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CHAPTER

Breath analysis for respiratory infections

21

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Chapter outline

21.1 Overview 335
21.2 Bacterial infections 336
21.3 Viral infections 339
21.4 Fungal and other eukaryotic infections 342
21.5 Summary 344
References 346

21.1 Overview

Respiratory pathogen diagnostics are an important area of current breath research, and researchers have taken various tactics to approach studies on this topic.[1] Any studies of infectious diseases in humans have relied on subjects in preidentified cohorts who tested positive for the diseases under normal standards of care, using gold-standard confirmatory methods. Breath samples, collected with informed consent, have been concentrated and/or directly analyzed in a variety of ways. Room air analytical controls are sometimes used as a best practice. A "control" cohort of subjects is usually employed as a comparison and typically is comprised of uninfected age-matched and gender-matched healthy individuals, although in more ideal circumstances, this would be a group that was suspected of having the infection but was later ruled out (see Chapter 35 for further details on clinical study design). Indeed, recruiting those with the same symptoms in the control groups, including noninfectious disease subjects, such as sarcoidosis patients in the case of breath

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sampling for tuberculosis, is an increasingly essential parameter in a study design. Once breath samples are obtained by an appropriate sampling method (see Chapter 2), a common theme has been to use different forms of mass spectrometry (MS) analysis of the breath samples to provide complete metabolomic chemical identification of the sample contents. Using an untargeted approach to statistical analysis, researchers then applied various data analysis techniques to try to identify candidate breath biomarkers of that specific pulmonary infection.

Parallel animal model studies have provided insight into human breath biomarker studies. The animals may be chosen from natural disease occurrence, similar to the human studies, or laboratory animals in carefully controlled studies that target a specific pathogen at different doses of inoculation. These animal models should be—and often are—of high human relevance and have included macaques, mice, ferrets, and swine. Animal model studies have played a key role in developing and maturing biomarker identification, as well as providing fundamental information, such as how biomarkers change over an infection time course postinoculation.

Breath biomarkers of infection from human and animal studies can arise from the host, generated by the immune system[2] or by cellular responses of infected tissues, or they can arise from the microbial pathogen as a function of virulence or as a consequence of normal cell cycles and proliferation. Thus, there is also a role for in vitro cell culture models, which can produce translatable biomarkers or be used to understand the origin of biomarkers during disease progression (see Chapter 26).

Together, this triad of approaches (human, animal, and in vitro cell culture studies) has allowed researchers to identify candidate breath biomarkers that can be carried forward into larger studies. A summary of these advances is outlined in this chapter.

21.2 Bacterial infections

Tuberculosis (TB) is the largest infectious disease killer in the world and the biggest killer of people with human immunodeficiency virus (HIV) infection and/or acquired immune deficiency syndrome (AIDS). TB is one of the respiratory diseases for which breath analysis studies have been performed in both humans and animal models. Traditional means of TB detection, still used in many countries,[3] are slow and require weeks-long sputum culturing to confirm diagnosis and assess antibiotic resistance. Nucleic acid amplification approaches generate useful diagnostic data faster, typically in three days, but this timeline is problematic as many patients do not return for their result and thus are lost to follow up. And, children and immunodeficient patients generally do not produce sputum. Thus, breath diagnostics are poised to provide a much more rapid timeline for physicians, especially to allow patient treatment to begin rapidly.

Breath studies to diagnose bacterial infections caused by *Mycobacterium tuberculosis* (*Mtb*) are more likely to succeed than any other approach due to the extensive groundwork done by the Belgian-Tanzanian group APOPO (Anti-Persoonsmijnen

Ontmijnende Product Ontwikkeling) who have evaluated the volatiles from tens of thousands of sputum samples using trained giant Gambian rats.[4] Morozov and colleagues performed a proof-of-principle study aimed at developing noninvasive diagnostics for pulmonary TB based on potential TB biomarkers in microdroplets exhaled by TB patients and collected on electrospun fibers.[5] This study involved 42 TB patients (including recent and chronic TB cases) and 13 healthy volunteers. Samples were tested for the presence of *Mtb* cells, *Mtb* DNA, and protein biomarkers. While no *Mtb* cells or *Mtb* DNA could be detected, an ultrasensitive immunoassay was developed that detected immunoglobulin A (IgA) in >90% of samples from both TB patients and at higher rates than healthy volunteers. Antigen-specific IgA was detected at higher rates in the patient samples compared with the healthy control samples. Although the authors report and comment on the relatively low sensitivity and specificity, they suggest that expanding this method to include inflammation-specific biomarkers in addition to the TB-specific antibodies promises to increase the level of discrimination in the future.

