Tampere University

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ACADEMIC DISSERTATION
To be presented, with the permission of
the Faculty of Medicine and Health Technology
of Tampere University,
for public discussion in the auditorium F114
of the Arvo building, Arvo Ylpön katu 34, Tampere,
on 27 January 2023, at 12 o'clock.

ACADEMIC DISSERTATION
Tampere University, Faculty of Medicine and Health Technology
Finland

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Cover design: Roihu Inc.

ISBN 978-952-03-2720-0 (print)
ISBN 978-952-03-2721-7 (pdf)
ISSN 2489-9860 (print)
ISSN 2490-0028 (pdf)
http://urn.fi/URN:ISBN:978-952-03-2721-7

Carbon dioxide emissions from printing Tampere University dissertations have been compensated.

PunaMusta Oy – Yliopistopaino
Joensuu 2022

To my family

ACKNOWLEDGEMENTS

First, I would like to express my sincere gratitude to my supervisor Docent Ilkka Kivekäs MD, PhD. You introduced me to this subject and encouraged me to carry out this project. You also guided me along this scientific path and always had time for me, even though you had many other projects, too. I truly admire your enthusiasm for research and evidence-based medicine.

I am deeply grateful to my other supervisor Professor Markus Rautiainen MD, PhD. You provided insightful comments and supportive guidance during the whole project. It has been an honour to have had you supervise this dissertation.

I also want to express my gratitude to my other supervisor Professor Niku Oksala MD, PhD, DSc (Med). You provided valuable instructions for the project. You also gave lightning-fast responses to my e-mail enquiries. Your innovative approach to research is intriguing.

I am greatly indebted to Antti Roine MD, PhD who played a major part in this project. You introduced me to differential mobility spectrometry technology, and you were the one I contacted when I encountered problems. You always found time to answer my questions. Your knowledge and guidance regarding the methodological parts of each study was extremely important.

I would like to express my gratitude to Markus Karjalainen MSc (Tech) and Anton Kontunen DSc (Tech). Your expertise in the field of engineering was invaluable. You provided practical solutions when I encountered technical problems. I would also like to express my thanks to Anna Anttalainen MSc (Tech) and Jaakko Ormiskangas MSc (Tech) for their efforts in this project. It was a great pleasure to work with you.

I wish to express my thanks to Docent Jura Numminen MD, PhD for his expertise and guidance in rhinology, both in this research project and in clinical work.

I would like to thank Lauri Hokkinen MD and Docent Risto Vuento MD, PhD for their contribution to the project.

I am deeply grateful to research nurses Piitu Oksanen and Merja Rumpunen for their invaluable help in recruiting the study patients and collecting the samples. You constantly have such positive energy. Thank you.

I am extremely thankful to my pre-examiners Docent Samuli Hannula MD, PhD and Perttu Halme MD, PhD for the time they spent reviewing this dissertation and for their constructive comments which helped to improve the quality of the dissertation. I am greatly honoured to have Professor Heikki Irjala, MD, PhD as my opponent.

I want to thank all my colleagues in the department of otorhinolaryngology for their support and interest in the project. Especially, I want to thank Olli Valtonen MD, PhD for his efficient guidance during the finishing phase of this dissertation.

My thanks also go to Peter Heath, MA for the official language editing of the manuscripts and this dissertation.

I also want to express my gratitude to all the volunteers and patients who participated in this study. Without them, this dissertation could not have been completed.

I am deeply grateful to my parents Arto and Tuula for all the love and support they have given me during my whole life. You have given me everything I ever needed. I want to thank my brother Lauri for often asking: "*What is taking so long to finish your dissertation?*" It helped a lot.

Finally, I would like to thank my amazing and loving wife Miia for her support and understanding during this project. Without your efforts in our daily life, this project would not have been completed. I am extremely thankful for our two wonderful sons Eeli and Alvar who are the light of my life.

This study was financially supported by the Consociatio Rhinologica Fennica, the Doctoral School of Tampere University, the Finnish Medical Foundation, the Finnish ORL-HNS Foundation, and Tampere Tuberculosis Foundation. This study was also financially supported by the Competitive State Research Financing of the Expert Responsibility area of Tampere University Hospital.

Tampere, August 2022

Jussi Virtanen

ABSTRACT

The sense of smell can potentially be used to diagnose diseases. However, the sense of smell in humans has lower sensitivity and discrimination capability compared to animals such as dogs. Probably the most well-known example of using a dog's sense of smell is drug detecting dogs at airports. Research studies have demonstrated that dogs can identify samples acquired from patients with cancer or infection.

The molecules detected by sense of smell are volatile organic compounds (VOCs). Nowadays, it is possible to measure these compounds, for example, with an electronic nose (eNose). An eNose is a device that analyses gas-phase mixtures and produces a measurement signature that represents the spectrum of the molecules found in the mixture. Differential mobility spectrometry (DMS) is not a traditional eNose but produces comparable information and, in addition, has a higher sensitivity. DMS has not previously been used in otorhinolaryngologic studies.

Acute rhinosinusitis (ARS) is typically caused by a virus or bacteria. As both produce similar symptoms, differentiating them based on symptoms or clinical examination is a challenging task, which often leads to the overprescription of antibiotics. Chronic rhinosinusitis (CRS) involves, for instance, nasal blockage and discharge lasting at least 12 weeks. As many other rhinologic diseases cause similar symptoms, the definite diagnosis of CRS warrants computed tomography imaging, which is not available in primary care. Therefore, there is a need for a rapid, accurate and non-invasive method to diagnose ARS and CRS.

This dissertation examines the diagnostics of ARS and CRS with DMS and consists of four studies. First, five common rhinosinusitis bacteria *in vitro* were analysed with DMS. Second, maxillary puncture and aspiration of the contents were performed on patients with ARS. The acquired pus was analysed with DMS, and the results were compared to the traditional bacterial culture of the pus. Third, nasal air from volunteers was aspirated into collection bags using a pump built for this purpose. The results were then compared to room air samples and the feasibility of the method was evaluated. Fourth, patients with CRS without nasal polyps and patients with deviated nasal septum were studied. Aspirated nasal air was collected in the same way as in the third study and analysed with DMS. The ability of DMS to distinguish patients in different groups was then evaluated. The data analysis

employed in the studies involved machine learning methods which were used to examine the sensitivity and specificity of DMS to distinguish samples.

The results reveal that DMS can separate common rhinosinusitis bacteria *in vitro* with very good accuracy. Furthermore, DMS shows very good accuracy to distinguish bacterial positive and bacterial negative samples compared to bacterial cultures. The method used for aspirating the nasal air and subsequent analysis with DMS proved to be a useful method. The nasal air samples were perfectly distinguished from room air samples. In addition, DMS demonstrates good accuracy to discriminate patients with CRS without nasal polyps from patients with deviated nasal septum by analysing nasal air.

The studies in this dissertation were pilot studies and the results are affected by small sample size. However, cross-validation provides confidence of the reliability of the classifier. The studies demonstrate that DMS can analyse different sample types and distinguish groups from each other. The aspiration of nasal air was shown to be practicable and can be used in further studies of rhinologic diseases.

TIIVISTELMÄ

Sairauksia voidaan mahdollisesti diagnosoida hajuaistin avulla. Koiran hajuaisti on ihmisen hajuaistia parempi ja kenties tunnetuin esimerkki koirien hajuaistin hyödyntämisestä on huumekoirien käyttö lentokentillä. Tutkimukset ovat myös osoittaneet, että koirat pystyvät tunnistamaan esimerkiksi syöpiä ja mikrobien aiheuttamia infektioita erilaisista näytteistä.

Hajuaistilla aistittavat molekyylit ovat haihtuvia orgaanisia yhdisteitä. Nykyään niitä voidaan analysoida tarkoitukseen soveltuvilla laitteilla kuten elektronisella nenällä, joka tuottaa kokonaiskuvan kyseisen näytteen sisältämien molekyylien seoksesta. Liikkuvuuserospektrometria (*differential mobility spectrometry*, DMS) ei ole elektroninen nenä alkuperäisen määritelmän mukaan, mutta tuottaa vastaavanlaista informaatiota kuin perinteiset elektroniset nenät. Lisäksi se erottaa molekyylejä paremmalla herkkyydellä. DMS:n käyttöä ei kuitenkaan ole aikaisemmin tutkittu korva-, nenä- ja kurkkutautien saralla.

Äkillisen nenän sivuontelotulehduksen aiheuttaa tyypillisesti virus tai bakteeri. Näiden aiheuttama oirekuva on kuitenkin hyvin samankaltainen ja vaikea erottaa oireiden tai kliinisen tutkimuksen perusteella. Äkillistä viruksen aiheuttamaa nenän sivuontelotulehdusta hoidetaan liian usein antibiootilla. Pitkäaikaisen nenän sivuontelotulehduksen oirekuvaan kuuluu muun muassa yli 12 viikkoa kestäneet nenän tukkoisuus ja niistämisen tarve. Kuitenkin monet muutkin nenän ja nenän sivuonteloiden sairaudet tai anatomiset syyt voivat aiheuttaa vastaavanlaisia oireita. Pitkäaikaisen nenän sivuontelotulehduksen diagnoosin varmentaminen vaatiikin nenän sivuonteloiden kuvantamista tietokonetomografialla, jota ei kuitenkaan ole käytettävissä perusterveydenhuollossa. Äkillisen ja pitkäaikaisen nenän sivuontelotulehduksen diagnosoimisen tueksi olisi hyödyllistä saada nopea, luotettava ja potilaaseen vähän kajoava keino.

