EDITORIAL

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Driving progress in exhaled breath biomarkers: Breath Biopsy Conference 2019

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Abstract

November 2019 saw Cambridge, UK play host to the second Breath Biopsy Conference, a community-focused event aimed at sharing and supporting advancements in the collection and analysis of volatile organic compounds in exhaled breath. The event expanded upon the previous year's format, spanning two days and concluding with an expert panel discussion. Presentations covered detection, monitoring and precision medicine studies examining diseases including asthma, cirrhosis, cancer and tuberculosis. The meeting attracted representatives from diverse backgrounds, such as metabolomics, artificial intelligence, clinical research and chemical analysis. This meeting report offers an overview of what was presented and discussed during the conference.

1. Introduction

The first Breath Biopsy Conference was organized in November 2018 by Owlstone Medical (Cambridge, UK) as a user community platform to exchange ideas and progress in the field of breath biomarker research. The success of the inaugural one-day event led to the organization of a second conference the following year—the subject of this meeting report—that featured an expanded length and format; it is planned to make this an annual feature in the breath research networking calendar. Overall, the Breath Biopsy Conferences seek to bring together expertise from across the breath research space to explore the latest advances, share challenges, and generate new ideas and multi-disciplinary collaborations to accelerate progress towards a greater variety of clinically-applied, non-invasive breath tests for the early detection, monitoring, and precision treatment of various medical conditions. Now in its second year, the conference attracted academic and biopharma researchers from across Europe, America, Asia and Australia. This editorial aims to offer an overview of the topics and themes of the conference.

The 2019 Breath Biopsy Conference was attended by close to 150 delegates, including some of the leading names in breath research and related fields. The gathering aimed to stimulate new ideas and raise awareness of breath research by attracting specialists from a wide range of related fields to give their input on the latest research, progress and future directions. The conference was held in central Cambridge, UK and spanned two full days. The programme included three sessions examining applications of breath testing, approaches to breathomics, and the growth of targeted breath testing methods. Keynote presentations were given by Professor George Hanna from Imperial College London, UK, Professor Jane Hill from the Thayer School of Engineering at Dartmouth in Hanover, NH, USA (now at University of British Columbia, Vancouver, BC, Canada), and Prof. Jessica Lasky-Su from Brigham and Women's Hospital in Boston, MA, USA. The programme concluded with an expert discussion session chaired by Owlstone Medical CEO, Billy Boyle, which made predictions and looked at key challenges in the field over the next five years.

The emergent theme from the conference was the strong desire for greater collaboration leading to large-scale biomarker identification and validation studies to demonstrate the clinical utility of high quality and broadly applicable breath biomarkers in a robust and reproducible manner. Many speakers picked up on this theme, pointing to the abundance of single-centre and pilot studies that have reported prospective biomarkers for diverse conditions

and disease states. Many of the sessions from the conference were recorded with permission by the speakers to make these available to the community. These recordings, as well as digital versions of posters, are available to view online. [3]

Several speakers shared disparate views on the need for more work to identify the biological origins of breath biomarkers. Some hold the view that a strong statistical relationship between given biomarker patterns and a specific condition is sufficient for these patterns to become the basis for a clinical test. Meanwhile, others voiced the opinion that more resources need to be invested in identifying the metabolic origins and biochemical pathways of individual biomarkers in breath in order to ensure full confidence in their biological relevance, and that only then can breath research achieve full acceptance in clinical practice. This discussion is of particular relevance in relation to the use of sensor-based technologies, often used in breath analysis, which deliver sensor-response patterns rather than detect individual compounds.

2. Keynotes

The keynote presentation by Jane Hill focused on ongoing research into breath tests for the detection of infectious diseases [1, 2], particularly tuberculosis (TB) [3–5]. Prof. Hill asserted that most diagnostic procedures have improved little since the time of Louis Pasteur and highlighted the need for tests that give faster results, particularly for multi-drug resistant infections. And, she emphasized, there are currently no adequate tests for many paediatric respiratory infections, including TB. Her research includes a focus in South Africa where TB is rampant and a point-of-care diagnostic, such as that potentially achievable via breath analysis, could be a game-changer. In addition, Prof. Hill has been investigating how to collect reliable breath samples from macaques, an important clinical model for testing new anti-TB drugs and vaccines. In the macaque model, she showed promising results suggesting that breath testing could detect the early stages of TB in advance of existing diagnostic methods, as well as prospectively predicting infection severity. TB breath analysis in humans is ongoing, with her team and others feeling cautiously optimistic about the use of breath biomarkers in clinics around the world. The session helped to demonstrate the complementary value of animal studies in the breath field and illustrated the clear need and great potential that breath research has in helping to control the spread of infectious diseases.