Beccaria and colleagues conducted two studies evaluating the use of human breath collected and stored on thermal desorption tubes and analyzed by comprehensive gas chromatography−time-of-flight mass spectrometry (GC×GC-TOFMS) to diagnose active TB in subjects with confirmed *Mtb* infection.[6,7] The control samples used in each study included room air, as well as either a group presenting to the clinic with the same symptoms (South Africa) or in subjects with nonrespiratory infectious diseases (Haiti). About 84 subjects were evaluated, of which 34 were patient controls. Both studies present a chemometric pathway, utilizing statistical and machine learning tools to translate thousands of analytical signals to a putative biomarker set in the context of an underpowered study design.

Hill and colleagues also investigated the potential of breath analysis for detecting mycobacterial infections using a murine model and *Mycobacterium bovis* bacillus Calmette−Guerin (BCG),[8] as well as *Mtb* in macaques.[9,10] This was the first report of breath being collected from mice or nonhuman primates infected with a pathogen from the *Mycobacterium tuberculosis* complex (MTBC). A related study on mice used breath collected at two time points for eight mice infected with *M. bovis* BCG and eight healthy mouse controls exposed to instilled phosphate-buffered saline (PBS). Room air samples were collected as controls. The breath of ventilated mice was collected in Tedlar bags and then concentrated onto thermal desorption tubes. Analysis of the breath samples was performed using GC×GC-TOFMS. Statistical analysis using random forest identified 23 features in the data that discriminated between the breath samples of infected mice and the controls. Tentative identification of these 23 compounds was provided by mass spectral matches to a reference library. Four of these markers were also reportedly found in previous breath studies on animals with MTBC infections. This study showed that this overall methodology can differentiate between healthy and infected mice using breath analysis. It also indicated that a mouse breath model might be useful to study TB pathogenesis and evaluate preclinical drug regimen efficacy.

A design for the collection of breath in gently anesthetized macaques in a biological safety level three laboratory was published by Mellors et al., in 2017.[8] A chemometric process for evaluating the repeatability of breath samples in terms of chemical composition was given, and a pilot level determination of 37 putative biomarkers to distinguish *Mtb* lung infections from healthy animals using breath was generated. In a follow-up study,[10] the same group looked for and found substantial synergy between cell culture volatile molecules of *Mtb* in the breath of nine cynomolgus macaques with infection by the same strain (Erdman). Thirty-seven molecules found in culture could distinguish the breath of infected and healthy macaques with an area under the receiver operating characteristic curve (AUROC) of 87%. The authors point out, however, that the origin of the molecules in the breath of the macaques was unknown; therefore, the translation from culture to breath should be considered precarious unless supported by unique metabolism, as can be found in some fungal infections (see Section 21.4).

Bergmann and colleagues investigated the in vivo volatile organic compound (VOC) signatures of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) in goats[11] in a study that involved 26 MAP-inoculated animals and 16 healthy controls. Volatile molecules were collected from the animals' breath using an automated alveolar sampling device and needle trap microextraction, as well as from feces using solid-phase microextraction (SPME), with sampling performed over various time points spanning up to 48 weeks after inoculation. All samples were analyzed by GC-MS, with tentative substance identification accomplished using a reference library. In parallel, blood was sampled and analyzed for MAP-specific antibodies and MAP-specific interferon-γ response to correlate measured breath molecule patterns to measurements of these blood-based biomarkers. The observed VOC patterns differed between MAP-inoculated and noninoculated animals. Although the measured VOC patterns evolved during the infection, notable differences in observed VOCs between inoculated and noninoculated animals remained throughout the course of infection. A total of 28 VOCs were identified as potential markers for MAP infection in vivo, some of which have been found in previous in vitro studies in the headspace of MAP cultures.[12] Some of the observed VOC markers in breath were attributed to host response, and the authors cautioned that results from in vitro VOC studies (bacterial headspace sampling) may not simply transfer to in vivo conditions. The results of this work also indicated the potential usefulness of VOC profiles from feces to detect the presence of mycobacteria in the gut of ruminants. Further details of this and other studies with ruminants are given in Chapter 27.