Tämä väitöskirja keskittyy äkillisen ja pitkäaikaisen nenän sivuontelotulehduksen diagnostiikkaan DMS:llä ja koostuu neljästä osatyöstä. Ensimmäisessä osatyössä DMS:llä analysoitiin viittä nenän sivuontelotulehduksen aiheuttajabakteeria elatusmaljoilta. Toisessa osatyössä tutkittiin äkillistä nenän sivuontelotulehdusta sairastavia potilaita. Heille tehtiin poskiontelopunktio ja tällä menetelmällä saatu märkäerite analysoitiin DMS:llä, minkä jälkeen tuloksia verrattiin samasta

märkäeritteestä tehtyyn bakteeriviljelyyn. Kolmannessa osatyössä selvitettiin hengitysilman keräämistä nenäontelosta näytteeksi ja sen analytiikkaa DMS:llä. Neljännessä osatyössä verrattiin hengitysilmanäytteitä DMS:llä pitkäaikaista nenän sivuontelotulehdusta sairastavien potilaiden ja nenän väliseinän vinoudesta kärsivien potilaiden välillä. Väitöskirjan kaikissa osatöissä data-analyysi tehtiin koneoppimismenetelmin, joiden avulla selvitettiin DMS:n herkkyyttä ja tarkkuutta erottaa näytteitä toisistaan.

Tutkimuksissa havaittiin, että DMS erottaa erittäin hyvin toisistaan yleiset sivuontelotulehduksen aiheuttajat bakteerimalja-analyysin perusteella. Lisäksi havaittiin, että DMS erottaa erittäin hyvällä osuvuudella poskiontelon märkäeritteestä bakteeripositiivisen ja -negatiivisen näytteen verrattuna perinteiseen viljelymenetelmään. Hengitysilman keräys ja analysointi DMS:llä osoittautui käyttökelpoiseksi menetelmäksi. Pitkäaikaisesta nenän sivuontelotulehduksesta kärsivät potilaat pystyttiin erottamaan hyvin potilaista, joilla nenän tukkoisuuden aiheutti nenän väliseinän vinous.

Alustavien tulosten perusteella DMS toimii hyvin nenän sivuontelotulehduksen diagnostiikassa. Tutkimukset osoittivat, että DMS soveltuu hyvin erilaisten näytetyyppien mittaukseen ja se erottaa ryhmiä varsin hyvin toisistaan. Ilman keräys nenäontelosta keräyspussiin osoittautui käyttökelpoiseksi menetelmäksi, jota voidaan hyödyntää tulevaisuudessa etenkin nenään ja nenän sivuonteloihin liittyvissä tutkimuksissa.

CONTENTS

ABBREVIATIONS

ARS	acute rhinosinusitis
ABRS	acute bacterial rhinosinusitis
ATS/ ERS	American Thoracic Society & European Respiratory Society
BMI	body mass index
CBCT	cone-beam computed tomography
CI	confidence interval
COPD	chronic obstructive pulmonary disease
CP	conducting polymer
CRS	chronic rhinosinusitis
CRSsNP	chronic rhinosinusitis without nasal polyps
CT	computed tomography
CV	cross-validation
DMS	differential mobility spectrometry
DS	deviated nasal septum
EDMMC	endoscopically derived middle meatal culture
eNose	electronic nose
FAIMS	field asymmetric ion mobility spectrometry
GC-MS	gas-chromatography mass-spectrometry
IMS	ion mobility spectrometry
LDA	linear discriminant analysis
LOOCV	leave-one-out cross-validation
LM	Lund-Mackay
kNN	*k*-nearest neighbour
MOS	metal-oxide semiconductor
NPV	negative predictive value

PCA	principal component analysis
PPV	positive predictive value
QCM	quartz crystal microbalance
SAW	surface acoustic wave
SNOT-22	22 item Sinonasal Outcome Test
U_{CV}	compensation voltage
U_{SV}	separation voltage
VOC	volatile organic compound

d	distance
E	electric field strength
K	mobility coefficient
v	drift velocity

LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following original publications referred to in the text by their Roman numerals I to IV.

Publication I Virtanen J, Hokkinen L, Karjalainen M, Kontunen A, Vuento R, Numminen J, Rautiainen M, Oksala N, Roine A, Kivekäs I. In vitro detection of common rhinosinusitis bacteria by the eNose utilizing differential mobility spectrometry. European Archives of Otorhinolaryngology. 2018;275(9):2273-79.

Publication II Manuscript. Virtanen J, Roine A, Kontunen A, Karjalainen M, Numminen J, Oksala N, Rautiainen M, Kivekäs I. The Detection of Bacteria in the Maxillary Sinus Secretion of Patients with Acute Rhinosinusitis Using an Electronic Nose—A Pilot Study (submitted).

Publication III Virtanen J, Anttalainen A, Ormiskangas J, Karjalainen M, Kontunen A, Rautiainen M, Oksala N, Kivekäs I, Roine A. Differentiation of aspirated nasal air from room air using analysis with a differential mobility spectrometry-based electronic nose: a proof-of-concept study. Journal of Breath Research. 2021;16(1):016004.

Publication IV Virtanen J, Kontunen A, Numminen J, Oksala N, Rautiainen M, Roine A, Kivekäs I. Identifying chronic rhinosinusitis without nasal polyps by analyzing aspirated nasal air with an electronic nose based on differential mobility spectrometry. Acta Otolaryngologica. 2022;142(6):524-531.

AUTHOR'S CONTRIBUTION

The author was the sole main author of all the publications.

Publication I	The author has taken part in the study design. The author has performed the majority of the measurements of the bacterial plates. The author drafted the initial manuscript and submitted the manuscript as the corresponding author.
Publication II	The author has taken part in the study design. The author enrolled the majority of the patients and collected the data. The author performed all the measurements of the samples and part of the statistical analyses. The author drafted the initial manuscript and submitted the manuscript as the corresponding author.
Publication III	The author has taken part in the study design. The author enrolled all the participants and collected the data. The author performed all the measurements of the samples. The author drafted the initial manuscript and submitted the manuscript as the corresponding author.
Publication IV	The author has taken part in the study design. The author enrolled all the patients and collected the data. The author performed all the measurements of the samples and a part of the statistical analyses. The author drafted the initial manuscript and submitted the manuscript as the corresponding author.

1 INTRODUCTION

Physicians have used sense of smell in disease diagnostics for millennia. However, the sense of smell in humans has lower sensitivity and discrimination capability when compared to animals such as dogs. The molecules detected by sense of smell are volatile organic compounds (VOCs) which consist of chemicals that are volatile in ambient temperatures. Some VOCs can serve as possible biomarkers of diseases (Sethi et al., 2013; Wilson, 2015). Such VOCs can potentially be identified from gas-phase mixtures, such as human breath, and can therefore be used as non-invasive methods to diagnose diseases.

An electronic nose (eNose) is a device that performs a qualitative analysis of gas-phase mixtures. The device consists of an array of nonselective gas sensors, pre-processing electronics and a computer that interprets sensor signals (Gardner & Bartlett, 1994). During the analysis, VOCs react with the sensors, resulting in a measurement signature describing the VOCs in the sample. Thereafter, the signatures can be compared using pattern recognition software which can reveal the VOC pattern of a certain disease. During the last twenty years, eNose technology has gained interest in the field of medical research. For example, the technology has been studied in the diagnostics of airway obstructions, respiratory infections, inflammatory diseases and cancer (Farraia et al., 2019).

Although not a traditional eNose, ion mobility spectrometry (IMS) provides analogous information from gas mixtures (Röck et al., 2008). It measures time-of-flight, which is the time an ion spends in the drift tube (D'Atri et al., 2018). The separation power of IMS can be further enhanced with differential mobility spectrometry (DMS). In DMS, ion swarms are exposed to intermittent high- and low-electric fields which affect the mobility of the ion swarm (Borsdorf et al., 2011). Unlike with IMS, it is possible to detect ions of the same size and charge using DMS. As the ions hit the detector, an electrical current is generated and recorded (Borsdorf et al., 2011). The result is a dispersion plot that depicts the measurement signature of the chemical mixture. DMS has been studied in the medical field and shown to be able to discriminate pancreatic cancer from healthy controls by analysing urine samples with a sensitivity and specificity of 79% (Nissinen et al., 2019). Furthermore,

DMS can analyse smoke from a diathermy knife and distinguish samples with breast cancer from benign ones with a sensitivity of 80% and specificity of 90% (Sutinen et al., 2019). To date, however, DMS has not been studied in the field of otorhinolaryngology.

Rhinosinusitis is a symptomatic inflammation of the paranasal sinuses and nasal mucosa. Symptoms include nasal blockage, nasal discharge, facial pressure and reduction of smell. In acute rhinosinusitis (ARS), the symptoms will have lasted less than 12 weeks (Fokkens et al., 2020). Although it is known that ARS is usually caused by a virus, the prevalence of bacteria is unclear (Chow et al., 2012; Orlandi et al., 2021). The differential diagnostics between viral and bacterial aetiology is difficult as both viruses and bacteria cause similar symptoms (Autio, Koskenkorva, et al., 2015). Imaging findings do not differentiate the aetiology either (Autio et al., 2016; S. Smith et al., 2015). A reliable way to detect bacterial aetiology would be to perform maxillary sinus puncture and aspiration of the sinus contents for bacterial culture. However, this procedure is not routinely performed. As a result, antibiotics are often overprescribed in cases of viral ARS (Benninger et al., 2016; Pouwels et al., 2018; Sharma et al., 2017).

