatile organic compound (VOC) patterns are showing potential to distinguish between those with and without a diagnosis of chronic obstructive pulmonary disease (COPD) from those with asthma and healthy controls (Kuo *et al.*, 2024). Integration of microbiome, metabolome and host immune signatures using AI powered models may allow for more precise classification of endotypes of patients with chronic obstructive pulmonary diseases.

Clinical Translation – challenges, and barriers

While blood eosinophils and SP-D have been shown to have clinical utility, most candidate biomarkers are at the discovery/validation stage. Translation to clinical use is limited due to heterogeneity of various populations, lack of standardization of the assay and lack of sufficient multicenter trials. One of the most challenging aspects is the dynamic nature of the disease, as biomarkers can change depending on smoking status, treatment and frequency of exacerbation of symptoms (Agustin-Menca & Amadas, 2015). Future studies should focus on longitudinal validation, if they are possible in the real world, and incorporating biomarkers in clinical decision algorithms that can demonstrate utility and cost-effectiveness.

Bronchiectasis: Microbial & Host Biomarkers

Challenges in Pathobiology and Clinical

Bronchiectasis is a long-term disorder of the airways in which there is irreversible dilatation of the bronchi, with chronic infection and inflammation. Its incidence is increasing worldwide, particularly in older patients and in patients with comorbidities (chronic obstructive pulmonary disease / asthma) (Chalmers *et al.*, 2021). Despite management based on guidelines, disease heterogeneity is still high: in some patient's disease is stable with few exacerbations, in others there are recurrent infections, rapid decrease of pulmonary function and poor quality of life. Current clinical assessment tools such as the Bronchiectasis Severity Index (BSI) and (F) ACED score are of prognostic value, but only to some extent - and these tools fail to cone the biological drivers of the variability in disease processes. This gap clearly demonstrates the need for microbial and host biomarkers which guide individualized care.

Microbial Biomarkers

Microbial colonization and dysbiosis are important in pathogenesis of bronchiectasis. A chronic colonization with *Pseudomonas aeruginosa* especially is a strong predictor of the frequency of exacerbations and hospitalization, as well as mortality (Lonergan *et al.*, 2022). Other bacteria, such as *Haemophilus influenzae*, *Moraxella catarrhalis* and *nontuberculous mycobacteria* (NTM) contribute to disease progression. High throughput sequencing has led to the discovery of lesser microbial diversity and virulence of pathogenic microbes in severe disease (Woo *et al.*, 2020). Dynamic changes to the microbiome during exacerbations raise the potential for microbial signatures to help serve as early-warning signs of what is coming. Sputum metagenomics and 16S sequencing are becoming a popular way to profile microbial communities in the airways to answer questions of endotypes that represent individual pathogen dominance versus commensal resilience.

Host Biomarkers

Compensatory information is provided by host inflammatory markers. Increased sputum neutrophil elastase activity has been confirmed as a biomarker of the risk of exacerbation and lung function decline (Lonergan *et al.*, 2021). Blood eosinophils, having been traditionally investigated in asthma and chronic obstructive pulmonary disease (COPD), have likewise been associated with different bronchiectasis endotypes, possibly helping to guide corticosteroid response (Shoemark *et al.*, 2022). Serum and sputum cytokines such as IL-8 and TNF & orderly with disease activity but do not have disease-bond specificity. Recent proteomic studies propose novel candidates such as calprotectin and matrix metalloproteinases (MMPs) as potential prognostic biomarkers (Keir *et al.*, 2022).

Multi-Omics and New Mirrors

Integration of microbiome and host-response information has presented a promising method for stratification of bronchiectasis phenotypes. Transcriptomic and proteomic profiling have detected molecular endotypes based on neutrophilic (neutrophilic vs. eosinophilic - "eazea" IL-8 Flammarion)" neutrophilic" vs mixed" clear" (i.e., neutrophilic asthma acthetroleilia (Polverino *et al.*, 2023). Breathomics, less well developed in bronchiectasis than in asthma or in similar disease states such as chronic obstructive pulmonary disease, has recorded some success in differentiating between stable and exacerbated states.