Several human studies have targeted chronic *Pseudomonas aeruginosa* (PA) in subjects with cystic fibrosis. Cystic fibrosis (CF) is a genetic disease that results in polymicrobial lung infections, with the presence of PA, in particular, being linked to poorer outcomes.[13] Extricating a breathprint from the milieu of complex microbial ecology and host comorbidities is one of the most complicated undertakings in breath research. Gilchrist and colleagues evaluated hydrogen cyanide (HCN) in breath using selected ion flow tube-mass spectrometry (SIFT-MS) as an early

biomarker for PA infection in 233 patients over 2 years (2055 total breath samples).[14] Grounded in microbiology confirmation, which revealed 71 PA-positive cultures, the specificity of the approach was estimated to be 99%, although the low sensitivity (estimated at 33%) makes the current test inadequate as a screening tool. Španěl and colleagues evaluated the breath of 20 CF patients with confirmed *P. aeruginosa* infection and contrasted those with 38 CF patients who were PA-free. Using a panel of 16 molecules obtained from a linear logistic model, they achieved an AUROC of 0.91. Further details of these studies with SIFT-MS are given in Chapter 9. Nasir and colleagues evaluated a lung sample, bronchoalveolar lavage (BAL), from patients with CF, seeking potential biomarkers for *P. aeruginosa* and *Staphylococcus aureus*.[15] Using 154 samples, they identified breathprints for each infection type, with good negative predictive values for *P. aeruginosa* (0.92) and good positive predictive values for *S. aureus* (0.86).

Two mouse studies also provided insights into lung infections by bacterial pathogens. Purcaro and colleagues evaluated a core breathprint for PA in mouse lung infections, the contribution of the host to the breathprint, and explored which molecules could be used to distinguish mice infected with the major clades of *P. aeruginosa* (PAO1, PA14, PAK, PA7).[16] Sham infection controls (PBS) were employed. Nine compounds from the headspace of those cultures were sufficiently consistent and frequently observed, and were thereby considered core in the in vivo model at 24 and 48 h infection time points. A breathprint containing ten molecules for mice infected with different strains could somewhat reflect the phylogenetic groups to which the strains belong. Barbour and colleagues developed a method to sample nose-only breath from mice and performed VOC analysis of breath from mice with systemic bacterial infections caused by *Borrelia hermsii*.[17] While inflammatory and antiinflammatory cytokines were found to be elevated in infected mice compared with uninfected controls, no significant difference was found in the profiles of 72 different VOCs from nasal sampling, although carbon monoxide (CO) was notably elevated in the breath of infected mice. Control experiments of headspace sampling from the cultures of *B. hermsii* indicated that bacteria themselves did not produce CO and thus were unlikely to be the source of elevated CO in mouse breath; however, in vivo verification is still required to confirm this hypothesis. The findings of this work indicate the potential utility of CO concentration in exhaled breath during systemic infection and inflammation. Exhaled CO in inflammatory pulmonary diseases is treated at length in Chapter 6.

21.3 Viral infections

Numerous viruses can infect the human airway, with common examples being influenza viruses and respiratory syncytial virus (RSV). Most respiratory viruses cause similar symptoms of a fever and aches, but can be quite severe for patients of certain ages or who are immunocompromised, leading to more developed maladies, such as pneumonia or bronchitis. RSV leads to nearly 14,000 deaths annually in US adults, a number that is likely underestimated.