If rhinosinusitis symptoms last at least 12 weeks, the condition is called chronic rhinosinusitis (CRS). According to a survey-based study, the prevalence of CRS has been estimated to be 11% in Europe (Hastan et al., 2011). Furthermore, the diagnostics of CRS are challenging. A diagnosis that is based only on symptoms can lead to a high risk of false positive findings (Bhattacharyya & Lee, 2010). Nasal endoscopy with rigid or flexible endoscopes improves the reliability of the diagnostics of CRS (D. H. Kim et al., 2020). A definite diagnosis of CRS would require confirmation of inflammatory findings in paranasal sinuses using computed tomography (CT) imaging (Fokkens et al., 2020). However, neither endoscopes or CT are readily available in primary care.

Today, there is a need for rapid, non-invasive and reliable techniques as an addition to the current instruments used in the diagnostics of ARS and CRS. This dissertation describes how eNoses and DMS work and demonstrates their potential in the medical area. The diagnostic methods currently employed for ARS and CRS are covered in detail. The studies in this dissertation evaluate the ability of DMS to be used in the diagnostics of ARS and CRS. First, the detection of common rhinosinusitis bacteria *in vitro* is examined. Second, patients with ARS are examined and evaluated as to whether bacterial presence in the maxillary sinuses can be detected with DMS. Finally, aspirated nasal air is analysed with DMS and the feasibility of the method and whether it can detect patients with CRS are evaluated.

2 REVIEW OF THE LITERATURE

2.1 Detection of diseases by smell

Physicians have used sense of smell to diagnose disease for millennia. A well-known example is the sweet smell of acetone on the breath, which is associated with diabetes but also diet (Španěl et al., 2011). The human nose, however, has low sensitivity and discrimination capability when compared to animals such as sniffer dogs that are used at airports to detect drugs and other contraband.

The ability of dogs to detect cancer was first published in the Letters to the Editor section of the Lancet by Williams and Pembroke in 1989. They wrote about a case where a pet dog had started to pay special attention to a mole on a patient's leg. The patient visited the doctor, and the mole was excised. A pathology examination later identified it as a malignant melanoma. In another study, a dog was able to identify lentigo maligna, malignant melanoma in situ or malignant melanoma in six out of seven cases. A second dog was also tested with four of these patients and concurred with the results of the first dog (Pickel et al., 2004). Willis et al. (2016) found that in 20 double-blind tests, each having one melanoma sample and ten controls, the dog was able to distinguish melanoma on nine (45%) occasions. Other studies have investigated the ability of dogs to detect other cancers, such as ovarian cancer (Horvath et al., 2008), breast cancer (McCulloch et al., 2006), lung cancer (Amundsen et al., 2014; Ehmann et al., 2012; Fischer-Tenhagen et al., 2018; McCulloch et al., 2006), colorectal cancer (Sonoda et al., 2011) and prostate cancer (Cornu et al., 2011; Taverna et al., 2015). The results have varied significantly with sensitivity and specificity rates of 56-100% and 8-99%, respectively.

Interestingly, dogs can detect not only cancer but also other diseases. A trained beagle showed promise in detecting *Clostridium difficile* in both stool samples and hospital patients (Bomers et al., 2012). In a recent study, eight detection dogs trained to detect the saliva or tracheobronchial secretions of patients infected with SARS-CoV-2 were examined. The dogs could discriminate infected samples from the samples of healthy patients with an overall average detection rate of 94%, a sensitivity of 83% and specificity of 96% (Jendrny et al., 2020). In another study, twelve dogs were trained to detect SARS-CoV-2 infection. The dogs could

discriminate SARS-CoV-2 swab samples from the samples of other common respiratory viruses with a sensitivity of 74% and specificity of 95% (ten Hagen et al., 2021). In a Finnish study, a pet dog was trained to detect paediatric urinary infection with an accuracy of 89% (Koskinen et al., 2017). Furthermore, another study found that two out of three dogs could detect obstructive sleep apnoea from urine samples two thirds of the time (Koskinen et al., 2019).

However, the use of "detection dogs" has some drawbacks. For example, dogs require extensive training, which needs special expertise and is expensive, and have a limited lifespan. Moreover, even though the dog is trained properly, the dog's motivational level may affect detection accuracy. Breeds behave differently and have different characteristics, meaning that some breeds are more favourable for the task than others (Jamieson et al., 2017). Dogs might also be distracted by other stimuli (Bomers et al., 2012), which might result in the examination coming to an end. In addition, some people might consider diagnostics dogs as unhygienic in a hospital setting.

2.2 Volatile organic compounds

Volatile organic compounds (VOCs) are a wide range of chemicals that are volatile at ambient temperature. Endogenous VOCs are released as by-products of normal cell metabolism, but also directly by microbes or due to an inflammatory response to infection (Sethi et al., 2013). Exogenous VOCs are inhaled or absorbed through the skin from the environment and may indicate the exposure of an individual to toxic chemicals (Sethi et al., 2013; Wilson, 2015). It is relevant to consider which VOCs are part of normal physiological processes in humans to distinguish them from pathological ones. In 1971, the Nobel laureate Linus Pauling and his colleagues measured 250 different compounds in human breath samples (Pauling et al., 1971). Since then, many more VOCs have been identified in healthy humans, including 1488 from breath alone. VOCs from bodily fluids include 549 from saliva, 444 from urine and 379 from blood (Drabińska et al., 2021). In contrast, many of the VOCs identified can be possible biomarkers of diseases (Sethi et al., 2013; Wilson, 2015). Thus, identification of VOCs from human breath, urine, faeces, and other bodily fluids is a potential and non-invasive method for disease diagnostics.

2.3 Gas chromatography and mass spectrometry

Scientists have tried to measure aromas since the 1910s (Hogewind & Zwaardemaker, 1919). Today, thanks to advancements in technology, analytical instruments, such as gas chromatography-mass spectrometry (GC-MS), and other technical instruments, for example, laser spectroscopic techniques (Wang & Sahay, 2009), it is possible to conduct higher level investigations. These instruments can measure specific VOCs and probably identify disease-specific biomarkers, and thereby serve as diagnostic tools. They also allow the measurement of biomarkers in human samples, such as breath, blood, urine and pus.

Gas chromatography separates the molecular constituents of a chemical compound when the compound goes through a column. Each constituent has different delays before reaching the end of the tube, creating a series of peaks. In GC-MS, a gas-chromatography tube separates the mixture into its molecular constituents and mass spectrometry separates them based on their mass-to-charge ratio, creating a spectrum for each peak. A database allows storage of the labelled spectra for the identification of each chemical compound in a specific peak (Nagle et al., 1998). GC-MS can also perform quantitative analysis to provide information on the components of the sample. However, these devices are expensive and require time-consuming sample preparation, training and data interpretation (Vaks et al., 2014). Therefore, not yet suitable for clinical use. A simpler and cheaper instrument would serve better than GC-MS in point-of-care use.

2.4 Electronic nose

Persaud and Dodd (1982) first described the concept of a group of sensors to classify odours. Later, Gardner and Bartlett (1994) defined the term "electronic nose" (eNose) to describe a device that comprises an array of nonselective chemical sensors, pre-processing electronics and a computer that interprets signals from the sensors. Unlike GC-MS that identifies the specific components of a sample, the eNose investigates the sample as a whole. Therefore, it performs a qualitative analysis of gas-phase mixtures. The eNose is a device that attempts to mimic the highly complex mammalian olfactory system. The original definition states that the device recognises odours (Gardner & Bartlett, 1994). However, this definition is misleading because odourless compounds can also be detected by the device. The eNose measures chemical interactions of VOCs and a gas sensor. The result is a signal

pattern that can be called a measurement signature. Sometimes it is also referred to as a "smellprint", but the device does not smell VOCs. Furthermore, the term "chemical fingerprint" is somewhat inaccurate, as the signal pattern may not be unique to the measured gas mixture. Different gas mixtures may produce a similar signal which the computer cannot differentiate. If the same sample is measured repeatedly, slight shifts in composition might alter the measurement signature (Boeker, 2014).

In addition to being investigated in the medical field, many areas, such as agriculture (Wilson, 2014) and the food industry, (Suman et al., 2007) can utilise eNose technology. The armed forces or the police can also exploit eNoses to detect explosives (Brudzewski et al., 2012; López et al., 2017) or toxic gases (Laquintinie et al., 2018; Olguín et al., 2014; Stassen et al., 2016). In environmental monitoring, eNoses allow the detection of toxic waste (Kalinowski et al., 2013) or the monitoring of air quality (Wang et al., 2018). The application of eNose technology in different fields has been thoroughly reviewed in the published literature (Berna, 2010; Capelli et al., 2014; Hu et al., 2019; Wilson & Baietto, 2009).

Traditionally, an electronic nose detection system consists of an array of 8-32 different sensors, which are tuned to different chemical groups. The gases are then presented to the sensors through sample delivery system (S. Chen et al., 2013). The air above the sample is called the headspace from which VOCs are introduced to the sensors. When the headspace is injected into the eNose, the measured response varies between sensor types and subsequent changes in voltage, current, resistance, mass or temperature can occur (James et al., 2005; Wilson & Baietto, 2009).

2.4.1 Gas sensors

The principle of gas sensors is a change in the physical properties in the active layer of the sensor when exposed to VOCs, which is then converted to an electric signal by a transducer (Ollé et al., 2020; Patel, 2014). Classification of sensors can be made according to the types of transducers, which can be electrochemical, gravimetric, optical or thermal (James et al., 2005; Ollé et al., 2020). Different characteristics exist between sensor types that affect their sensitivity, selectivity, reproducibility and cost. The literature thoroughly covers the different sensor types, their working mechanisms, and their advantages and disadvantages (Arshak et al., 2004; James et al., 2005; Nagle et al., 1998; Nazemi et al., 2019; Ollé et al., 2020; Wilson & Baietto, 2009; Zohora et al., 2013). Thus, traditional sensors are described only briefly here.