Clinical Translation and Barriers - an article discussing barriers within the field of clinical translation.

However, problems remain in translating this to routine care. Chronic *P. aeruginosa* colonization has been the best biomarker with immediate clinical value. Other candidates such as neutrophil elastase have showed promise but need to be validated in multicenter studies. The major obstacles are heterogeneity of patient populations, variability of sampling (sputum vs. BAL), and lack of standardization of assays. In order to have both a clinical impact and biomarker-guided therapeutic strategies (including the stratification of patients to receive long-term antibiotics/biologics), they must be tested in randomized control trials. (Chalmers *et al.*, 2023).

Pneumonia and Lower Respiratory Tract Infections (LRTIs): Prognostic Biomarkers: Microbiological and Host Biomarkers

Pathobiology and Clinical Issues

Community acquired pneumonia (CAP) and hospital acquired pneumonia including ventilator associated pneumonia (VAP) are important causes of morbidity and mortality around the world. Accurate identification of pathogens is essential for antimicrobial therapies but traditional culture methods can be less sensitive and take a long time for the results to come back (Metlay *et al.*, 2019). In the critically ill patient, it is associated with inappropriate or delayed therapy, and poor outcomes, including mortality. This makes a compelling need for microbial biomarkers and host biomarkers that allow the means to more rapidly diagnose, and to predict prognosis and antimicrobial stewardship.

Microbial Biomarkers

Molecular diagnostics have progressed far ahead in the matter of pneumonia. Metagenomic next-generation sequencing (mNGS) studies of bronchoalveolar lavage (BAL) and sputum have shown higher sensitivity than culture and PCR which allows the detection of microbial (bacterial, viral and fungal) pathogens in one assay (Chen *et al.*, 2025). Studies in intensive care units (ICUs) show that mNGS brings about clinical actionable results that result in change in clinical management in up to 50% of cases (Jiang *et al.*, 2024). Multiplex PCR panels identifying common respiratory pathogens are also fast, but are constrained by predefined targets. Microbial diversity indices obtained by sequencing are correlated with disease severity suggesting that they may be used as predictive markers of disease severity (Zheng *et al.*, 2024).

Host Biomarkers

Host immune responses offer other diagnostic and prognostic information. Classical biomarkers volume of procalcitonin [PCT] and C-reactive protein [CRP]: While they have a wide range of applications in clinical practice, they are not 100% specific, especially concerning discriminative bacterial from viral infections. (Self *et al.*, 2022). Some peripheral blood-derived gene signatures have been found to distinguish between bacterial and viral pneumonia (Tsalik *et al.*, 2021), and they could possibly be used to drive antibiotic stewardship. In addition, several proteins such as soluble triggering receptor expressed on myeloid cells (sTREM-1) and presepsin have been explored as early sepsis and severity signs in patients with pneumonia (French *et al.* 2022).

Multi-Omics and Approaches to the Novel

Breathomics is a promising new frontier in the diagnosis of pneumonia, but it is not invasive (Tian *et al.*, 2025). Metabolomic signatures of serum and BAL fluid are being investigated in order to assess the severity of a disease and the effects of treatment Magnano *et al.*, (2024). Integration of microbial, transcriptomic, and metabolomic data, as well as ML, have led to an increase in the accuracy of predictive models in terms of ICU outcomes, demonstrating the promise of multi-omics in clinical decision making.

Clinical Translation and Barriers

Despite promising findings, attempts to implement microbial and host biomarkers in pneumonia have been hampered by important issues. mNGS suffers from high costs, the need for bioinformatics and the potential detection of colonizing rather than causative organisms. Host biomarkers need additional validation in different populations including resource limited settings. Nevertheless, coupling rapid diagnostics to stewardship protocols is increasingly being recognized as an important strategy to limit unnecessary antibiotic consumption but to also optimize outcomes (Metlay *et al.*, 2019). Further work should be done on multicenter validation studies, cost-effectiveness studies and on the implementation of biomarker-informed care into ICU workflow.