As with bacteria, there have been significant advancements in breath tests for viral infections. While bacteria and fungi can generate their own metabolite signatures, viruses rely on the metabolic machinery of their host to propagate. Thus, viral detection often looks for direct evidence of the virus, accomplished by hunting for nucleic acid segments specific to a virus in a bodily fluid sample, such as blood or mucous. This technique of nucleic acid screening, known as polymerase chain reaction (PCR), is very sensitive but often includes a lag time with samples being shipped to offsite laboratories and additional time for the analysis to be conducted. More rapid tests for viral infections are needed, as correct diagnoses by even a day sooner could save lives. In addition, specifying between viral versus bacterial infection is rather important for treatment, as prescription of antibiotics is ineffective against viruses. Finally, methods to collect bodily fluids for PCR can be quite invasive, especially for respiratory infections. Bronchoscopies are a common technique, in which an instrument is threaded through the mouth or nose and down into the lungs, for instance, to collect liquid biosamples for analysis, as in BAL. As breath is collected noninvasively, it is a much more attractive technique, especially for those already suffering from pulmonary disorders.

Around the turn of the millennium, pigs in industrial farm settings were contracting Aujeszky's disease, caused by Suid herpesvirus 1 (SHV-1). The disease starts with respiratory symptoms that often become severe, leading to death. Mortality rates were as high as 100% for piglets, and at the time it was not understood how the virus spread. Gillespie et al. posited that as infected pigs coughed or sneezed, they would produce aerosols that could travel long distances, carrying the virus and infecting nearby pigs. In their study, they inoculated a set of pigs with Aujeszky's disease. Once they showed symptoms, an infected pig was paired with a healthy "recipient" pig. A mouthpiece was placed over each pig's snout, which was then connected with tubing. This allowed the recipient pig to inspire the exhaled breath of the infected pig, without physical contact between animals. Afterward, nasal mucus was collected from all pigs. Each infected pig had detectable levels of SHV-1 in their mucus samples, but no virus was detected from the recipient pigs. Yet, all recipient pigs developed clinical symptoms of Aujeszky's disease[a] The authors made two conclusions, namely (1) that viruses can be carried by respiratory aerosols and infect others nearby and (2) that pigs are much more sensitive indicators of airborne SHV-1 virions than laboratory aerosol detection methods at the time.[18] This work demonstrates what is commonly known today—that respiratory viruses can be spread through exhaled aerosols.

Influenza infection is a major cause of seasonal morbidity and mortality in humans worldwide, and efforts to detect influenza viruses and virus infections rapidly and specifically are evolving. Phillips and colleagues conducted a before-and-after study looking at exhaled metabolites emitted by subjects receiving live attenuated influenza vaccine (LAIV).[19] Confounding biologic

[a] Since this study was first reported, a vaccine has been developed for Aujeszky's disease.

variables were likely minimized because each subject was his/her own control. LAIV breath metabolites were measured and compared pre- and postvaccination. The authors hypothesized that oxidative stress products (e.g., 2,8-dimethyl-undecane and 4,8-dimethyl-undecane) were seen in post−LAIV-treated subjects because influenza virus characteristically causes increased reactive nitrogen oxide species. Indeed, oxidative stress products were observed with changing concentrations over time post-LAIV inoculation, and these products did not appear in the LAIV headspace. The breath test performances in classifying subjects as pre- or post-LAIV inoculation ranged from a C-statistic of 0.82 (day 2) to 0.95 (day 7) to 0.95 (day 14), thereby demonstrating the feasibility of this approach. This study provides an important framework for future studies assessing influenza virus infection in its elegant design.

Aksenov and colleagues assessed metabolites from several different influenza strains that infected cultured human B-lymphoblastoid cells.[20] Lymphoblastoid cells were used to minimize the contribution of apoptosis to metabolite production. Several metabolite patterns were increased significantly over background and were used to identify specific influenza virus strains (e.g., H1N1 vs. H6N2) and strength of virus inoculation (e.g., H1N1 multiplicity of infection [MOI] 1 vs. MOI 10). This proof-of-concept study has many implications for influenza detection at both an individual level and for epidemiologic studies (consider pandemic influenza strain testing). Importantly, there were many overlapping alkene and other compounds seen in the Aksenov and Phillips studies. This provides phenomenological significance in the metabolites generated from influenza-infected cells, despite not knowing the exact mechanism of metabolite production.