Electrochemical sensors

Electrochemical sensors can be further divided into chemoresistive, potentiometric and amperometric sensors (James et al., 2005). The first sensor contains metal-oxide semiconductors (MOS) and conducting polymer (CP) sensors, which are probably the most common sensors used in medical studies (Baldini et al., 2020; Farraia et al., 2019; van der Sar et al., 2021). In MOS, VOCs interact with the sensing layer, which is a metal oxide. This interaction alters the conductivity of the oxide. Sensors are highly sensitive but typically require a high operating temperature and high power consumption. They also have poor long-term stability (Arshak et al., 2004; Nagle et al., 1998; Nazemi et al., 2019; A. P. F. Turner & Magan, 2004).

CP sensors have a polymer surface that interacts with VOCs, resulting in an alteration of the electrical conductivity of the polymer, which creates a measurable signal. (Nagle et al., 1998; Patel & Kunpara, 2011; A. P. F. Turner & Magan, 2004). The sensors have low power consumption, easy synthetisation and are sensitive and inexpensive. However, they are also sensitive to humidity and sensor drift occurs over time. In addition, there are variations between batches (Arshak et al., 2004; Zohora et al., 2013).

Potentiometric sensors include metal oxide silicon field effect transistors which have catalytic metals as a sensitive material. The working principle is based on a change in the threshold voltage of the sensor when in interaction with VOCs. Metal oxide silicon field effect transistors are of low cost and reproducibility is good. However, the operating temperatures affects selectivity and sensitivity. The sensors also suffer from baseline drift (S. Chen et al., 2013; Nagle et al., 1998; Patel, 2014).

Amperometric sensors measure the current between electrodes produced by the transfer of an electron to or from the analyte (Patel, 2014). Sensors have low power consumption, are resistant to humidity but are quite selective (James et al., 2005; Ollé et al., 2020).

Gravimetric sensors

Gravimetric sensors are known as piezoelectric sensors and two types are commonly used: the surface acoustic wave (SAW) device and the quartz crystal microbalance (QCM) (Chang et al., 2000; Patel & Kunpara, 2011). The function of piezoelectric sensors is based on the mechanical stress within a crystal caused by electric potential. The acoustic wave is used as the sensing mechanism. The wave goes through (QCM) or on the surface (SAW) of the sensor coating material (Arshak et al., 2004). VOCs

bind to the coating material which then causes mass change and the frequency of the sensor shifts (Chang et al., 2000). The sensors have high sensitivity and short response time, but they have complex electronics and suffer from sensor aging (Nagle et al., 1998; Zohora et al., 2013).

Optical and thermal sensors

In optical sensors, the optical properties of the sensing layer go through a change when exposed to VOCs (Ollé et al., 2020). For example, variation in light absorbance or fluorescence can be measured (James et al., 2005). Sensors are highly sensitive and do not suffer from low signal-to-noise ratio but, in turn, are complex, have a short lifetime and low portability (Ollé et al., 2020; Patel, 2014).

The working principle of thermal sensors is the detection of heat produced by catalytic oxidation of flammable gases. These devices are not widely employed in eNoses as they are optimized to detect high concentrations of VOCs. Furthermore, they have high power consumption and short lifetime (James et al., 2005; Ollé et al., 2020).

2.4.2 eNose in the medical field

Electronic noses have been widely investigated in the medical field because they are non-invasive, simple and cheap devices. Although a variety of possible biomedical applications exist (Wilson & Baietto, 2011), the main focus is probably on disease diagnostics. Many review articles have covered studies dealing with the biomedical applications of eNoses (Azim et al., 2019; Baldini et al., 2020; Dragonieri et al., 2017; Farraia et al., 2019; Hintzen et al., 2021; Krilaviciute et al., 2015; Scarlata et al., 2015; van der Sar et al., 2021). In their review article, Farraia et al. (2019) covered the eNose studies in disease diagnostics and broke them down into five categories. These categories included airway obstructions, respiratory infections, inflammatory diseases, cancer and other diseases. In total, 81% of the studies used the Cyranose 320 (Sensigent, California, USA), which is based on CP sensors.

In the category of airway obstruction, the most studied diseases are chronic obstructive pulmonary disease (COPD) and asthma (Farraia et al., 2019), which is probably due to the prevalence of these diseases and the potential ease of diagnostics via breath analysis with an eNose. For example, Fens et al. (2009) utilised the Cyranose 320 and examined 20 asthma patients, 30 COPD patients and 40 healthy

controls divided into two subgroups based on whether they smoked or not. Asthma was distinguished from COPD with an accuracy of 96% after leave-one-out cross validation (LOOCV). However, when all groups were compared in one model, the accuracy fell to 56%.

Studies on respiratory infections include the detection of pneumonia (Hockstein et al., 2004; Schnabel et al., 2015) and mycobacterium tuberculosis (Fend et al., 2006; Saktiawati et al., 2019). Furthermore, a recent review evaluated VOC-based breath analysis for the detection of COVID-19 infection using both eNoses and GC-MS. The cumulative sensitivity was 98% and specificity 74%. Subgroup analysis showed a higher sensitivity for eNoses, but the specificity was better with GC-MS (Subali et al., 2022).

The detection of cancer by eNose is a potential field for further research, as the eNose could facilitate early diagnosis. In their review article, Baldini et al. (2020) included 60 studies that covered the detection of cancers from exhaled breath with eNoses. Of these, most studies focused on lung cancer. Again, the most used eNose was the Cyranose 320 followed by MOS-based sensors such as the Aeonose (The eNose Company, Zutphen, The Netherlands). Overall, the ability of eNoses to detect cancers is good, although wide heterogeneity exists. For instance, an electronic nose can determine patients with colorectal, bladder and lung cancers from healthy controls with a sensitivity and specificity rates of between 71-85% and 76-100% (Bassi et al., 2021; D'Amico et al., 2010; de Meij et al., 2014; Di Natale et al., 2003; Machado et al., 2005; van de Goor et al., 2018). Furthermore, according to a recent meta-analysis, eNoses can detect cancers from exhaled breath with a sensitivity of 90% and specificity of 87%. Most of these studies, however, were feasibility studies with substantial heterogeneity (Scheepers, Al-Difaie, Brandts, et al., 2022).

In a systematic review and meta-analysis evaluating the overall accuracy of eNoses in detecting diseases via breath analysis, 44 studies were included covering 5728 patients. The pooled sensitivity was 90% with moderate heterogeneity, the pooled specificity was 88% with high heterogeneity and the diagnostic odd ratio was 40.7, suggesting high accuracy. However, the reporting of the accuracy was not standardized (Yang et al., 2021).

Some studies have investigated the use of the eNose in upper airway disease diagnostics. Table 1 and Table 2 present data on the studies related to otorhinolaryngologic diseases and eNoses based on gas-sensors. As can been seen in Tables 1 and 2, most of the studies are case-control types that evaluate the accuracy of an eNose in disease detection. The majority of the studies utilise CP-based or MOS sensors.

Although eNose technology is commercially available for use in several different fields, such as detecting toxic gases (environics.fi), the application of the technology in the medical field is lacking. To the author's knowledge, to date, only a few devices (April 2022) are available. The DSA BreathPass™ device produced by the Finnish company, Deep Sensing Algorithms (Tampere, Finland), has recently received medical CE certification. The device uses nanosensors and is designed to detect COVID-19 in exhaled human breath (dsa.fi). In the USA, the Food and Drug Administration (FDA) has given emergency use authorisation for the InspectIR COVID-19 Breathalyzer (InspectIR Systems, LLC, Frisco, Texas, USA) to detect COVID-19 in an authorised setting. The device is based on GC-MS (inspect-ir.com). Another mass spectrometry device for the detection of COVID-19 is the BreFence™ Go (Breathonix, Singapore) which has received Singapore's Health Sciences Authority's Provisional Authorisation (breathonix.com).

Table 1. Studies concerning otorhinolaryngologic diseases (excluding cancers) and gas sensor-based electronic noses.

Reference	Aim	Device	Participants	Sample	Accuracy
Aronzon et al., 2005	To distinguish CSF from serum	Cyranose 320 (CP-based sensor)*	*n* = 10	CSF fluid and serum	N/A
Broza et al., 2018	To diagnose CRS	Nanomaterial-based sensor	CRSsNP = 17 CRSwNP = 24 HC = 30	EB	CRS vs HC = 90% CRSsNP vs. HC = 86% CRSwNP vs. HC = 81%
Bruno et al., 2008	To diagnose CRS	zNose™ (GC and SAW-based sensor)	CRS = 14 HC = 14	EB + a tampon held in the middle meatus	N/A
Dragonieri et al., 2019	Discrimination between AR with and without asthma and controls	Cyranose 320	Validation set: AAR = 7 AR = 7 HC = 7	EB	AAR vs AR = 83% AAR vs HC = 67% AR vs HC = 77%
Dutta et al., 2005	To distinguish swab samples of MRSA, MSSA and C-NS	Cyranose 320	MRSA = 50 MSSA = 50 C-NS = 50	Swab samples	96-100%
Lai et al., 2002	To distinguish different bacterial species	Cyranose 320	In vitro study	Swabs containing bacterial isolate	N/A

Table 1. Continued.

