Chapter 1

General introduction and outline of this thesis

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Decisions about the right treatment for the right patient and individual prognostic trajectories depend on an accuracy diagnosis. The goal of the diagnostic process is to determine an individual’s condition or disease based on relevant variables (such as signs and symptoms) of the patient. Innovations in the measurement of (new) variables and/or their interpretation can lead to important advances in medical diagnosis and hence healthcare in general. In this thesis, we investigate the utility of volatile organic compounds (VOCs) in exhaled breath as predictive variables for diagnostic outcomes in the intensive care unit (ICU). The focus is on an electronic nose (eNose) that can measure the VOCs in exhaled breath continuously.

Below we provide preliminaries on intensive care, blood glucose monitoring, exhaled breath analysis, and others. Next, we state our research questions and provide an outline of the thesis chapters.

**Intensive Care**

Patients are admitted to the intensive care unit if they are critically ill. Patients in the ICU are often intubated and mechanically ventilated, and vital signs are monitored constantly. Ideally, biological signals are also monitored continuously in this patient population, but most measurements can only be taken intermittently. The intermittent character of many biological tests limits their monitoring value on the intensive care unit and makes that this patient group may benefit greatly from technological advancements. The identification of a non-invasive alternative to blood draws is desirable as continuous monitoring requires large of amounts of measurements per patients.

**Blood glucose monitoring**

Alike patients with diabetes mellitus, critically ill patients require frequent monitoring of blood glucose levels. Due to critical illness, glucose metabolism deranges, which is treated with intravenous insulin administration. This potentially deadly intervention requires intensive monitoring of the blood glucose levels to prevent hypoglycemia. Often, glucose is measured intermittently, leaving periods of unmonitored insulin administration and the possibility of hypoglycemia. There is a possible benefit in monitoring glucose continuously to prevent hyper- and hypoglycemia [1,2]. Continuous glucose measurement (CGM) devices have been found safe and beneficial in patients with diabetes mellitus and are frequently used in that population [3]. Use of CGM in the ICU however, is still in its early stages. Therefore, thorough testing of accuracy and reliability of different methods for CGM in ICU patients is important.
Exhaled Breath

Exhaled breath is mainly made up of carbon dioxide, oxygen, nitrogen and water vapor [4]. In addition, a small percentage of breath is composed of thousands of volatile organic compounds (VOCs). In some cases, VOCs can be indicative for disease. The most famous example is the sweet odor of the breath of a person going through an episode of keto-acidosis [5]. In this metabolic process, low blood glucose levels lead to degradation of ketone bodies causing a rising concentration of acetone, which ends up in exhaled breath. While we know the origin of acetone and its connection to changes in glucose status, the origin of most VOCs is unknown. In addition to markers of systemic origin, VOCs can come from a pulmonary source. This could be due to inflammation or local metabolism of the lung, or could have exogenous sources such as inhaled VOCs or resident bacteria [6]. The field of exhaled breath analysis has been expanding quickly over the last years and has been investigating a growing number of health conditions and diseases. These include pulmonary conditions such as asthma and chronic obstructive pulmonary disease [7,8], lung cancer [8-11] and acute respiratory distress syndrome [12,13], and non-pulmonary conditions such as kidney disease [14] and pregnancy [15]. Although numerous studies have been conducted, few had led to technology being used in practice. Numerous breath analysis techniques are currently used. These include gas chromatography and mass spectroscopy (GC-MS) and electronic noses (eNose), which are discussed below.

Gas chromatography and mass spectroscopy

GC-MS is likely the most used breath analysis technique in clinical research and is considered the gold standard. Typically, breath is collected in a storage device (e.g. a bag or a sorbent tube) for a specified amount of time using a gas sampling pump. The breath sample is then introduced into the GC-MS device. Then, the sample is inserted in the gas chromatograph into a long column. Due to the differences in chemical properties, different molecules reach the end of the column after different periods of time: This is called the retention time of the compound. Then, these molecules are ionized and the fragment ions are detected by a mass spectrometer, resulting in a mass/charge ratio. The fragmentation pattern of a molecule in combination with the retention time can be used to identify compounds in the breath sample. Thus, with GC-MS, VOCs can be separated, quantified and identified to get a very detailed snapshot of the content of breath. However, because of the size of GC-MS machines, the time-consuming nature of GC-MS analysis and the expertise needed, GC-MS has a low clinical applicability. This holds especially for intensive care medicine as results are required as soon as possible.
Electronic nose
A typical eNose contains an array of sensors that are sensitive to a combination of molecules [16]. While metal oxide sensors are often used, optical sensors, conducting polymer sensors and surface or bulk acoustic wave sensors are also used in eNoses [16]. When molecules pass over eNose sensors and bind to them, the resistance of the sensors changes depending on the sensitivity to the molecule. The combination of the reactions of the sensors leaves a certain fingerprint, or breathprint, for each combination of molecules. Using pattern recognition techniques, these breathprints can be linked to certain events. eNoses are used in many fields and are actively investigated for use in clinical settings. While typical eNoses take single measurements, and are often used to diagnose a single event, continuous measurement is possible with adapted devices. In contrary to GC/MS, eNoses can be miniaturized, can provide continuous signals and analysis can be performed at bedside. Electronic noses are especially promising in the ICU setting since many patients in the ICU are intubated and mechanically ventilated, which provides constant and non-invasive access to the exhaled breath.