Zoonotic reservoirs are common for influenza A viruses, and efforts to determine breath metabolites from animals may shed light on understanding emerging pandemic influenza strains. Traxler and colleagues assessed breath metabolites from swine during a complete H1N1 infection cycle.[21] The study reported six metabolites that classified swine as infected, as measured against standard diagnostic testing. Although the animals showed biochemical evidence of infection, they did not show clinical evidence of infection, such as decreased food or water intake, lethargy, and so on. This suggests that breath metabolite analysis may identify subclinical influenza A infection in animals, which may ultimately become more virulent when they bridge from animals to humans. As mentioned, a limitation of these studies is that they do not address the biologic origin or mechanism(s) of metabolite production, which will be important considerations of future studies.

Lower respiratory tract infections, such as pneumonia, are more challenging to diagnose compared with upper respiratory infections. Doctors are required to order invasive tests for the patients with suspect infections, including oropharyngeal swabs, induced sputum collection, and BAL. Toward less invasive techniques for lower respiratory tract diagnoses, PneumoniaCheck is a commercially available tool to collect aerosol particles from exhaled breath patients. Users simply cough into a handheld device, which diverts away breath exhaled from the upper respiratory tract and allows air from the lower respiratory tract to collect onto a filter.

The filter can then undergo molecular and biochemical analyses for disease detection. While previously demonstrated as a successful tool for bacterial infections, Patrucco et al. investigated its utility for viral diagnostics. Using PneumoniaCheck, samples were collected from persons with pneumonia infections, in addition to BAL. Using PCR, both sets of specimens were screened for a panel of common respiratory viruses, such as influenza, rhinovirus, and bocavirus. A high concordance was observed among the paired samples collected by BAL and PneumoniaCheck. Specificity rates were 100%, but sensitivity was 66%. Thus, while more investigation needs to be done, this work shows that this approach has high potential for noninvasive respiratory viral diagnostics.[22]

Other ways in which breath could be used as a viral detection matrix have also been explored. Etiological studies have recently demonstrated a relationship between certain viruses and the development of lung cancers. Specifically, two herpes viruses have been of great interest. The Epstein−Barr virus (EBV), a herpes virus, is a recognized carcinogen. Another herpes virus, cytomegalovirus (CMV), has been associated with maladies of the brain, lung, and colon. Early diagnosis of these viral infections could lead to lifestyle changes to reduce the associated cancer risk. Carpagnano et al. investigated breath as a sample matrix for detection of EBV and CMV. PCR was used to investigate if EBV and/or CMV DNA were present in exhaled breath condensate (EBC) from 110 patients, among which 70 had some type of lung cancer and 40 were healthy controls. These studies demonstrated that these two viruses were directly detectable in breath samples and increased in positivity among lung cancer patients, especially those in advanced stages. This technique could be a huge relief from the current gold-standard approaches, whereby viral nucleic acid is screened from bronchial brushings during fiber-optic bronchoscopies.[23]

Detection paradigms using breath have shown specificity to certain pathogens. Bacterial infections of PA, for example, are especially problematic to patients when co-infected with RSV. Purcaro et al. modeled the human airway by culturing human epithelial cells, a technique covered more in depth in Chapter 26. Cultures were inoculated into four classes: (1) PA only, (2) RSV only, (3) coinfection with PA and RSV, and (4) no infection (healthy controls). Detection of VOCs released by the cells was used to create a volatile profile of each category. Using higher statistics, the study demonstrated that a coinfection of the virus did not inhibit the ability to identify cells with the bacterial infection. In this particular case, however, cells inoculated with just RSV did not yield a sufficient change to the volatile profile for accurate diagnoses, providing evidence that each respiratory viral infection may have to be independently evaluated for its ability to be detected directly in breath samples.[24]

21.4 Fungal and other eukaryotic infections

Over 150 million people worldwide have serious fungal disease and more than 1.6 million people die from fungal infections annually, a rate similar to TB.[25]

Risk factors for fungal infections include comorbidities, such as HIV/AIDS, TB, cancer, asthma, and chronic obstructive pulmonary disease (COPD), as well as immunosuppressive treatments for chronic conditions, such as transplant rejection and rheumatoid arthritis.[25] As the incidences of these risk factors and comorbidities are rising globally, so too are the incidences of fungal infections.[25]

In the clinic, the diagnosis of a fungal infection is usually made based on fungal growth on selective media or the detection of fungal antigens or antibodies in patient specimens. While these tests can be highly sensitive, if a good-quality lung specimen is obtained, e.g., via biopsy, bronchoscopy, or sputum induction, these procedures are contraindicated in many of the patients who are at high risk for fungal pneumonias. Therefore, the detection of fungal infections via biomarkers in breath or EBC would significantly enhance the surveillance and detection of mycoses.