Tuberculosis: Microbial & Host Biomarkers

Pathobiology and Clinical Issues

Tuberculosis (TB) is one of the leading infectious causes of death in the world with more than 10 million cases every year. Diagnosis is still very much based on sputum smear microscopy, culture and nucleic acid amplification tests (NATs). These approaches while being widely used have their limitations regarding sensitivity specifically in extrapolation of Paucibacillary TB, are often inaccessible in the low-resource settings (World Health Organization, 2022). Furthermore, the fact that latent TB infection (LTBI) has a lot in common with the active disease has been a forever point to distinguish. There is a pressing need for biomarkers that can lead to faster diagnosis, prediction of disease course and therapy, in accordance with the WHO's TPPs for TB diagnostics and prognostics.

Microbial Biomarkers

Microbial detection is still the important for TB diagnosis. Metagenomic next-generation sequencing (mNGS) and targeted sequencing have recently increased the possibilities of the diagnostic toolbox, especially in extrapulmonary TB (Zhou *et al.*, 2023). The molecular tests for MTB from sputum samples like Xpert MTB/RIF ultra gives rapid and rifampicin resistance results but cannot distinguish between live and dead bacilli (Dheda *et al.*, 2022). Emerging approaches involve the detection of cell-free *Mycobacterium tuberculosis* DNA in blood and urine and this approach shows promise for the non-sputum-based diagnosis of tuberculosis (Zhu *et al.*, 2024).

Host Biomarkers

Host-response biomarkers have received a boost as complementary or alternate diagnostic tool.

Transcriptomic studies have revealed blood gene expression signatures for differentiation between active TB and LTBI and respiratory diseases (Muwanga *et al.*, 2024). Other host biomarkers such as cytokines profiles (IFN-g, TNF-alpha, IL-2) and markers of immune activation (IP-10, Soluble CD14), have shown potential but need broader validation. Scriba *et al.*, (2021) Proteomic markers including C1q and serum amyloid A (SAA) have emerged as possible candidates for specifying TB prognosis (Penn-Nicholson *et al.*, 2022).

Prognostic and Predictive Biomarkers

Beyond diagnosis, biomarkers are being developed that can identify disease progression and treatment outcome. Blood transcriptomic signatures have been found to be useful for identifying subjects who are at high risk of progressing from LTBI to active disease (Mendelsohn *et al.*, 2024). Early on-treatment changes in gene expression and metabolomic profiles may be predictive of therapeutic response and risk of relapse - suggesting the role for biomarkers in treatment monitoring (Suliman *et al.* 2023).

Multi-Omics and Translational Chances

Integration of multi-omics data, such as transcriptomics, metabolomics, proteomics, etc. holds promise for the definition of robust biomarker panels. Breathomics is emerging as a non-invasive form of diagnosis with profiles for volatile organic compounds (VOC) demonstrating early promise of differential diagnosis of TB from non-TB pneumonia (Tian *et al.*, 2025). Combining biomarkers from the host and the microbes via machine learning methods should bring about a faster translation into clinical actionable tests.

Clinical Translation and Barriers to Clinical Translation

Despite quick progress, most TB biomarkers are still at the discovery/validation level. Barriers are at the population level, lack of standardization of assay and this is not being implemented in the high burden, resource constrained regions. Biomarkers fitting the TPP criteria: non-sputum based, highly sensitive, cheap, and point of care (POCT) are needed by WHO (WHO, 2022). Further research should concentrate on multicentre prospective validation and cost-effectiveness studies, and implementation of biomarker-guided diagnostics in TB control programmers.

Breathomics: Emerging Field of Non-Invasive Biomarkers for Respiratory Disease

Rationale and Clinical Application

Breathomics, the science of volatile and non-volatile compounds contained in exhaled breath, has been shown to be a potential, non-invasive tool for diagnosis and monitoring of many respiratory disorders. Exhaled breath contains thousands of volatile organic compounds (VOC) and non-volatile molecules such as protein, lipid and metabolites that reflects the interactions between the host and microbes, metabolic and airway inflammation (Dragonieri *et al.*, 2022). Its non-invasive character and possibilities for real-time analysis make breathomics an interesting alternative/complement for sputum and blood biomarkers, especially in the pediatric, elderly and critically ill patient.

