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NMR-based analysis of metabolites in urine provides rapid diagnosis and etiology of pneumonia

"In this new and exciting field called metabolomics, where infections by specific microbes produce unique urinary metabolite patterns in their hosts, the promise of enhanced clinical chemistry diagnostics will allow physicians to diagnose and monitor health in a more quantitative way."

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Community-acquired pneumonia is an infection of the lower respiratory tract caused by bacteria, viruses, fungi and parasites. Globally, pneumonia is responsible for more deaths than any other infectious disease, and kills more children than AIDS, malaria and measles combined [1]. Indeed, even within the USA, pneumonia ranks as one of the leading causes of death. Unfortunately, diagnosis and identification of the causative agent for pneumonia has been difficult owing to the wide diversity of culpable pathogens coupled with inadequacies in current diagnostic methods [2–4].

Severe viral respiratory infections that have emerged in the past few years, such as the severe acute respiratory syndrome pandemic in 2002–2003 and the H1N1 pandemic in 2009, have focused attention on the need for rapid, accurate and cost-effective diagnostic tests to rapidly establish the causative pathogen(s) to enable better control of the spread of disease. In addition, viral infections can be superinfected by, or synergize with, bacterial infections, rendering diagnosis and management more complex. More rapid, accurate and comprehensive diagnosis would allow clinicians to focus on appropriate intervention(s), leading to a better clinical outcome for patients with reduced consumption of limited resources.

Current diagnostics

Pneumonia has generally been defined as having symptoms of an acute lower respiratory tract illness (cough, chest pain and difficulty breathing), which includes new focal chest signs on examination, at least one systemic feature (e.g., sweating, fever, aches and pains, and/or a temperature of 38°C or more) and no other explanation of the illness.

Diagnosis of pneumonia is primarily based on the skill of the attending physician, where a patient's age, medical history, physical

examination and corroboratory laboratory investigations are done, and the physician makes an initial judgment as to the diagnosis. Patients are often administered broad-based antibiotics and their response is monitored. Blood or sputum cultures can take several days before they give a definitive result and are often negative. Urinary antigen tests are already used for the identification of certain bacterial and fungal pathogens. Use of PCR testing for viral causes is expanding rapidly, but does not exclude bacterial superinfection. PCR testing for bacterial pathogens is limited by the regular detection of organisms colonizing the upper airway. Thus, as with many diseases, diagnosis of pneumonia by a physician is somewhat subjective, with the physician informally using a combination of qualitative and quantitative variables to statistically diagnose a patient. Ideally, if there were one test that could screen for a number of conditions and provide additional, statistically processed information, diagnosis and the correct treatment could be started earlier and potentially save a greater number of lives. Moreover, treatment of pneumonia could be more specific, thus minimizing adverse effects to the patient and the consumption of excess healthcare resources.

Metabolomics

Metabolomics is a field of study that attempts to identify all small-molecule chemicals and metabolites within a cell, organ, organism or panorganism, and applies chemometrics to identify key, but potentially subtle, chemical changes that may be indicative of a disease state, such as an infection or a change in response to external stimuli. It is considered a relatively new field of research, with recent emphasis on disease diagnosis and personalized medicine [5].

The fundamental idea behind metabolomics is that disease can be reflected by changes in metabolite concentrations in biological fluids and

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tissues. Unsurprisingly, this concept is not new. In ancient times, qualitative measures of color, taste and smell of urine were used to aid physicians in diagnosis. Just over 100 years ago, the development of analytical tools to measure small-molecule metabolites, as well as other substances, were brought about as tools to assist medical doctors when diagnosing disease. This branch of medical science was termed clinical chemistry, and has been invaluable to modern medicine. However, most clinical chemistry tests available today rely on old technologies that only measure a single chemical in blood, urine or other biofluid, and these tests are generally neither sensitive nor specific for any one particular disease. Comprehensive chemical analyses of urine or blood have simply never been done in a clinical setting before.