One of the most prevalent causes of pulmonary fungal infections, globally, is *Aspergillus* spp.,[25] which can cause invasive disease in immunosuppressed individuals. Koo and colleagues designed a study to identify putative breath biomarkers of invasive aspergillosis (IA) by taking an "in vitro−informed" approach to selecting classes of secondary metabolites to analyze in their clinical experiments.[26] The majority of cases of IA are caused by *Aspergillus fumigatus*, and therefore they focused their efforts on identifying VOCs that discriminate *A. fumigatus* from other *Aspergillus* spp., first by culturing the fungi in vitro. The study design included two important steps for the in vitro experiments to enhance the translation of results to clinical samples. First, oxygen and temperature conditions that favor hyphal growth (the dominant *Aspergillus* infection morphology) over conidia formation (the dominant environmental morphology) were established and benchmarked by comparing the in vitro transcriptome to the murine lung infection transcriptome. Second, *Aspergillus* spp. VOCs were characterized in a wide range of different media, from an all-purpose rich fungal medium (yeast extract peptone dextrose (YPD) broth) to media that are low iron, low nitrogen, alkaline stress, and *Aspergillus* minimal media. *A. fumigatus*−specific VOCs that were produced in most of these conditions were identified to enhance robustness of the approach, with several terpenes named as putative *A. fumigatus* IA biomarkers.

To validate the IA biomarkers, Koo et al. prospectively collected breath from 64 patients with suspected IA and analyzed the samples by GC-MS, focusing on the detection and identification of the terpenes observed in the in vitro studies. Blinded to the breath sample data, two clinicians reviewed the clinical data to classify the subjects as IA (having proven or probable aspergillosis, based on signs and symptoms) or non-IA (having possible IA, or another cause of invasive fungal disease), resulting in 34 IA and 30 non-IA breath samples. Several of the *Aspergillus* in vitro terpenes were observed in the breath of patients with or without IA, which were thus eliminated as possible biomarkers. Nevertheless, four terpenes and terpenoids specific to in vivo samples were identified that distinguished IA from non-IA with 94% sensitivity and 93% specificity. In one case, of the two false-positive samples, one was from a patient whose respiratory specimens were fungal culture negative and antigen negative, but upon autopsy, pulmonary nodules were recovered that

stained positive for *Aspergillus*. This not only highlights the difficulty in benchmarking new biomarkers against a clinical diagnostic with poor sensitivity but also underscores the potential impact of adding breath tests to the diagnostic armamentarium.

Fungi can also play a significant role in noninfectious respiratory disease. Fungi are implicated in more than 11 million annual cases of allergic asthma and 12 million annual cases of rhinosinusitis globally,[25] but studying the causal relationships between fungal colonization and disease severity is impeded by respiratory specimen access. Carpagnano and colleagues sought to determine whether fungi could be cultured from EBC from asthma patients, with the ultimate goal of supplanting sputum induction, which is not advised for asthmatics with moderate-to-severe disease.[27] EBC was collected from 28 atopic (i.e., allergic) asthma patients, 19 nonatopic asthma patients, and 20 controls, and these samples were paired with induced sputum, where clinically permissible. In addition, data on disease severity, anthropometric variables (body mass index, age, sex, etc.), and fraction of exhaled nitric oxide (F_ENO) were collected. Using fungal growth on selective media as a positive result, 70% of EBC from asthmatic subjects were found to be positive for fungi, whereas none of the control subjects were fungal-positive. Where a paired sputum sample was available, the same culture results were obtained from induced sputum and EBC, yielding a 100% sensitivity for the EBC test. Interestingly, EBC fungal positivity correlated with disease severity, with 100% of moderate-to-severe asthma patients culturing fungi versus 90% of mild and 63% of intermittent asthmatics, but there was no correlation to F_ENO. A weakness in this study was the lack of gold-standard specimens (induced sputum) in the moderate-to-severe cases of disease, but this again highlights the significant clinical need that EBC can fill for tracking fungal disease and colonization.