Volatile Organic Compounds or VOCs

Many publications have shown that profiles of VOCs can be used to discern between health and disease states. In asthma, the patterns of specific VOCs are linked to eosinophilic inflammation and are predictive for risk of exacerbations (de Vries *et al.*, 2022). In the case of the people with chronic obstructive pulmonary disease (COPD) they found that VOCs pattern separated frequent exacerbators and distinguished between being suffering from a gastrointestinal disease (such as asthma) and having a chronic lung disease (COPD) from Kuo *et al.*, (2024). In pneumonia, VOCs have been identified to differentiate the pathology of bacterial pneumonia and viral pneumonia which could be useful for antibiotic stewardship (Tian *et al.*, 2025). Methyl nicotinate and cyclohexane derivatives are currently being explored as breath volatile organic compounds (VOCs) in tuberculosis as diagnostic biomarkers (Beccaria *et al.*, 2019).

Non-Volatile Compounds (nVOCs) & Exhaled Breath Condensate (EBC)

In addition to the volatile organic compounds (VOCs), exhaled breath condensate contains non-volatile molecules which have been shown to be reflective of airway inflammation and infection. Cytokines (IL-6, IL-8, TNF-alpha), markers of oxidative stress [hydrogen peroxide, 8-isoprostane] and lipid mediators have been identified in EBC and shown to be related to asthma severity and exacerbation of active CD4 T-cell-mediated asthma. Proteomic and metabolomic profiling of EBC is progressing, although technical issues of reproducibility and standardization still exist (Horváth *et al.*, 2020).

Technologies and Analytical Methods

Breathomics makes use of various analytical platforms such as gas chromatography-mass spectrometry (GC-MS), proton-transfer reaction mass spectrometry (PTR-MS) and electronic nose (eNose) devices. Compared to GC-MS and PTR-MS, though, spectrometer systems have not yet been marketed in industrial or laboratory applications due to the high sensitivity and legend specificity required, as well extra laboratory environments. In contrast, eNose can be rapidly implemented for point-of-care profiling as a diagnostic tool, and can be clinically implemented at the cost of lower specificity (Rock *et al.*, 2021). Classification accuracy and disease prediction are now significantly better by involving machine learning algorithms.

Interpreting the Clinically and Blocks

Although breathomics is highly promising, identification in clinical practice is hindered for the following reasons:

Standardization: Inconsistent sampling protocols, storage and analytical pipelines make it difficult to compare studies.

Validation: most results are taken from small cohorts from single centers; multicenter validation is urgently needed

Interpretability: The deployment of complex VOC and nVOC datasets needs highly efficient computation sources to augment the complexity leading to clinical acceptance issues.

Regulation and scalability: Diagnostic tests based on breath will need to meet high standards for analytical validity and clinical utility, as well as being cost-effective and gaining regulatory approval.

Future Directions

Breathomics will surely become a cornerstone of precision respiratory medicine, especially in conjunction with multi-omics and profiling of the microbiota. Trending areas include palm portable eNose devices for point-of-care diagnostics, classification of VOCs using artificial intelligence and integration into telemedicine platforms for remote monitoring. Large-scale initiatives including the European Respiratory Society Task Force on exhaled biomarkers will be of foremost importance for translation into the clinic.

Validating and Putting Microbial Biomarkers into Clinical Practice

From Discovering to Implementing in Practic

The bench to bedside translational pipeline for microbial and host-response biomarkers involves discovery, analytical validation, clinical validation and once more, demonstration of clinical utility. While discovery provides biomarker candidates through the use of high-throughput omics, only a minor proportion reach the stage where they may see end use clinically, because of inherent barriers around reproducibility, assay robustness and cost efficiency (Hamburg and Collins, 2019). The U.S. Food and Drug Administration (FDA)-National Institutes of Health (NIH) BEST (Biologic Effectiveness, Safety Testing) Resource has established a common taxonomy for the classification of biomarkers (diagnostic, prognostic, predictive, monitoring, pharmacodynamic, and safety) to facilitate the comparison of studies in the field (U.S. FDA-NIH Best Biomarker Working Group, 2021).

