

Meeting Report:

Non Invasive Biomedical Analysis - Breath Networking Session at PittCon 2011, Atlanta, Georgia

Joachim D. Pleil
National Exposure Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency

Background

This was the second year that our breath colleagues organized a networking session at the Pittsburgh Conference and Exposition, or "PittCon" (<http://www.pittcon.org/>). This time it was called "Non-invasive Biomedical Analysis" to broaden the scope a bit, but the primary focus remained on exhaled breath diagnostics. As reported last year in the Journal of Breath Research, PittCon continues to be one of the largest international conferences for analytical chemistry and instrumentation, typically attracting about 25,000 attendees and 1,000 commercial exhibitors (Pleil 2010). This year the conference was held in Georgia, USA at the Georgia World Congress Center in the city of Atlanta.

Breath Networking Session at PittCon 2011

For the 2011 meeting, our networking session was organized and facilitated by Dr. Wolfram Miekisch, Ph.D. (wolfram.miekisch@uni-rostock.de) from the Rostock University Hospital in Rostock Germany. Dr. Miekisch develops analytical methods for measuring biochemicals in exhaled breath from patients to develop non-invasive diagnostic tests for critical care medicine.

To start the meeting off, Dr. Miekisch briefly introduced the session and explained that the focus this year would be on data interpretation; that is, how can we assure ourselves that the measurements we make and the biomarker patterns we observe are actually valuable and probative for their intended purpose. Dr. Joachim Pleil (pleil.joachim@epa.gov) from the U.S. Environmental Protection Agency then briefly took the floor and introduced the affiliations of the session with the International Association of Breath Research (IABR) and the Institute of Physics - Journal of Breath Research (JBR). He also reminded the attendees about the book "***Breath Analysis for Diagnosis and Therapeutic Monitoring***" from World Scientific Publishing Co., Singapore (2005) edited by Prof. Anton Amann (Medical University of Innsbruck, Austria) and Prof. David Smith (Keele University, Stoke on Trent, UK). This book has served as a central resource for breath researchers for the past 6 years; Dr. Pleil mentioned

that a new book encompassing advances in breath research is currently in preparation with an anticipated publication date in 2012.

Dr. Miekisch then continued at the podium and presented his thoughts on the increasing complexity and sensitivity of breath instrumentation, especially in the multidimensional GC x GC – ToF-MS (2-dimensional gas chromatography – time of flight mass spectrometry) applications where a single sample can yield thousands of analyte responses. He observed that in all of the newer methods, there is an increasing data density that requires more sophisticated interpretation tools. He then introduced the other presenters and how their respective topics would fit into this overall theme of data interpretation and ultimate use. As in last year's meeting, the intended character of the session was informal; attendees were encouraged to ask questions of the presenters and enter into discussions.

The first invited speaker was Dr. Jens Herbig, Ph.D. (jens.herbig@ionimed.com) from Ionimed Analytik, Innsbruck, Austria who presented his thoughts regarding the concept of “Voodoo Correlations” in complex data structures. He initially described his company's instrumentation for breath analysis, the PTR-MS (proton transfer reaction – mass spectrometer) that is capable of rapidly providing a great amount of real-time breath data. The “technological hazard” presented by such new fast, sensitive, and specific analytical instruments is that in capturing the complexity of the true breath spectrum, they also create a high data density. This leads to an inevitable problem; the number of measured variables quickly overwhelms the number of samples that can feasibly be taken and distinct patients that can be recruited. As such, the chances of finding coincidental (or voodoo) correlations grows proportionally especially when sophisticated data-mining tools are used that combine complex sets of markers to separate even the weakest features. He discussed how we, as a community, should develop rigorous mathematical tests for screening true effects from random correlations. He suggested some approaches wherein one replaces small sets of real data with random numbers to test whether correlations can still be found indicating that the original correlations might also be coincidental. This is the equivalent of measuring the “statistical background noise”. Dr. Herbig concluded with three suggestions: 1) reduce the number of variables (remove known biomarkers for environmental exposures and normal metabolism), 2) challenge the diagnostic procedures with random data, and 3) practice validation with independent data sets (provide corroboration of proposed correlations).

This topic engendered a lengthy discussion among the participants regarding data interpretation. The subject of lung cancer diagnosis was at the center of the concern; it was brought up that there are over 300 journal articles that have touched upon the use of breath-borne biomarkers to diagnosis pre-symptomatic lung cancers and that they all seem to use different discriminator mechanisms. As in the previous year's discussions, the consensus of the group was that the underlying biochemistry and the metabolic pathways should be known before groups of compounds are invoked to assign cancer status. Furthermore, meta-data effects such as environmental and medical history, and host factor data such as gender, age, weight, age, etc. should all be considered to assess whether or not an individual displays an unremarkable (control) biomarker profile.