While not specific to the airway, malaria is a well-known viral infection, with hundreds of millions of worldwide cases each year. Early diagnosis is crucial to prevent death, but diagnosis is still contingent on a century-old test: visualization of the parasite in stained blood films. Berna et al. collected breath samples from subjects involved in controlled malaria studies for the purposes of developing vaccines and treatments.[28] Astonishingly, nine VOCs were not only elevated in the breath of the malaria cohort but also the concentration of these volatiles tracked with parasitemia levels measured in their blood. When subjects began their antimalarial treatments 8 days after being infected, the levels of these VOCs decreased, still correlating with the measured level of blood parasites. Thus, the interaction of the parasite with the body produced volatile compounds in human breath that could be used for malaria diagnostics. Detection of malaria infection via breath is covered in depth in the next chapter.

21.5 Summary

Over the last decade, there have been tremendous advances in breath analysis techniques applied to respiratory infection and colonization, which represents one of the

most clinically relevant applications for the field of breath diagnostics and monitoring. The studies outlined in this chapter present a paradigm of untargeted biomarker discovery, when study design is employed to uncover breath compounds that are statistically correlated with specific clinical conditions. As with all breath studies, pitfalls exist. In most human studies in this area, there have been limited subject numbers, and the breath biomarkers have not been confirmed in independent cohorts. Currently, there is no adequate understanding of how confounding factors or other unrelated clinical conditions may affect biomarker profiles. It is possible that breathborne biomarker signals may change over time and may vary with pathogenicity and microbial load. It is also possible that animal studies could be performed to inform pilot human studies, but that is not yet a common practice, and there have not yet been exhaustive comparisons of animal and human trials to cross-correlate and confirm results between the two.

There is a tremendous amount of ongoing parallel research to elucidate biomarker signals that emanate from in vitro cell culture models—in addition to the human/animal studies work (as outlined in Chapter 26). Most of this in vivo and in vitro work has been accomplished using various types of confirmatory analytical chemistry methods (e.g., mass spectrometry). While bringing novel sensors into this research area will eventually be an important step toward clinical translation, unequivocal chemical identification is critical during this untargeted exploratory phase of biomarker discovery. The field is now on the cusp of profiling candidate breath biomarkers of infection that can be pursued further. The best clinical studies to date have compared human/animal breath data paired with studies of in vitro organism cell models, especially those that examined biomarkers from the actual microbial clinical isolates involved in the clinical work.

Applications to aid modern emerging infectious disease epidemics or pandemics truly represent a holy grail of breath research—the chance to provide meaningful early, real-time, noninvasive diagnostics and distinguish trivial conditions from grave infectious pathogens. Given that so many respiratory infections result in nonspecific clinical presentations (e.g., elevated body temperature, malaise, etc.), it would be extremely valuable to differentiate patients who are critically ill with a specific circulating pathogen of interest. It would also allow clinicians to focus on truly ill patients and distinguish them from the "worried well" in western nations who may descend on hospital emergency departments during an infectious disease outbreak. In the far future, it would also allow public health officials to use potential technology to help limit or prevent disease spread and could provide information to help during quarantine endeavors in extreme situations. For epidemiologists, asymptomatic biomarker detection of infection could be very valuable while tracing contacts during an outbreak. While the breath research community works toward these goals, more research is needed before any of these scenarios is possible. Ideally, researchers would have immediately available high-fidelity in vivo cell culture and animal models to generate candidate breath biomarkers without the need for parallel human discovery efforts. This would save the most time during an infectious disease outbreak or in the unlikely event of intentional or engineered biological pathogen release. None of this is possible at present, but as in vitro cell culture models

advance and begin to increase in fidelity, it is possible this could ultimately occur. This paradigm would have been very beneficial three times since the turn of the century, during the 2002 severe acute respiratory syndrome (SARS) pandemic, the 2009 swine influenza H1N1 pandemic, and the 2019 coronavirus disease (COVID-19) pandemic currently underway.

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