Reference	Aim	Device	Participants	Sample	Accuracy
Mohamed et al., 2003	To diagnose CRS	LibraNose (QCM-based sensor)**	CRS = 5 HC = 5	EB + a tampon held in the middle meatus	60%
Saidi et al., 2015	To diagnose AR	eNose based on semiconducting sensors	AR = 5 HC = 16	EB	99%
Shykhon et al., 2004	To identify pathogens associated with ear, nose, throat infections	Cyranose 320	*n* = 90	Swab samples	71-88%
Thaler et al., 2000	To distinguish CSF from serum	eNose based on semiconducting sensors	*n* = 19	CSF fluid and serum	95%
Thaler & Hanson, 2006	To diagnose bacterial rhinosinusitis	Cyranose 320	Second period: ARS = 34 HC = 34	EB	72%
Thaler et al., 2008	To distinguish biofilm-producing bacteria from non-biofilm-producing bacteria of the same species	Cyranose 320	*In vitro* study	Glass vials containing bacterial strains	72-100%

AAR: allergic rhinitis with astma; AR: allergic rhinitis; ARS: acute rhinosinusitis; C-NS: patients with an infection caused by coagulase-negative *staphylococci*; CP: conducting polymer; CRS: chronic rhinosinusitis; CRSsNP: chronic rhinosinusitis without nasal polyps; CRSwNP: chronic rhinosinusitis with nasal polyps; CSF: cerebrospinal fluid; EB: exhaled breath; eNose: electronic nose; GC: gas-chromatography; HC: healthy control; N/A: not reported; MRSA: patients with an infection caused by methicillin-resistant *Staphylococcus aureus*; MSSA: patients with an infection caused by methicillin-susceptible *Staphylococcus aureus*; QCM: quartz crystal microbalance; SAW: surface acoustic wave.

*Cyranose320 (Sensigent, California, USA);

**LibraNose (University of Rome Tor Vergata, Italy);

***zNose™ (Electronic Sensor Technology Inc., California, USA).

Table 2. Studies concerning head and neck cancers and gas sensor-based electronic noses. Exhaled breath was used as a sample in all studies.

Reference	Aim	Device	Participants	Accuracy
Scheepers, Al-Difaie, Wintjens, et al., 2022	To differentiate TC from benign thyroid disease	Aeonose*	TC = 48 Benign thyroid disease = 85	71%
Gruber et al., 2014	To diagnose HNSCC	GC-MS and nanomaterial-based sensor	HNSCC = 22 BT = 21 HC = 19	HNSCC vs. HC = 83% HNSCC vs. BT = 84% BT vs. HC = 73%
Hakim et al., 2011	To distinguish HNC cancer from LC and HC	NA-NOSE GC-MS	NA-NOSE set: HNC = 16 LC = 20 HC = 26	HNC vs. HC = 95% LC vs. HC = 96% HNC vs. LC = 100%
Lang et al., 2016	To distinguish HNSCC (before and after surgery) from HC	Nanomechanical membrane sensors	HNSCC = 3 HC = 4	N/A
Leunis et al., 2014	To distinguish HNSCC from HC	Metal-oxide sensor	HNSCC = 36 HC = 23	88%
N. Mohamed et al., 2021	To distinguish OSCC from HC	Aeonose	OSCC = 49 HC = 35	79%
van de Goor et al., 2017	To discriminate HNSCC from BC and CC	Aeonose	HNSCC = 100 BC = 40 CC = 28	HNSCC vs. CC = 81% HNSCC vs. BC = 84% BC vs CC = 84%
van de Goor et al., 2019	To diagnose locoregional recurrent or new primary HNSCC after curative treatment	Aeonose	recurrent HNSCC = 20 HNSCC without recurrence = 20	83%
van de Goor et al., 2020	To distinguish HNSCC from HC	Aeonose	HNSCC = 91 HC = 72	72%
van Hooren et al., 2016	To distinguish HNSCC from LC	Aeonose	HNSCC = 53 LC = 34	85%
Witt et al., 2012	To distinguish HNC from HC	Metal-oxide sensor	HNC = 10 HC = 13	82%

BC: bladder cancer; BT: benign tumour; CC: colon cancer; GC-MS: gas-chromatography mass spectrometry; HC: healthy control; HNC: head and neck cancer; HNSCC: head and neck squamous cell carcinoma; LC: lung cancer; NA-NOSE: Nanoscale Artificial Nose; OSCC: oral squamous cell carcinoma; TC: thyroid cancer.

*Aeonose (the eNose Company, Zutphen, the Netherlands).

2.5 Ion mobility spectrometry and differential mobility spectrometry

2.5.1 Ion mobility spectrometry

Ion mobility spectrometry (IMS) is not considered to be an eNose in the strictest sense because it is not based on a traditional multisensor array (Wen et al., 2018; Wilson & Baietto, 2009). IMS provides analogous information from gas mixtures (Röck et al., 2008). It is widely used for the detection of chemical warfare agents and explosives (Ewing et al., 2001). It is also exploited in the food industry, for example, for the detection of pesticides, veterinary drugs and bacteria, and the control of food freshness and quality (Hernández-Mesa et al., 2017; Vautz et al., 2006).

The working principle ion mobility spectrometry (IMS) is separation of ionic mixtures and characterization of ions by some property of their transport through a carrier gas in an electromagnetic field (Shvartsburg, 2008). First, the sample molecules are ionized. Usually, the ionization is done by Nickel-63, but other methods do exist (Borsdorf & Eiceman, 2006). Ions are then injected into an electrical field (E) where they move with a certain drift velocity (v) towards a detector. The movement is against a counterflow of dry, neutral drift gas, which is usually nitrogen or air (Borsdorf & Eiceman, 2006). IMS measures time-of-flight, which is the time an ion spends in the drift tube. Time-of-flight depends on the mass, shape, cross-sectional area and charge of the ion. Those ions that collide with buffer gas have more often greater friction and, therefore, lower terminal velocity (D'Atri et al., 2018.). Thus, these ions arrive at the detector later than the smaller ions. Although drift tube IMS is considered the classic IMS, other instrumentation types, such as travelling wave IMS and trapped IMS, exist (Cumeras et al., 2015; D'Atri et al., 2018; Dodds & Baker, 2019).

IMS has many advantages. For example, power consumption is low, and the device can be miniaturised and can operate at ambient pressure. Ambient air serves as the carrier gas. Response time is fast, and IMS has high sensitivity (Borsdorf & Eiceman, 2006). A major drawback of IMS is that when different ions have a similar size, mass and charge, they may not be separated from each other. Background noise can also complicate separation specificity (Borsdorf & Eiceman, 2006).

2.5.2 Differential mobility spectrometry

Differential mobility spectrometry (DMS), also known as field asymmetric ion mobility spectrometry (FAIMS), is a technology that can enhance the separation power of IMS. Although the terms DMS and FAIMS are used synonymously, DMS refers to an analyser with a planar micro-fabricated design and FAIMS to a cylindrical design (Kolakowski & Mester, 2007). Nevertheless, ion separation in both designs is based on field-dependent mobilities that are essentially the same (Borsdorf & Eiceman, 2006).

The history of DMS and FAIMS is covered in an article by Schneider et al. (2016). In a drift tube IMS, the drift velocity v of ions can be expressed as follows:

$$v=KE \tag{1}$$

where K represents a mobility coefficient (Anttalainen et al., 2018). However, K is only constant in low electrical fields, whereas in strong electric fields with high-frequency asymmetric waveforms, it is field-dependent (Cumeras et al., 2015; Schneider et al., 2016). Thus, in strong electric fields the drift velocity of ions becomes a non-linear function of the electrical field, which is the basis of the separation of ions in DMS (Anttalainen et al., 2018).

In practice, sample vapour is ionised, and the ions are passed into a channel along which they travel as in IMS. The carrier gas is usually ambient air which is provided from a pure air generator with a mass flow controller. The channel is called the separator and is formed by two plates which are metal electrodes on ceramic plates (Borsdorf & Eiceman, 2006). A separation voltage (U_{SV}) is applied and an asymmetric radio frequency field, containing a high-amplitude positive phase and a low-amplitude negative phase, is created between the plates in an asymmetric fashion. The high-field phases have shorter duration than the low-field phases, resulting in their net voltage being similar (Borsdorf et al., 2011). Ion motion is perpendicular to the electric field. In one phase, ions move toward one electrode and then toward an opposite electrode in another phase. As a result, ions will zig-zag in the channel. If the mobility of the ions is different between the high- and low-fields, they will move toward one electrode. If the difference is great enough, the ions will hit the electrode and be lost. If the mobility is the same in the phases, the ions will pass through the channel to the detector, which is an electrometer. When the ions reach the detector, they generate an electrical current. The detection of positive and

negative ions can be done simultaneously (Borsdorf et al., 2011; Schneider et al., 2016).

The oscillating motion of the ions will eventually lead to a collision with the electrodes and the annihilation of the ions (Borsdorf et al., 2011; Krylov et al., 2007). To counter this effect, a compensation voltage (U_{CV}), which is superimposed on the electrical field to allow the transverse drift velocity of the ions to be zero, is applied (Krylov et al., 2007). Different U_{SV} and U_{CV} values are scanned and the ion current emerging from the channel at each value can be recorded. This enables the detection of certain ions and the exclusion of others (Krylov et al., 2007). Also, the electric field strength can be altered, and similar scanning as with the compensation voltage performed. These scans and ion current recordings at each scan should reduce the signal-to-noise ratio and improve detection limits (Kolakowski & Mester, 2007). The working principle of DMS is shown in Figure 1.