The second keynote was given by Jessica Lasky-Su, who looked at the role of metabolomics and breathomics in the multi-omics era. Prof. Lasky-Su examined the effect that omics research has had on our overall perspective on biological systems and presented work from large-scale multi-omics trials, emphasising the potential that could be achieved through greater collaborative thinking in the breath field. In particular, she outlined the complementary benefits of studying systems through multiple parallel omics approaches with large sample sets and rigorous validation to demonstrate robust results. Prof. Lasky-Su highlighted the outcomes of several large-scale studies on childhood asthma, including VDAART [6], COPSAC [7], COMETS [8] and EPIC-Norfolk [9]. Her work has helped to show that maternal vitamin D intake during pregnancy can reduce asthma risk, but that this relationship is disrupted by an ORMDL3 mutant allele [10]. Other results show that the caffeine-derivative theophylline—formerly a widely used anti-asthma drug—in maternal blood can help to prevent asthma. She also showed findings based on samples from young children suggesting that diet may contribute to asthma risk [11]. In work like this, Prof. Lasky-Su highlighted the inherent challenges in comparing data for meta-analysis and referenced the COMETS analytics tools that have been developed to aid comparison of non-standardized datasets. She concluded her talk by discussing the need for, and benefits of, standardization in study design and the advantages of breath testing in enabling easy-to-use and accessible sample collection that could be applied for health monitoring.

As an experienced voice in breath research, George Hanna used his keynote talk to advocate for adaptive trial design and external validation of results as the keys to progress in the field. Prof. Hanna discussed the promising results from existing breath research trials, which have consistently produced models with high sensitivity and selectivity metrics. He went on to highlight the importance of careful trial design and rigorous quality control in achieving reliable results in the field [12]. In particular, Prof. Hanna discussed the impact of oral hygiene routines, the diversity of breath collection methods in existing studies, and the need for appropriate standards in analysing breath samples [13]. He emphasized the importance of using relevant target populations and including suitable controls—not just healthy individuals, but those affected by clinically related conditions—as a means to identify biomarkers with real clinical utility. Finally, Prof. Hanna explored the need for validation to be more widely included in breath studies, in particular calling for greater independent, external validation of results, collecting samples from multiple centres, and analysing samples in different laboratories.

3. Breath Biopsy Applications

The opening session, *Breath Biopsy Applications*, set the scene for the conference by examining potential

[3] https://support.owlstonenanotech.com/hc/en-us/community/topics/36000097157-2019-Breath-Biopsy-Conference-Presentations.

applications for breath testing, with speakers Christopher Mayhew, Olaf Holz, Maxim Wilkinson, and Kayleigh Arthur. As well as exploring the diversity of applications, this session highlighted the varied collection and analysis approaches currently used in the field. The session included work on asthma, inflammation, liver disease, diabetes and lung cancer. Non-medical applications, such as finding trapped people in disaster zones, and detecting people smuggling in cargo, were also mentioned. A key message from the session was the importance of specificity in breath biomarkers as many compounds can indicate different conditions depending on context. This was illustrated by showing how, using some predictive models, an individual can seem to transition from healthy to having lung cancer simply by changing their breathing behaviour.

The presentations provided a broad overview of breath test applications, including breath studies on muscle activity, lung cancer and type 1 diabetes. Christopher Mayhew shared his work on liver disease—a prime target for breath research due to its metabolic significance. Bad breath (*fetor hepaticus*) has been linked with advanced liver disease for centuries [14] and Prof. Mayhew's work has helped to show that liver disease leads to elevated limonene in exhaled breath as the ailing liver fails to process dietary limonene [15, 16]. Using exogenous compounds as breath biomarkers was explored further in a later session by Isabel Orf, as is discussed below.

Finding biomarkers of inflammation is another key application of breath research. Olaf Holz's research involves introducing irritants into the lungs of healthy volunteers to measure inflammation responses using breath samples, MRI and collection of exhaled particles. His presentation emphasised the importance of understanding the biology underlying breath biomarkers. Dr Holz discussed the impact of ambient chemicals on breath sampling and how environment specific compounds, like hospital cleaning products, can influence results—a common issue when comparing healthy and hospitalized individuals. Dr Holz reported several aldehydes as interesting compounds that could be the product of oxidative stress linked to lung inflammation.

One challenge in breath collection is the humidity of exhaled air, which can interfere with sample analysis. Samples collected directly into sorbent tubes are typically dry purged to remove water before analysis. Maxim Wilkinson focused his presentation on the effects of humidity on different sorbent tubes in the context of studying asthma [17]. Dr Wilkinson's results show that three common tube types (Tenax, 1TD and 5TD) are affected similarly by humidity, which causes particular challenges in recovering large hydrophobic molecules. Notably, 5TD tubes require a longer purge than other tubes. The presentation by Dr Kayleigh Arthur also explored technical aspects of breath research by sharing recent work integrating Orbitrap™ mass spectrometry capabilities into the Breath Biopsy platform to offer enhanced breath biomarker discovery and analysis capabilities.