The next speaker was Prof. Jochen Schubert, M.D. (jochen.schubert@med.uni-rostock.de) from the University of Rostock (Germany) Hospital where he serves as the director of critical care medicine. Prof. Schubert discussed the topic “From research to diagnostics” wherein he emphasized that diagnostic medicine has an imperative responsibility to be correct because patients' lives are at risk. He used the example of the measurement of blood dextrose level

where an error could result in the incorrect administration of sugar (measurement too low) or insulin (measurement too high) where either mistake could harm a diabetic patient. He went on to discuss "CSM" which he defined as common statistical mistakes comprised of: 1) insufficient number of patients, 2) non-blinded and non-randomized trials, 3) biased study populations with unrealistically high disease prevalence, and 4) too many random measurement variables. All of these CSMs could result in erroneous interpretations of data with serious repercussions when applied to critically ill people. He closed with the thought provoking comment to the assembled researchers: *"Basic research is indispensable for medical progress, but always remember: YOU may be the one whose life is depending on the test you proposed for clinical application!"*

The subsequent discussions were animated. The general consensus of the group was in agreement with Prof. Schubert regarding the importance of "being correct". We discussed that one of the biggest issues facing exhaled breath research in diagnostic medicine is the proliferation of pattern recognition schemes for identifying pre-symptom and pre-clinical cancers (prostate, lung, breast, etc.). We reiterated the primary concern for these tests as to specificity and sensitivity; false positives may be almost as damaging to a patient as a false negative result. In fact, the consensus from the group was that *"...the more vital the (medical) problem, the more reliable the test results must be."*

Dr. Joachim Pleil, Ph.D. then presented his thoughts entitled "Dealing with analytical results from breath analysis: what is real, what is background, and what is random?". He first posed a series of questions that breath analyses and different categories of compounds could potentially answer for three client communities: Environmental (what have you been exposed to? What is your absorbed dose?), Medical (Do you have cancer? Are you getting better?), and Intelligence (Where have you been? Are you infected or stressed? Are you a threat?). He then demonstrated some new breath data visualization techniques based on heat mapping wherein the researchers can quickly ascertain patterns, data outliers, and develop new hypotheses. He also cautioned the attendees regarding factors that could affect the quality of breath data such as subject breathing technique, previous and co-exposures to compounds of interest in breath, and random effects from over-modeling. He concluded that the heat map approach could provide a fast qualitative overview of complex data and help discern data patterns, but that rigorous mathematical and statistical evaluations are always required to draw sound conclusions.

The ensuing discussions of the attendees revolved around clinical and environmental experimental designs such as case-control and cross-sectional experiments and how to properly interpret correlations from (unrealistically) balanced data groups (e.g. 50% affected/exposed vs. 50% control patients) to assess "wild - type" subject cohorts where the disease prevalence is random and rare or environmental exposure is intermittent. Another important factor discussed was the typical availability of a small "n" of patients, and the actual need for large numbers of patients to achieve valid statistical results especially in context of complex observed data structures. Furthermore, we have to realize that isolated cases of discriminating observations may merely be a random effect and that sometimes we can make a bad decision based on random patterns. A case brought to the attention of the group was a recent assertion in the breath community wherein exhaled acetone was suggested as an indicator of cancer disease status that was summarily dismissed by some of the researchers as a voodoo correlation. Another example discussed was recent work wherein dogs were trained to "sniff out" cancer patterns in patients, and how some of the data visualization techniques might be applied to simulate such a process with analytical measurements. Finally, the attendees discussed how interference from

environmental factors, including putatively natural products, could affect patients similarly to prescribed drug regimens. The consensus was that we need to do as much as possible to collect meta-data and make ancillary measurements that can help identify real patterns in breath biomarkers and thus avoid interpreting random effects as diagnostic.

Concluding remarks

At the conclusion of the session, Dr. Mickisch summed up the articulated concepts and concluded that the natural progression of breath analysis should be: Laboratory research to clinical transition to data interpretation. He emphasized that the third tier, data interpretation, is critical and that every effort should be made to implement data cross-testing and statistical validation along this progression. He also expressed his opinion that the use of qualitative data visualization techniques such as heat mapping and three-dimensional graphics are an important part of assessing the eventual efficacy of diagnostic breath measurements. The group concluded that we should continue our efforts with these networking sessions and that in the coming years we should strive to add an organized technical session focused on breath that could serve as a lead-in. The consensus of the group was that PittCon is an excellent venue for disseminating breath analysis research in that it is a very eclectic conference and that there is great access to analytical instrumentation expertise. We, the participants in this networking session, plan to continue our association with PittCon in the future and encourage others in the breath community to take advantage of the breadth of analytical chemistry science represented there.

Reference:

Pleil JD, 2010. "Meeting Report: Breath Biomarkers Networking Sessions at PittCon 2010, Orlando Florida", Journal of Breath Research, 4:029001 (5pp).

The United States Environmental Protection Agency through its Office of Research and Development has subjected this article to Agency administrative review and approved it for publication.