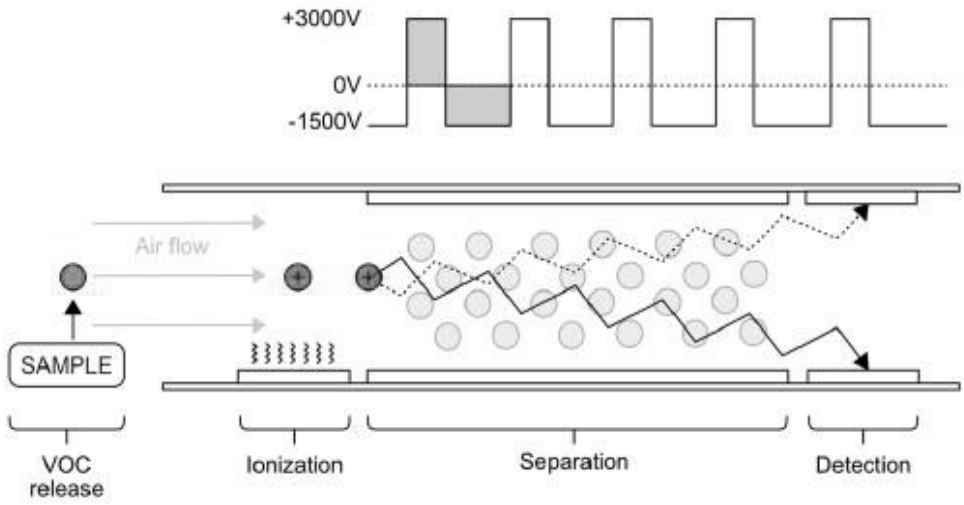

Figure 1. The working principle of differential mobility spectrometry. Volatile organic compounds (VOCs) that evaporate from the sample are driven with a flow of air. They are given an electric charge by ionisation, allowing discrimination of the molecules according to their charge. The collisions with the medium also discriminate the molecules according to their size and shape. The molecules then enter the separation phase, where they are exposed to intermittent high- and low-electric fields. Ionised molecules move towards the oppositely charged plate at different speeds in the low and high fields, resulting in another dimension for separation. After separation, the molecules collide with the detector, resulting in a signal. V: volt. (I)

The result of a scan is a multidimensional signal print, also known as a dispersion plot, that depicts the measurement signature of the chemical mixture. It is a data

matrix, where every pixel represents an electrical current produced by ions colliding with the detector, each with a certain value of electric field voltage and U_{CV}. The x-axis shows the U_{CV} values and the y-axis the U_{SV} values. Examples of dispersion plots are shown in Figure 2.

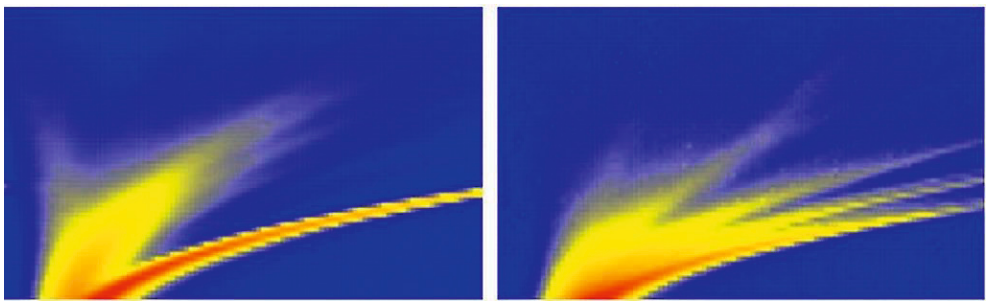

Figure 2. Example of a dispersion plot and both positive (left) and negative (right) ions are shown. Nasal air was used as a sample material.

As with traditional eNoses, the output of DMS is a qualitative analysis of the sample and does not reveal the specific components. DMS detectors are highly sensitive and specific and have sufficient intra device stability. Although DMS is more expensive than many eNoses, it is still relatively cheap. Moreover, as with most eNoses, it can perform a fast analysis at room temperature. Miniaturized DMS devices, which allow better field use, have been built (Wilks et al., 2012).

2.5.3 Studies in the medical field

Like traditional eNoses, both IMS and DMS have gained popularity in studies regarding disease diagnostics. For example, Roine et al. (2014) used the ChemPro®100 device (Environics, Mikkeli, Finland), which is an eNose based on IMS and MOS. They analysed urine samples from 50 patients with prostate cancer and 15 patients with prostate hyperplasia. The eNose could distinguish samples with a sensitivity of 78% and specificity of 67%. In another study, the same device was used to discriminate the four most common urinary tract infection pathogens. After LOOCV, the bacterial plates could be distinguished from sterile plates with a sensitivity of 95% and specificity of 97% (Roine, Saviauk, et al., 2014). Furthermore, the ChemPro®100 could distinguish six relevant wound infection pathogens *in vitro*, including methicillin-sensitive *Staphylococcus aureus* (*S. aureus*) and methicillin-resistant *S. aureus,* with a sensitivity of 83% and specificity of 100% (Saviauk et al., 2018).

A formerly commercial FAIMS apparatus called the Lonestar VOC Analyzer (Owlstone Medical Ltd., Cambridge, UK) was able to distinguish patients with diabetes from healthy ones. If samples were stored for less than a year, sensitivity was 92% and specificity 100% (Esfahani et al., 2018). Furthermore, FAIMS was used to analyse urine samples collected from patients with pancreatic cancer, pre-malign lesion of the pancreas, acute and chronic pancreatitis, and healthy controls. FAIMS distinguished pancreatic cancer from healthy controls with a sensitivity of 79% and specificity of 79%. All the pancreatic diseases were distinguished from healthy controls with a sensitivity of 91% but with specificity of only 47% (Nissinen et al., 2019). Colorectal cancer has been discriminated from healthy controls by analysis of urine samples with a sensitivity of 88-100% and specificity of 60-92% (Arasaradnam et al., 2014; Mozdiak et al., 2019). When colorectal cancer samples were compared to three categories (low, intermediate, and high risk) of adenomas, the sensitivity was 75-83% and specificity was 75-100%. However, the sample size was only 7-12 patients in each group (Mozdiak et al., 2019). Urine was also analysed with FAIMS in a study by Niemi et al. (2018) in which samples from patients with malignant ovarian tumours (n = 33) were distinguished from controls (n = 18) with a sensitivity of 91% and specificity of 63%.

The ENVI-AMC™ (Environics Ltd., Mikkeli, Finland) is a differential mobility spectrometer which was originally designed for the ultra-low-level detection of ammonia and acids in clean room facilities. Two studies examined whether an analysis of diathermy smoke with the device could distinguish tumours when *ex vivo* samples were incised (Haapala et al., 2019; Sutinen et al., 2019). A specific smoke analysis system was used in these studies (Kontunen et al., 2018). Diathermy smoke from benign and malignant brain tumours could be discriminated from a control sample containing haemorrhagic or traumatically damaged brain tissue with a sensitivity of 78% and specificity of 89% (Haapala et al., 2019). In a study by Sutinen et al. (2019), the ENVI-AMC™ device was able to discriminate breast cancer from benign samples with a sensitivity of 80% and specificity of 90%. Recently, the feasibility of an analysis of diathermy smoke with the ENVI-AMC™ device in tissue identification was tested *in vivo* during breast tumour surgery. The accuracy of the device to distinguish four tissue types was 44% and showed high variation between surgeries (Kontunen et al., 2021).

As mentioned previously, several studies have been published on DMS/FAIMS in the medical area. To date, however, no studies have been made on diseases in the field of otorhinolaryngology.

2.6 Data-analysis

2.6.1 Pre-processing

An electronic nose produces a digital output of the sample. The digital output comprises raw data that need to be further analysed and interpreted (Wilson & Baietto, 2009). Usually, some sort of data pre-processing and dimensionality reduction is performed before classification of the sample can be performed (Scott et al., 2006). Pre-processing may include compensation of sensor drift, noise filtering and data normalisation (Gutierrez-Osuna, 2002; Zhao et al., 2008). To find descriptive parameters from a high dimensionality sensor signal, some sort of dimensionality reduction is preferable to transfer the signal to a low dimensional feature space (Zhao et al., 2008).

2.6.2 Classification

In a similar way to data processing, a vast number of techniques for the classification of the analysed data exist. These include principal component analysis (PCA), linear discriminant analysis (LDA), *k*-nearest neighbour (kNN) and artificial neural networks. These techniques are described thoroughly in the literature (Berrueta et al., 2007; Gutierrez-Osuna, 2002; Scott et al., 2006; Zhao et al., 2008). However, no single method for dimension reduction and classification is superior to eNose technology in breath analysis (Leopold et al., 2015). Therefore, depending on the sensor and application, different methods should be tested.

Data classification, also known as model building, can be divided into supervised learning and unsupervised learning processes (Zhao et al., 2008). In the former, the classifier is given known examples of the data. With these examples, the classifier learns to map the data into classes and a model is built. In contrast, in unsupervised learning, class labels in the training set are unknown and the classifier aims to find similarities in the structure of the data and to make decisions based on these similarities (Alpaydin, 2014).

2.6.3 Validation

An error in a machine learning model can be due to bias or variance. When there is bias, the model oversimplifies the data and does not work well with new data. Bias usually decreases when the complexity of the model increases, meaning that the training error decreases. However, variance can also increase and the model may concentrate too much on the training data and may learn features that do not represent real-life data. In such cases, the model is poorly generalisable. This problem is called overfitting. If there is bias, the model is underfit to the data, and this is called underfitting (Alpaydin, 2014).

To avoid underfitting and overfitting and to find the optimal model complexity, validation is executed. The most robust method of validation is external validation, where the data set is divided into training and test sets (a so-called holdout method) (Han et al., 2011; Marco, 2014). The training set is used for the model building and the classification is done with the separate test set (Broadhurst & Kell, 2006). Usually, two-thirds of the data are used in the training set and the remaining one-third in the test set. Sometimes the terms "validation" and "test" sets are interchangeable. However, this can lead to confusion because the validation set is also meant as a set that is used to optimize the model structure and, therefore, is a part of the model building. The test set is completely independent of the model building (Broadhurst & Kell, 2006; Marco, 2014).