Analytical Validation

Analytical validation of biomarker assays provides an assurance that whether or not the intended signal can be detected reliably in defined conditions. It contains reproducibility, sensitivity, specificity, and limit of detection. For microbial biomarkers there is the problem of distinguishing colonization from infection, modification in specimen types (e.g, sputum vs. BAL) as well as contamination in sequencing workflows. Transparency and reproducibility of such studies are enhanced by the adoption of guidelines, such as STORMS for the reporting of microbiome studies (Mirzayi *et al.*, 2021) and STARD 2015 for the reporting of diagnostic accuracy studies (Bossuyt *et al.*, 2015).

Clinical Validation

Clinical validation studies help determine if a biomarker is an accurate measure of future clinical outcome in the targeted population. For respiratory diseases, this involves the use of multicentre cohorts that are representative of patient diversity with respect to geography, age, comorbidities and without regard to treatment exposures. Transcriptomic TB signatures validated across African cohorts were recently published by Muwanga *et al.*, (2024) as well as breathomics datasets ranging over asthma, bronchiectasis and COPD by Kuo *et al.*, (2024). Multicohort validations are needed to avoid biases caused by one center discovery studies.

Proving that result is clinically useful

Clinical utility requires demonstration that the biomarker-directed intervention is improved in terms of patient effect or benefit, decision-making and/or cost effectiveness. For example, the number of eosinophils in the blood (blood eosinophils) is used as a guide for the use of inhaled corticosteroids in COPD, although this has only recently been supported by randomized controlled trials (Agusti *et al.*, 2023). In the case of pneumonia, mNGS has been able to change the selection of antibiotic treatment and optimize the diagnostics yield in ICU cohorts Jiang *et al.*, (2024). However, most biomarkers have been discovered but are in discovery or validation without future human evidence of patient benefit. Randomized or pragmatic clinical trials--such as trials that incorporate biomarker-guided care into routine workflows--are typically needed to acquire regulatory approval.

Implementation into the Clinic

For successful implementation, biomarkers must fulfil requirements that typically must be practical:

Turnaround time: With acute cases such as pneumonia it is vital to obtain results quickly.

Cost-effectiveness: Health-economic studies are of the essence for adoption, especially where resources are scarce.

Laboratory integration: Assays must be able to integrate into existing infrastructure and to meet established standards of excellence.

Integration with EHRs and AI-powered Decision Support Systems: Integration with electronic health records (EHRs) and AI-based Decision Support Systems eases the adoption process.

Challenges and Ethnic Studies for Tomorrow

Barriers include variability of assays, absence of harmonious protocols and uncertainty of regulatory paths for multi-omics diagnostics. Ethical and equity considerations are also critical: biomarkers that are validated in high-income countries might not perform in highly burdened and low-resource countries. Appropriate future efforts should include a priority for:

1. Prospective, multicenter, validity studies.
2. Development of international consensus consortiums of biomarkers
3. Regulatory frameworks for artificial intelligence (AI) based, multi-omics based diagnostics

4. Health-economic studies to prove cost-effectiveness on scaling up.

Artificial Intelligence and Machine Learning based Multi-Omic Integration

Rationale

Respiratory disorders are biologically complex, relating interactions of host immunity, microbial community, and environmental exposures. No one biomarker is able to represent this complexity. Artificial intelligence (AI), and specifically machine learning (ML), approaches have the potential to incorporate multi-omics data, such as genomics data, transcriptomic data, proteomics data, metabolomics data, microbiome profile and breathomics data, into clinically actionable models (Topol, 2019). Such integration provides stratification of endotypes of a disease, prediction of treatment response and early detection of exacerbations or disease progression.

Applications to Respiratory Medicine

AI-powered methods have already shown their usefulness in several types of respiratory diseases:

Asthma: ML classifiers using transcriptomic and microbiome data for better prediction of corticosteroid responsiveness than classical markers such as FeNO, allergy findings or eosinophils (Thorsen *et al.*, 2023).