4. Targeted breath research

Targeted methods for breath analysis, such as exogenous VOC probes (EVOC® Probes) and stable isotope tests, are attracting growing interest. They operate on the basis that it is easier to optimize analysis techniques to examine preselected biomarkers than it is to discover new ones. Additionally, introducing target compounds into a subject's body ensures a stronger signal for detection. The efficacy of this approach is reflected in the several targeted breath tests that have achieved clinical integration [18]. Existing tests largely make use of isotope labelled (typically ^{13}C) compounds, such as ^{13}C-methacetin use for liver function monitoring [19, 20].

There are several shared challenges encountered by both labelled isotope and VOC breath tests—specificity, selectivity and clinical relevance. Douglas Morrison introduced these as well as the specific challenges of working with isotopes, including cost and access to labelled compounds. Using isotope labelled peas, Dr Morrison has investigated digestion in the gut, demonstrating the complex breakdown profile of isotope labelled compounds and the effect of changing the structure of starch on human health. Similar approaches are also useful in studying the microbiome and exploring nutritional health [21, 22]. He also discussed how technological advances are broadening applications enabling richer datasets and more point-of-care use.

Isabel Orf delivered a presentation on EVOC® Probes, which have been developed recently as part of the Breath Biopsy platform [23]. EVOC® Probes are compounds that are classified as generally recognized as safe and so are easy to implement for widespread use. These compounds and/or their metabolic products can be detected and monitored in exhaled breath. EVOC® Probes develop on the work of Prof. Mayhew studying limonene in breath as a measure of liver function. Current understanding is that limonene is not produced by the body and its presence in exhaled breath originates mainly from diet. The presentation by Dr Orf summarized new results obtained using Breath Biopsy to detect increased levels of limonene as a biomarker for advanced liver diseases (cirrhosis and hepatocellular carcinoma). In her presentation, Dr Orf proposed that a prescribed dose of limonene could be administered as an EVOC® Probe in advance of breath collection, which would provide a standardized VOC signal that can be collected in breath samples and measured over time as a means to assess liver function. This approach is expected to enhance the consistency and sensitivity of assessing liver function through breath testing.

Anil Modak has long been a proponent of targeted breath analysis, and he closed the session by speaking about advances in the field, highlighting the diversity of ongoing trials and the tests approved for clinical use. While these successes are great, he noted that tests based on isotope labelling still face challenges in gaining regulatory approval. Dr Modak discussed the application of targeted breath analysis for pharmacokinetics, including measuring enzymatic rates in patient metabolism. Knowing how a patient will respond to a treatment is one of the biggest challenges in medicine and estimates suggest as many as 75% of treatments are ineffective. Using breath tests to identify suitable treatments could save both money and lives in many areas.

5. Breathomics and Breath Biopsy

The final session explored deeper aspects of breath research, particularly in the context of breath analysis to phenotype asthma patients. Joao Rufo and Renaud Louis both spoke on the use of breath tests to predict treatment responses in asthma, while Jose Torrecilla discussed the role of artificial intelligence in developing models for diagnostic tests.

In the opening of his talk, Joao Rufo shared his journey into the breath field, and how he came to identify breath as an optimal medium for biomarker collection due to its non-invasive nature and its potential for point-of-care applications. He alluded to well-known examples of scent being used to detect biological changes, including Oscar the Cat [24] and Joy Milne [25, 26], respectively noted for detecting death and Parkinson's disease. Dr Rufo works with electronic noses (enoses) [27] and believes that breath analysis can have medical use without knowing the biochemical specifics of the compounds being detected. His work has produced a model for screening asthma patients for response to inhaled corticosteroids.

Renaud Louis discussed various proxy methods that have been used to phenotype asthma patients. Sputum eosinophils continue to be the best predictor for treatment response [28, 29], despite efforts with blood eosinophils [30] and fraction of exhaled nitric oxide (F_ENO) [31]. One particular challenge in the area, he noted, is that medical systems do not use standardized diagnostic criteria for asthma exacerbations, which makes them a limited point of reference against which to judge new tests. Prof. Louis also shared results from a large breath research study [32], which goes some way to demonstrate what is needed for successful trial design and validation in breath research. This study generated a predictive model based on over 270 asthma patients and went on to validate it in an independent validation cohort.

Jose Torrecilla closed the session by arguing that quality data is the key to effective pattern recognition in diagnostic tests. Multiparametric data, he said, is needed for reliable application of artificial intelligence in the clinical setting—doctors use all of their senses to detect illnesses; we should expect the same from computers. Dr. Torrecilla asserted that there are many applications for artificial intelligence in breath research [33, 34] and medicine but concluded that more work is needed and this needs to be driven by collaboration between doctors, scientists and artificial intelligence experts.