In real life, there is not always enough data. In such cases, cross validation (CV) can be used to estimate the prediction error. It is also known as internal validation, meaning that the classifier is validated using the same data set that the classifier is built on. Thus, it may give overoptimistic results because the model building is made with the same data (Broadhurst & Kell, 2006; Marco, 2014).

In k-fold CV, the data is randomly divided into k (e.g., five or ten) approximately equally sized folds. Training and testing are performed k times. Each time, each fold is reserved as a test set and the rest as a training set. This means that each fold is used the same number of times as the training and test sets. The predictive accuracy estimate is the average of the correct classification from all the runs (Han et al., 2011). LOOCV is one type of k-fold CV in which the k equals n (the number of samples). The model is built with all but one sample (k-1), which is used to test the model. The procedure is repeated k times with every sample being the test sample once (Alpaydin, 2014; Han et al., 2011). LOOCV is almost unbiased, but it can have high variance. In contrast, ten-fold CV gives more biased but less variable estimates.

LOOCV is computationally intensive and requires many models to be built. Sometimes it may also give overoptimistic results (Marco, 2014).

Although CV is considered crucial (Marco, 2014), a literature review regarding the investigation of exhaled breath and eNoses yielded 46 studies in which internal validation was performed in 29 (63%) studies and external validation in only 7 (15%) (Leopold et al., 2015). Likewise, in their review, Farraia et al. (2019) found that external validation with a newly recruited population was done in only 10% of studies.

2.7 Rhinosinusitis

Rhinosinusitis is a symptomatic inflammation of the paranasal sinuses and nasal cavity mucosa. In adults, it is defined by prevalence of two or more symptoms, one of which should be either nasal blockage/obstruction/congestion and/or nasal discharge. Additionally, facial pressure or pain and/or reduction or loss of smell exist. In clinical definition, there should be either a CT scan showing mucosal changes in the osteomeatal complex and/or paranasal sinuses and/ or endoscopic signs such as nasal polyps, mucopurulent discharge primarily from the middle meatus or middle meatal oedema/mucosal obstruction (Fokkens et al., 2020). Rhinosinusitis is further divided to ARS and CRS according to the duration of symptoms.

In ARS, symptoms are of sudden onset and last less than 12 weeks (Fokkens et al., 2020). Other guidelines suggest that the duration of symptoms should be up to four weeks (Chow et al., 2012; Orlandi et al., 2021; Rosenfeld et al., 2015). When symptoms last 4-12 weeks the condition is sometimes called subacute rhinosinusitis, although the use of the term is rare. If the patient has four or more episodes of ARS per year without resolution of symptoms between episodes, the term recurrent ARS is used. (Rosenfeld et al., 2015.) If symptoms last at least 12 weeks, the condition is called chronic rhinosinusitis (CRS).

2.8 Acute rhinosinusitis

2.8.1 Epidemiology

The incidence of ARS is unclear. In a questionnaire study conducted in Asia, it was estimated that 6–10% of cases in outpatient practice are due to ARS. Respondents included general physicians, otorhinolaryngologists and paediatricians (D. Y. Wang et al., 2011). However, the percentage is quite high in comparison to other studies. For example, ARS was diagnosed in 0.5% of all outpatient visits among adults in the USA during a 2000-2009 study period (Fairlie et al., 2012). In Finnish primary care, ARS accounted for 0.4% of all visits in 2019 (Finnish Institute for Health and Welfare, 2019).

2.8.2 Socioeconomic aspects

Rhinosinusitis causes a significant burden on the healthcare system. For example, the decreased quality of life in patients with rhinosinusitis leads on average to 6 missed workdays annually (Bhattacharyya, 2009). Missed workdays and visits to the doctor do not come without costs. It has been reported that one episode of ARS has a direct cost of 266 € (converted from Swedish krona in May 2011). Indirect costs varied greatly between individuals, ranging from 0 € to 4752 €. (Stjärne et al., 2012). An evaluation of medical claims data in the United States of America between 2003 and 2008 showed that the direct costs of recurrent ARS are approximately 1100 dollars per patient-year. The indirect costs of ARS are also likely to be significant (Bhattacharyya et al., 2012).

2.8.3 Aetiology

The most frequent cause of ARS is viral, associated with upper respiratory infection, and is found in 27-84% of ARS cases (Chow et al., 2012; Orlandi et al., 2021). This wide variation may be explained by the different techniques used for viral identification. The most common causative virus for ARS is rhinovirus. Other causative viruses include influenza and parainfluenza viruses, respiratory syncytial virus, and coronavirus (Mäkelä et al., 1998; Monto, 2002).

A common statement is that bacterial ARS (ABRS) complicates 0.5-2% of viral upper respiratory infections. These numbers are citations as made clear in the meta-analysis in which they were traced to two original articles (S. Smith et al., 2015). In the first, a study by Dingle et al. (1964), 53 (0.5%) of 11 134 patients with a common cold had diagnosed sinusitis based on clinical criteria. In the second study, the authors examined 100 patients with a common cold or "other acute ENT infection" and found purulent fluid in sinus aspiration in two patients (2%) (Berg et al., 1986). Patients were, however, excluded if they had paranasal symptoms suggesting sinusitis. The prevalence of ABRS is probably higher than the commonly stated 0.5-2%. When using cultures from antral swabs as a reference, the bacterial prevalence was 61%. In contrast, the bacterial prevalence was 33% when endoscopic middle meatal sampling was used as a reference (S. Smith et al., 2015). A cohort study found bacterial cause in 40% of sinus aspirates if the patient with ARS symptoms had air-liquid level, gas bubbles, or total opacification of either maxillary sinus in cone beam computed tomography (CBCT) imaging. Of all the patients with ARS symptoms, however, the bacterial cause was found only in 16% of cases (Autio et al., 2016). In a meta-analysis by Ebell et al. (2019), the authors concluded that the prevalence of ABRS with clinically suspected ARS is 31% when bacterial culture from sinus puncture fluid is used as a reference.

Streptococcus pneumoniae (*S. pneumoniae*), *Haemophilus influenzae* (*H. influenzae*), *Moraxella catarrhalis* (*M. catarrhalis*) and *S. aureus* account for 84% of ABRS pathogens (Payne & Benninger, 2007). However, in odontogenic rhinosinusitis, the presence of anaerobic bacteria and oral microbial findings are the most prevalent (Brook, 2005; Wuokko-Landén et al., 2019). A recent Finnish study found dental origin to be the probable cause of ARS in 15% of patients (Wuokko-Landén et al., 2019). Moreover, based on imaging findings, the prevalence in unilateral rhinosinusitis is even higher at 40-73% (Vestin Fredriksson et al., 2017; Matsumoto et al., 2015).

2.8.4 Pathophysiology

The pathophysiology of ARS is unclear. A typical causative agent is a virus which attaches to the nasal epithelium. The attachment initiates a complex host defence mechanism and inflammatory response, which then leads to mucosal oedema, mucus production and sinus obstruction. If mucociliary function is impaired, it may be lead to secondary bacterial infection (Fokkens et al., 2020).

Guidelines consider that viral ARS precedes ABRS (Chow et al., 2012; Fokkens et al., 2020). However, it is a difficult task to distinguish these from each other. In a study in the Finnish population, abnormalities in paranasal mucosa and occlusion of the osteomeatal complex in CBCT scans already appeared in the first days of the disease and remained virtually the same at days 9 and 10 in both viral ARS and ABRS (Autio et al., 2016). A comparison of symptom scores and CBCT findings show a gradually increasing discrepancy between viral ARS and ABRS during the first few days of infections, suggesting a potential bacterial presence at an early phase of ARS (Autio et al., 2016). Findings of *H. influenzae* in the middle meatus early in an ARS episode led to worse disease scores and prolonged symptoms. Furthermore, it correlated with a wider spread of the disease to the paranasal sinuses. Therefore, bacteria may affect the pathogenesis from the beginning of ARS (Autio, Tapiainen, et al., 2015).

2.8.5 Diagnostics

It is important to differentiate viral and bacterial ARS to avoid unnecessary antibiotic treatment. However, due to difficulties in diagnostics, medical professionals overprescribe antibiotics in more than 80% of cases ARS (Benninger et al., 2016; Pouwels et al., 2018; Sharma et al., 2017). A Cochrane review found the effects of antibiotics on uncomplicated ARS based on clinical diagnosis were limited, which could be related to the difficulties in distinguishing ABRS and viral ARS (Lemiengre et al., 2018).

Symptoms and clinical examination

Typical symptoms related to ARS are nasal obstruction/congestion, anterior or posterior nasal discharge, facial pain/pressure, headache and reduction or loss of smell. Other systemic or distant symptoms are fever, fatigue, cough and dysphonia (Fokkens et al., 2020). Although unreliable, guidelines advise symptom-based differential diagnostics (Fokkens et al., 2020; Orlandi et al., 2021; Rosenfeld et al., 2015). However, a meta-analysis by Engels et al. (2000) found no study that compared bacterial culture from sinus aspirate and symptoms for ABRS diagnostics. A later meta-analysis investigated symptom duration and purulent rhinorrhoea in differential diagnostics and found no evidence to support their use (van den Broek et al., 2014). ABRS may develop earlier than 7-10 days as shown in the differences

in local and systemic biomarkers and CBCT and symptom scores (Autio et al., 2016, 2017). According to a systematic review, fever and facial or dental pain cannot be used to differentiate ABRS and viral ARS (Hauer et al., 2014). In another study, dental pain was specific for ABRS but was present in only 4% of patients (Autio, Koskenkorva, et al., 2015).