Continuous blood glucose measurement through eNose in the ICU
Following the clinical recognition of the acetone smell of a diabetic keto-acidosis, many researchers have studies exhaled breath analysis as non-invasive test for glucose control in diabetes patients and healthy controls. In general, these studies showed positive results on the predictive value of exhaled breath markers for blood glucose levels. However, these results have not been translated to the intensive care setting. A possible challenge with continuous exhaled breath analysis in intubated and mechanically ventilated ICU patients is the variance in ventilation modus in these patients, and the risk of noise being introduced to the signal due to the continuous nature of the measurements. Therefore, it must be investigated how continuous breath signals in critically ill patients should be handled with respect to de-noising and normalizing the signal. These cleaned signals should then be compared and correlated to blood glucose measurements.

Analysis challenges
In addition to the challenges in the analysis of continuous eNose signals described in the previous paragraph, it is not clear what the best approach for processing non-continuous eNose signals is. Researchers in the field use many different combinations of pre-processing, dimension reduction, classification and validation techniques. A summary of the used methods followed by an evaluation would shed a light on this topic. More importantly, many investigators do not validate their results. Generalizability of their findings therefore
remains unknown. Stressing the need for external validation by providing examples would stress its importance to the community.

**Analysis of VOCs through the extracorporeal circulation**

In some critically ill patients, pulmonary or cardiovascular conditions require extra-corporeal circulation for oxygenation (extracorporeal membrane oxygenation (ECMO)) or CO2 removal (extracorporeal CO2 removal (ECCO2R)). This effectively generates a second circulation in which gas-exchange occurs. In this specific scenario, exhaled breath is not the only source of air that can be analyzed. The two air sources theoretically share VOCs that are produced throughout the body and are normally carried to the lung via the circulation. The main difference between the artificial gas exchange and the lung is that VOCs are also produced locally in the lungs. Comparison of the simultaneous signals from both sources allows for the identification of VOCs and breathprints that are generated locally as compared to systemically.

**HYPOTHESES AND RESEARCH QUESTIONS**

There are several hypotheses that will be tested in this thesis. First, we hypothesized that several devices can be used for continuous glucose monitoring in critically ill ICU patients with adequate accuracy and reliability. Second, we hypothesized that that continuous exhaled breath analysis using an eNose could be used to accurately predict blood glucose levels in intubated, mechanically ventilated patients. Finally, we hypothesized that there is a correlation between VOCs in exhaled breath and VOCs coming from extracorporeal support devices know to be of non-pulmonary origin. To test these hypotheses, we subdivided this thesis into three parts, accompanied by three different aims. First, we aimed to compare and test different (continuous) blood glucose measurement methods (part I). Second, we aimed to investigate the use of exhaled breath to monitor glucose levels (part II). Finally, we aimed to investigate further development of eNose measurement techniques and data analysis methods (part III). Figure 1 illustrates the outline of this thesis.

**Research questions**

The following research questions are used to test the hypotheses:

- **RQ1.** What are the different techniques for CGM, and how should accuracy of these devices be assessed?
- **RQ2.** What is the accuracy of two different CGM devices developed for use in the ICU?
- **RQ3.** Is there an association between VOCs in exhaled breath and blood glucose levels?
RQ4. What are the sources of noise in continuously collected breath signals from ICU patients and how can these sources of noise be reduced?
RQ5. Can continuous exhaled breath analysis using an eNose be used to predict blood glucose levels in intubated ICU-patients?
RQ6. What are the data analysis techniques used in eNose studies and how do they compare?
RQ7. Is there a correlation between VOCs in exhaled breath and VOCs coming from extracorporeal support devices known to be of non-pulmonary origin?

Outline of this thesis

The above-stated research questions are answered in eight chapters, which are shortly summarized here. In chapter 2, an overview is given of the diverse continuous glucose monitoring (CGM) techniques and devices. In chapter 3 and 4 we hypothesized that two different CGM devices were point (and trend) accurate and reliable. In chapter 3, an interstitial CGM device is tested and in chapter 4, an intravenous CGM device is tested. Both studies were carried out in the same setting with a similar study design. In chapter 5 we hypothesized that there is an association between VOCs in exhaled breath and blood glucose levels. It encompasses a systematic review on the matter. The review focusses
on which exhaled metabolites correlate with changes in glucose levels. In chapter 6, we aim to investigate sources of noise in a continuous eNose breath signal measured in a non-controlled clinical setting. In addition, we discuss several approaches to reduce this noise. In chapter 7, we hypothesized that continuous exhaled breath analysis using an eNose could be used to accurately predict blood glucose levels in intubated, mechanically ventilated patients. We use the methods developed in chapter 6 and the data collected in chapter 3 & 4 in this study. In chapter 8, we compared classification methods in breath analysis by eNose used in literature. Our aim was to strengthen eNose research by evaluating which methods work best. These methods were tested on 4 different datasets we obtained from several researchers in the field of exhaled breath analysis. In chapter 9, we compared classification methods in breath analysis by eNose used in literature. Our aim was to strengthen eNose research by evaluating which methods work best. These methods were tested on 4 different datasets we obtained from several researchers in the field of exhaled breath analysis. In chapter 9, we discussed the safety of using and eNose and GC-MS measurements in extracorporeal life-support patients. In addition, we try to compare this data to exhaled breath data. The results described in this study are put into perspective in the discussion (chapter 10). The future directives are also discussed in that chapter.

References


2. Krinsley, J. S.; Preiser, J.-C. Moving beyond tight glucose control to safe effective glucose control. Crit Care 2008, 12, 149.


