Exhaled breath analysis

IMPACT PARAGRAPH

The present thesis analysed exhaled breath volatile organic compounds (VOCs) and their implementation in clinical settings regarding the diagnosis and prognosis of gastrointestinal diseases. Liver diseases, such as primary sclerosing cholangitis (PSC), are life threatening since no proper early diagnostic tools exist. Lack of early diagnostic tools leads to late treatment, which often results in a liver transplantation. In 2017, an estimated of 1,5 billion cases of chronic liver diseased individuals were reported worldwide [1]. Cirrhosis, the end-stage of liver impairment, accounted for approximately 1,32 million deaths in 2017. In the United States alone, estimated healthcare expenditures regarding hospitalizations reach $81,1 billions [2]. Moreover, the global liver disease treatment market size was valued at $20,673.70 millions in 2020, and it is estimated to reach $36,455.70 millions by 2030, growing at a compound annual groth of 5,7% from 2021 to 2030 [3]. PSC is a rare liver condition with unclear etiopathogenesis; it affects roughly 70.000 individuals in the western world. Nevertheless, it still remains the fifth most common indication for liver transplantation in the United States, and it remains a leading indication in several other countries as well [4]. Liver diseases are currently diagnosed through liver biopsy. Its invasiveness, costs, and relatively low diagnostic accuracy require new techniques to be sought. Colon diseases, such as inflammatory bowel disease (IBD), have dramatically increased over the years. In 2018, there were more than 36,8 million ambulatory visits for gastrointestinal symptoms and 43,4 million ambulatory visits with a primary gastrointestinal diagnosis in the United States [5]. IBD alone affects as many as 1,6 million Americans; 70.000 new cases are reported each year. In 2018, gastrointestinal disease healthcare expenditure totalled $119,6 billions; the estimated financial burden of IBD in the United States is more than $31 billions [6-8]. Colon diseases are presently diagnosed through colonoscopy, which has been the gold standard for diagnosing and monitoring disease activity. Alternative ways to diagnose and monitor disease activity are needed since colonoscopy is a considerably invasive and costly technique.

In human research, VOCs arise from different body matrices such as breath, faeces, urine, bile, breast milk, and blood. Based on research conducted in the present thesis, VOC analysis might greatly benefit gastrointestinal disease diagnosis and prognosis due to its promising use as a non-invasive, cost-effective, and easy-to-use diagnostic and monitoring tool. Exhaled breath VOC technology aims at replacing the current costly and invasive diagnostics with a noninvasive approach, using powerful algorithms, which can identify VOCs for accurate monitoring and diagnosis. Achieving this would reduce diagnosis and monitoring costs since exhaled breath analysis is cost-effective. At the same time, it would drastically improve patient treatment because it is patient-friendly due to its non-invasiveness. Moreover, it would also be convenient for clinicians since it can be applied directly at the point of care due to its potential portability. As discussed in the present thesis, bringing exhaled breath analysis into daily clinical settings would highly benefit research and treatment of gastrointestinal diseases since they require highly invasive, expensive, and often not very accurate

tools (e.g. biopsy or colonoscopy). Exhaled breath clinical implementation will have an immense impact on health insurance companies and hospitals because it will substantially decrease healthcare costs. Early diagnosis and proper monitoring will reduce, for example, the need for liver transplantations or the need for costly endoscopic equipment. In both cases, clinicians could also save up time to devote it into performing other medical care duties that might be lagging behind. Furthermore, it is believed that exhaled breath clinical implementation will allow patients to control the disease more efficiently; they will have to go to the hospital less often, which will save them energy and time. In return, this will make them more productive and more active members of the society; it will allow them to improve socially and financially, and make life more enjoyable for them and their families too.

However, implementation of the VOC analysis in gastrointestinal clinical practices is not ready yet for routine applications since more research is required in various aspects. Chapter 2 demonstrated that most VOC studies are either proof-of-concept studies or of a small sample size. Many studies did not perform any internal or external validation of their findings. The correction of possible confounding factors was also not considered, which might have affected the study results. Furthermore, most breath research has focused on endogenous VOC untargeted analysis; Chapter 2 showed that scientific interest should also shift towards exogenous VOC targeted analysis. Chapter 4 raised awareness regarding batch effects in exhaled breath VOC studies that do not allow for across-study comparisons. Chapters 2 and 4 showed that lack of a standardised framework in terms of clinical design, lack of a consensus in data handling and statistical tool availability and use, and wavering ideologies on whether targeted or untargeted approaches should be considered have hampered the exhaled breath clinical implementation. Therefore, Chapters 3 and 5 aimed to provide an overview of various pre-processing approaches suitable for volatilome data of diverse nature and to equip the reader with a basic overview of suitable techniques for treating and successfully exploiting volatilome data. Furthermore, from a VOC analysis standpoint, a diseased organ could release VOCs via the bloodstream in breath and other body excretion means (e.g. faeces, urine). Chapter 6 showed that fusing this complementary information could result in higher accuracy breath diagnostic tests. Given the complexity and size of volatilome data, more advanced fusion methods might be needed; Chapter 6 proposes such a method. Chapter 7 performed a case study that took into account knowledge gained in the present thesis (i.e. Chapters 2-6) and tested the assumption of using exhaled breath to differentiate primary sclerosing cholangitis patients from inflammatory bowel disease patients. The study results confirmed that assumption.

The findings of the present thesis might contribute to scientific advancement in several ways. Chapters 2 and 4 summarise and raise awareness regarding the lack of a standardised framework in terms of clinical design, lack of a consensus in data handling and statistical tool availability and use, and wavering ideologies on whether

targeted or untargeted approaches should be considered. Chapters 3 and 5 follow up on the lack of consensus in data handling and statistical tool availability and use by thoroughly discussing and proposing how volatilome data might be approached and analysed regarding VOC biomarker discovery. Chapter 6 provides insight on the concept of data fusion and why and how this concept can be applied to volatilome data. Data fusion is a known concept in computer sciences; however, little seems to be known and published regarding applications of data fusion in the field of exhaled breath research and VOC analysis as a whole. Chapter 7 shows that exhaled breath can be used to diagnose and monitor primary sclerosing cholangitis patients, which has been challenging clinicians to date. Additionally, the present thesis results and previous and future study results might help bring exhaled human breath research into daily clinical setups. Implementing exhaled breath analysis in the clinics would benefit not only gastrointestinal research but other medical fields since the same principle can be applied in any medical field when it comes to using VOC analysis. Therefore, the present thesis can interest scientific researchers in various fields aside from breath, and the presented statistical tools and ideas can be considered general guidelines for researchers who perform statistical modelling with complex biomedical data.

Finally, the work and results in the present thesis have been shared with other researchers since they have been presented at several international scientific conferences through poster and oral presentations. The work in Chapter 4 regarding the implementation of quality controls to prevent batch effects in breathomics data and allow for cross-study comparisons was awarded the Best Poster Prize by the Journal of Breath Research during the Breath Summit 2018 (June 17–20, 2018, Maastricht, The Netherlands). The work in Chapter 6 regarding advanced data fusion by using random forest proximities and the pseudo-sample principle was awarded the Best Presentation Prize at the 42nd Chromatographic Symposium (June 4-7, 2019, Szczyrk, Poland). All of the results have been or will be documented through scientific publications: Chapters 2-6 have been published, whereas Chapter 7 is in preparation.

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