COPD: Deep learning for sputum microbiome and plasma proteomics to help predict frequent exacerbator phenotypes with higher accuracy than clinical scoring systems. (Singhania *et al.*, 2022)

Pneumonia: Neural network models combining some host's gene expression pattern and also the ability to detect a pathogen increases the diagnostic accuracy and decreasing the inappropriate hunting of antibiotics (Tsalik *et al.*, 2021).

Tuberculosis: Predicting Disease Progression using Transcriptomic Risk Signatures Optimization of Biomarker Panels to Use in Resource-Limited Settings Severe disease has been shown to be applied using ML for the optimized risk sign panels (Mendelsohn *et al.*, 2024).

Standards and Reporting

To translate AI models to use in clinical settings, there are stringent methodological requirements. Information from Collins *et al.*, (2024) Specification solic Pattern Reporting for Machine Learning prediction Models (TRIPOD+AI extension): An Extension of the Reporting of Prediction Models Initiative Guidelines to Improve Reported ML Prediction Models. BMJ. Similarly, to this, STARD-AI is expanding diagnostic accuracy reporting to tests that have AI (Sounderajah *et al.*, 2021). These frameworks focus on transparency of the datasets, reproducibility, external validation, calibration metrics, and prevention of data leaking. Without conformity to these standards, AI-based biomarker models run the risk of overfitting, and lack of clinical credibility.

Technical Challenges

There are still a number of obstacles that need to be overcome before biomarker models are driven by Artificial Intelligence can be used widely:

1. Data quality and harmonisation: multi-omics data are generated through the use of a variety of platforms and pre-processing protocols that makes integration difficult.
2. Sample size limitations: High-dimensional omics data originated from a small cohort which often leads to overfitting.
3. Generalizability Models that are trained in high-income environments may not work in low-resource or diverse populations.

4. Interpretability: Black-box algorithms do not increase confidence among clinicians; explainable approaches to AI are required in order to establish links between biomarkers and biological mechanisms.
5. Data privacy: Federated learning is emerging as a promising approach to training cross-institutional networks without transferring raw data, and hence could also prevent privacy issues in omics and clinical/n Conversely, integration of these sensitive data types requires robust governance (Dayan *et al.*, 2021).

Integration of biomarker data using Artificial Intelligence is likely to revolutionize the field of respiratory medicine by:

Facilitating a real-time decision support via EHRS predictive analytics solutions.

Point-of-care Machine Intelligence Breathomics Device for Portable Diagnostics Applications

Supporting federated model training with global consortia pooling their omics data;

Toward adaptive trial designs driven by the use of AI to direct biomarker-based stratification and therapy selection.

Ethics, Equity, and Generalizability in the Translational Research of Biomarkers

Cultural Diversity and Prejudice

One of the central issues in biomarker research is getting the ability to generalise studies across different groups of people. Until now most studies have taken place in high income countries, and often examined cohorts not representative of the global disease burden. For instance, the tuberculosis biomarker signatures that have been validated in the African populations are distinct from those in the European discoveries in terms of their genetic, environmental and microbiome differences (Muwanga *et al.*, 2024). As with other areas of research, there is an underrepresentation of ethnic minorities, women and people from low resources in asthma and COPD biomarker studies (Jackson *et al.*, 2023). This lack of diversity threatens the basic care that depends on biomarkers if health disparities are not built into care unless models are widely validated.

Equity in Access

The new generation of biomarker tools including metagenomic sequencing or breathomics could capture an evolution of accessibility as well as affordability. In resource-constrained settings where the respiratory disease burden is highest, high costs, specialized equipment necessities and laboratory infrastructure make viability problematic (Pai & Behr, 2022). The greater the disparity in research returns by country, the more likely the revolution of biomarkers will lead to widening the global health gap, unless deliberate and strategic steps are taken for equitable distribution. Implementation science frameworks: Structural considerations to translate biomarkers assays to local work settings in an affordable, scalable and sustainable manner.