6. Poster presentations

The Breath Biopsy Conference attracted a small but wide-ranging collection of research posters. Several posters focused on cancer, including breath applications in lung cancer, colorectal cancer, and mesothelioma. Applications also included mental health and biomonitoring for environmental exposures. Other posters reported the development of breath analysis technologies or investigated the detection of specific VOCs from breath. The posters also reflected themes from the wider conference, including comments on study design and validation practices. Posters presented at the conference are available to download from the link in the footnote above.

7. Panel discussion

The conference closed with an expert panel discussion reviewing conference themes, exploring the challenges and opportunities for the next five years of breath research, and discussing ad hoc points from conference delegates. The session was chaired by Billy Boyle and the panel experts were Paul Thomas (Loughborough University, UK), Jonathan Beauchamp (Fraunhofer IVV, Germany), Frederik-Jan van Schooten (Maastricht University, Netherlands), Marc van der Schee (Owlstone Medical), as well as conference speakers Renaud Louis (University of Liège, Belgium) and Jose Torrecilla (Complutense University of Madrid, Spain).

The panel opened by making predictions for the years ahead, highlighting the need for a 'healthy breath profile' to understand natural variation in breath and the effects of environmental factors. Predictions also included understanding the biological origins of VOCs in breath, realizing selected clinical applications, and making advances in analysis technologies to enable point-of-care applications. The panel also expressed the desire to see progress on the kind of long-term, large-scale collaborative studies that will advance the field.

Targeted breath analysis was identified as an upcoming innovation, with EVOC® Probes offering a quicker route to identifying usable biomarkers, which may be of utility in benchmarking studies towards standardization [35]. In recent years, EVOCs have shifted from being viewed as contamination to emerging as potentially useful probes. Greater use of

existing metabolomics data also represents an opportunity to inform targeted 'bottom-up' investigations, particularly in relation to cancer where metabolism has been studied in depth.

While exploring the opportunities for breath research in different contexts, the panel compared the detection of liver diseases to the identification of lung and other cancers. Notably, the scale of the targets differs between a small early-stage tumour and the whole liver. The larger the source for a particular biomarker, the stronger the signal for detection. In cases such as cancer, this suggests there could be benefits to examining pre-cancerous changes that develop over time.

A lively debate over whether biomarker identities are important or not followed. Existing detection tests outside of breath research largely detect known target molecules, and this knowledge is a key part of demonstrating the safety and utility of the test when seeking regulatory approval. Yet, it was challenged, if a model consistently works in validated trials, is it necessary to know what is being detected? After all, the sense of smell works on pattern recognition and does not require chemical knowledge of the individual compounds involved. Further, the audience put forward the view that disease often includes many small deviations in homeostasis, which become more obvious through pattern recognition rather than the individual study of the contributing components. Overall, the debate of known markers versus patterns was a major point of discussion for the audience and amongst the panel, and is clearly an unresolved question for the field.

The audience also raised the persistent issue of (mis)use of the term 'biomarker' in publications, with the assertion that the term should only refer to validated indicators of a specific biological state. The literature contains many references to compounds that are reported as biomarkers but that lack sufficient validation. The need for studies to include positive controls from related conditions was also raised as a means to ensure that proposed biomarkers are specific, rather than reflecting wider biological responses.

The panel discussed that existing medical diagnoses can be relatively simplistic, and emerging technologies like breath research can be exploited to perform more biologically relevant investigations that may lead to precise medical interventions. The field was described as being in the process of coalescing, and that this is something that should be embraced to ensure breath research achieves its potential.

8. Summary

The breath community continues to grow in size and experience, having now produced a sizable body of evidence to support the value of breath as a sampling medium and source of metabolic biomarkers. While these initial studies have shown great promise, the field now needs to develop maturity, pulling together around larger, shared goals that can robustly demonstrate what breath analysis can achieve when applied in the clinical setting on a global scale.

Broad agreement on sample collection and data analysis standards that can be applied in a range of contexts is expected to facilitate the creation of meaningful datasets that can be shared and analysed by researchers in the field. Large adaptive trials should be favoured over smaller, predetermined pilot studies and care must be taken in study design to ensure that trial populations and controls produce the most meaningful and relevant results [36]. Targeted breath analysis is also emerging as a promising area within the field and more work is needed to explore the full potential here.

Overall, the Breath Biopsy Conference facilitated an open and progressive discussion of delegates keen for the community to come together and build upon past successes. By learning from each other, by agreeing to more extensive co-operation across the field, and by following the examples set by more established fields, breath research can make rapid progress towards its goals of changing the way of detecting, monitoring and treating some of the world's most prominent and prevalent health conditions. The next Breath Biopsy Conference will be held from 10-11 November 2020, this time as an online event. [4]

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