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The key difference between metabolomics and classical clinical chemistry lies in the significant advances in computational technologies coupled with advances in analytical instrumentation such as NMR spectroscopy and mass spectrometry. More chemicals than ever can be easily detected in samples of urine or blood, allowing researchers to detect subtle changes in many metabolites or metabolite families that provide clues as to the nature of disease and its effect on the overall body. These studies will allow more diagnostic and prognostic information to be obtained than has ever been realized before, and will potentially allow the development of more effective therapies for many diseases. It is these advances that will allow the fields of clinical chemistry and medicine to dramatically leap forward.

Metabolomics as a diagnostic tool for pneumonia

The use of NMR metabolomics in differentiating microbes of different genera and species is a modern extension of classical biochemical microbiology. Unsurprisingly, metabolomics is able to differentiate species of *Staphylococcus*, *Enterococcus* and *Streptococcus*, when these bacteria were grown on plates [6]. Different *Candida* species can also be differentiated by metabolomics [7,8]. However, of greater significance, changes in urinary metabolite patterns occur with urinary tract or parasitic gut infections [9–12]. In a well-controlled mouse

model of lung infection, *Streptococcus pneumoniae* and *Staphylococcus aureus* were demonstrated to produce different urinary metabolite patterns, which could be correlated with the genome of the pathogens as well as their effect on the host [13].

Perhaps the most encouraging study to date, illustrating the potential of metabolomics in a clinical setting, was the comparison of pneumonia caused by *S. pneumoniae* with a number of other health conditions and lung infections [14]. In this study, it was demonstrated that pneumonia caused by *S. pneumoniae* is distinct from lung conditions, such as asthma or chronic obstructive pulmonary disease, as well as pneumonia caused by other bacterial infections (e.g., *Mycobacterium tuberculosis*, *Legionella pneumophila*, *S. aureus*, *Coxiella burnetii*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *Escherichia coli*, *Enterococcus faecalis*, *Moraxella catarrhalis*, *Streptococcus viridans* and *Streptococcus anginosus*) or viral infections (e.g., influenza A, picornavirus, respiratory syncytial virus, parainfluenza virus, coronavirus, human metapneumovirus and hantavirus). Furthermore, recovery from pneumonia could be observed through the measurement of multiple longitudinal samples. This result is important as it would allow clinicians to have a better and earlier indication that a particular therapy is working. These results will have profound consequences for the pharmaceutical industry, by presenting novel biomarkers to more efficiently identify eligible patients, objectively monitor the efficacy of therapies and to specifically detect side effects.

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Thus, metabolomics has the capacity to provide a diagnostic tool for the rapid determination of the cause of community-acquired pneumonia from a single urine sample. The use of NMR for analytical clinical metabolomics is relatively inexpensive (after the capital cost of the equipment, costing only a few dollars per sample to run), and could easily be set up and used in hospitals and clinical laboratories. However, for use where portability is required, advancements in magnetic resonance equipment and computing technology will be required. Miniaturization of NMR is a new area of study in a number of academic and industrial settings [15].

Where, then, do we stand on the generation of a test that could potentially be used worldwide as an etiological diagnostic tool for pneumonia? In

this new and exciting field called metabolomics, where infections by specific microbes produce unique urinary metabolite patterns in their hosts, the promise of enhanced clinical chemistry diagnostics will allow physicians to diagnose and monitor health in a more quantitative way. This could lead to changes in the way we approach medical intervention of infectious diseases, allowing for earlier and more robust identification of pathogens, and potentially allowing for greater control over the spread of disease. These studies will also provide an understanding of the interaction between the microbe and the host, which may lead to the development of better treatment strategies by helping to pinpoint molecular targets for a new generation of antimicrobials.

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CM Slupsky currently holds equity interest in Metabolistics Inc., (www.metabolistics.com) a biotechnology company in the metabolomics domain, has a patent pending on urinary biomarkers for the determination of pneumococcal pneumonia, and holds an IP interest in this field. The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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