Data Governance and Privacy

Multi-omics integration and AI-driven biomarker discovery are possible thanks to an enormous exchange of data on a large scale, between institutions and countries. This raises ethical issues of data ownership, patient consent, patient privacy (Shabani *et al.*, 2021). A strong governance structure is required that will balance innovation whilst protecting patient rights. Approaches like federated learning enable cross-institutional AI model training without exposing the data at the individual level, and is a promising solution to privacy concerns (Dayan *et al.*, 2021).

Government and Ethics Regulation

The ability to identify biomarkers in a short time - especially in the case of biomarkers for AI-enabled diagnostics - has exceeded regulatory requirements. It is important that ethical oversight ensure issues of

algorithmic bias, transparency and explainability (Sounderajah *et al.*, 2021). Clinicians need to be able to interpret a biomarker-based decision-protecting human decision-makers from being off-load. In addition, equity assessments should be part of the regulatory review, with consideration given to making sure that biomarkers do not continue systemic health disparities.

Future Considerations

Ethics and equity need to be embedded at all levels of the biomarker pipeline (discovery to implementation). This includes:

1. Multicentric cohort recruitment to be able to capture global heterogeneity.
2. Affordability Frames to Provide Accessibility in Low-Resource Settings.
3. Recommendations for the report of demographic variables in biomarker studies:
4. Interpretable, fair and accountable AI frameworks.

By making diversity, equity and ethical governance a priority, biomarker-driven personalized medicine is on its way to a truly global and inclusive model of respiratory care.

Future Directions

The translation of microbial and host biomarkers to personalized respiratory medicine continues to accelerate although there are a number of important priorities:

Fabrication of Biomarker Pipelines

Variability in specimen collection and sequencing platforms, and the data analysis is a major barrier. Microbiome, breathomics and transcriptomic research should be seamlessly combined in international consortia, with a similar approach implemented as for biomarkers of cancer (Mirzayi *et al.*, 2021).

Validation Centre de Validation Multicentrique

The confirmation of reproducibility and generalizability of candidate biomarkers are important, and so are large prospective multi-ethnic cohorts. Real world utility is needed by incorporating biomarker-guided interventions in care pathways and will require a pragmatic clinical trial to establish utility (Agustí *et al.*, 2023).

Multi Omics- Integrated and Addressed with Integration of AI

Future research needs to be aimed at AI-based integration of multi-omics data for robust endotyping and prediction models. Federated learning frameworks have the potential to speed up the discovery, while preserving data privacy (Dayan *et al.*, 2021).

Non-Invasive Diagnostics

Breathomics and saliva-based biomarkers represent specific exciting and promising opportunities for point-of-care/diagnostics and DNA biomarkers. Standardization and miniaturization of eNose devices could make these technologies a feasible way to scale-up for routine practice (Dragonieri *et al.*, 2022).

Equity and Access

Implementation science needs to have an affordability and accessibility focus, especially in the low- and middle-income countries where the burden of respiratory diseases is high. It is essential to establish collaborations between academia, industry and policymakers in order to ensure demand equity in the implementation of innovative diagnostics (Pai & Behr, 2022).

Conclusion

Microbial and host response biomarkers are a paradigm shift in respiratory medicine, one that pushes respiratory medicine from one-size-fits-all therapy to detailed diagnoses and personalized therapy. The

candidates for candidate biomarkers are being generated through relative new approaches in the field of metagenomic sequencing, transcriptomic, proteomics, metabolomics and breathomics in the field of asthma, capacity, bronchiectasis, pneumonia and tuberculosis. Integration of these multi-omics approaches with artificial intelligence holds the potential to stratify patients based on endotype, predict response to a therapy, and optimise the management of a patient's disease. However, translation to clinical practice requires serious measures of validation, cost-effectiveness studies and regulatory oversight alongside ethical governance and/or equitable implementation strategies. Through conversations between bench scientists and clinicians, microbial biomarkers offer the opportunity to revolutionize the way we diagnose, predict and treat respiratory conditions, realizing the big picture of precision medicine.

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