In a Finnish study, the authors compared symptoms and physical findings to bacterial cultures from sinus aspirates. They found that none of the patient-reported symptoms at 10 days were ideal for identifying ABRS (Autio, Koskenkorva, et al., 2015). According to guidelines, a worsening of symptoms ("double sickening") could predict ABRS (Chow et al., 2012; Rosenfeld et al., 2015) but it only improves diagnostic accuracy slightly (Autio, Koskenkorva, et al., 2015).

Clinical examination may include anterior rhinoscopy, evaluation of the posterior pharynx and palpation of the skin over the sinuses searching for tenderness of the cheek or teeth (Fokkens et al., 2020; Rosenfeld et al., 2015; Sinusitis: Current Care Guidelines, 2018). These examinations are easy to perform in primary care. At 9-10 days after the onset of symptoms, a moderate amount or a profuse secretion present in either nasal passage in anterior rhinoscopy predict ABRS quite well. If no such secretion is present, ABRS can be ruled out. Furthermore, any secretion in the posterior pharynx increases the likelihood of ABRS. Additionally, cervical adenopathy can also predict ABRS (Autio, Koskenkorva, et al., 2015).

Nasal endoscopy, using a rigid or a flexible endoscope after a local anaesthetic and a decongestant, provides a great view of the whole nasal cavity, turbinates, middle meatus, sphenoethmoidal recess, sphenoid ostium and nasopharynx. Secretions seen endoscopically in the middle meatus at 9-10 days after the start of the symptoms predict ABRS (Autio, Koskenkorva, et al., 2015).

Bacterial cultures

Although maxillary sinus puncture and aspiration of the contents are the gold standard of ABRS diagnostics, they are not usually performed due to the discomfort caused to the patient and the possible lack of expertise in performing the procedure in primary care. Additionally, maxillary sinus puncture and aspiration only reveals pathogens in the maxillary sinus, which may differ from those in the other sinuses. International guidelines do not recommend the procedure be performed routinely (Chow et al., 2012; Desrosiers et al., 2011). Despite the relative discomfort and possible morbidity caused by maxillary puncture, it is well-tolerated by the patient (Blomgren et al., 2015). The Finnish guidelines suggest performing the puncture if

the patient has severe symptoms or when other treatments have failed. Moreover, in addition to obtaining cultural samples, the pain caused by pressure may be alleviated (Sinusitis: Current Care Guidelines., 2018).

As maxillary puncture produces some discomfort, alternative methods are desirable. A review revealed that in one study nasal swabs taken from the nasal cavity, inferior meatus or middle meatus yielded a correlation of 91% compared to the cultures acquired by maxillary sinus punctures (Jousimies-Somer et al., 1989), but only 42-65% in others (Benninger et al., 2002). In 57% of cases, cultures from the nasopharynx correlate with maxillary sinus puncture and culture of the contents (Thunberg et al., 2013). Possibly due to the mixed results, acquiring nasal swab cultures is not recommended (Chow et al., 2012; Desrosiers et al., 2011).

Endoscopically derived middle meatal cultures (EDMMC) are non-invasive compared to maxillary puncture. Additionally, they provide information on possible pathogens in other paranasal areas, such as the ethmoids and frontal sinuses (Benninger et al., 2006). Two meta-analyses support the use of EDMMC in the diagnostics of ARS and CRS. It was concluded that EDMMC had an accuracy of 73-76% when compared to cultures obtained by maxillary sinus punctures (Benninger et al., 2006; Dubin et al., 2005). Specifically, in ABRS, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 81%, 71%, 78% and 75%, respectively. (Benninger et al., 2006). When compared to CT-findings, EDMMC had a sensitivity of 93% and specificity of 80% (Elwany et al., 2012).

Imaging

Different imaging modalities, such as plain radiographs, CT, CBCT and magnetic resonance imaging, provide information on the nasal cavities and paranasal sinuses. When imaging is negative, ARS can be ruled out. However, positive findings, such as mucosal thickening or air-fluid level, do not help to differentiate ABRS and viral ARS (Autio et al., 2016; S. Smith et al., 2015). Indeed, positive findings are common even in upper respiratory infections (Gwaltney et al., 1994)). As a result, routine imaging is not recommended (Desrosiers et al., 2011; Fokkens et al., 2020; Orlandi et al., 2021; Rosenfeld et al., 2015).

Plain radiographs have limited usefulness and are not recommended (Fokkens et al., 2020; Rosenfeld et al., 2015). Magnetic resonance imaging is costly and time consuming and lacks bony detail and is not the first-line modality either (Kirsch et al., 2017).

CT imaging gives the best bony anatomic detail of the paranasal sinuses (Kirsch et al., 2017) and is recommended when planning surgical intervention or in cases of suspected complications (Bayonne et al., 2009; Fokkens et al., 2020; Rimmer et al., 2019). The downside of CT is the ionizing radiation dose. CBCT has a significantly lower radiation dose than traditional or low dose CT (Al Abduwani et al., 2016). Further, although it shows bony details efficiently, it lacks proper soft tissue resolution (Kirsch et al., 2017). CBCT is, however, a great alternative to CT in uncomplicated cases to assess sinus anatomy and pathology (Kirsch et al., 2017) or when planning surgical interventions.

Ultrasonography

An ultrasound device provides a way of showing fluid in the maxillary sinus with a sensitivity of 68-85% and specificity of 72-82% compared to maxillary sinus puncture or imaging findings (Ebell et al., 2016; Varonen et al., 2000). A positive finding does not predict ABRS well, however (Autio, 2017). Furthermore, ultrasound has limited usefulness in the diagnostics of ARS. The Finnish guidelines suggest relating the findings to patient symptoms (Sinusitis: Current Care Guidelines., 2018). Interestingly, international guidelines do not find this necessary (Orlandi et al., 2021) or fail to mention it at all (Chow et al., 2012; Desrosiers et al., 2011; Rosenfeld et al., 2015).

Inflammatory biomarkers

A systematic review found the sensitivity of C-reactive protein, white blood cell count and erythrocyte sedimentation rate in the diagnosis of ABRS to be 34%, 25% and 43%, respectively. Specificity was also better, varying between 83-88% (Ebell et al., 2016). In a review article, elevated C-reactive protein and erythrocyte sedimentation rate predicted bacterial ARS well, but sensitivity remained low. The authors of the review also stated that white blood cell count and procalcitonin are of no value in the diagnostics of ABRS. Nasal nitric oxide (NO) levels were lower in ABRS. However, further studies are needed on this subject as well as on the use of cytokines in diagnosing ABRS (Autio et al., 2018).

2.9 Chronic rhinosinusitis

2.9.1 Epidemiology

It is difficult to give an accurate estimate of the prevalence of CRS due to the heterogeneity of the disorder and the different diagnostic criteria used in the literature (Fokkens et al., 2020). According to a survey-based study, the self-reported prevalence of CRS in Europe is 11% (in Finland 7%). However, when evaluating CRS diagnosed by a doctor, the prevalence decreased to 5% (Hastan et al., 2011). The estimated prevalence obtained from an administrative database approach is 2-3% (Shashy et al., 2004; Xu et al., 2016). Bhattacharyya examined the United States Medical Expenditure Panel Survey database and found a prevalence of 5% (Bhattacharyya, 2011). If diagnosis is based on symptoms combined with endoscopic findings, prevalence was found to be 1% (J. H. Kim et al., 2016).

2.9.2 Socioeconomic aspects

CRS causes significant costs to society. Direct costs come mainly from office-based expenditures and prescription expenditures (Caulley et al., 2015). Bhattacharyya performed an analysis of the estimated national healthcare costs of CRS in the Unites States in 2007 and 2018 and found them to be 8.6 and 14.4 billion dollars, respectively (Bhattacharyya, 2011, 2021).

Indirect costs also result in a tremendous socioeconomic impact usually caused by missed workdays or reduced productivity. In a study assessing 322 patients with CRS with a survey instrument, CRS was found to cause an average of 4.8 missed workdays per year. The yearly economic cost of the missed workdays was 1539 US dollars per patient (Bhattacharyya, 2003). The total indirect costs of CRS due to absenteeism from work were estimated to exceed 20 billion dollars annually in the USA (Rudmik, 2017).

In addition to costs, CRS has a clear impact on individual health. Patients with CRS have worse general health and vitality than the general population (Gliklich & Metson, 1995). Furthermore, comorbid depression may present in up to 40% of patients with CRS (Schlosser et al., 2016).

2.9.3 Aetiology

The aetiology of CRS is complex and multiple environmental and host genetic factors have been implicated (Fokkens et al., 2020). In contrast to ARS, the role of microbes in the development of CRS is unclear. For instance, no evidence exists as to whether viruses and fungi are a direct cause in the aetiology of CRS (Fokkens et al., 2020; Orlandi et al., 2021).

A systematic review pooled bacterial culture results obtained from patients with CRS and found positive cultures in 64% of cases. Most (77%) of the cultures were obtained during surgery. The most common bacteria were coagulase-negative *staphylococcus* (25%), *S. aureus* (19%) and anaerobic bacteria (17%) (Thanasumpun & Batra, 2015). However, the bacteria may be just colonisation and not true pathogens. Healthy controls have S. *aureus* and coagulase-negative *staphylococcus* in 23% and 75% of the middle meatus, respectively (Uhliarova et al., 2013).

Bacteria may also form a biofilm, which is a community of bacteria in a protective extracellular matrix. It increases bacterial resistance to host defence and antibiotics. The prevalence of biofilms in patients with CRS varies between 15-97% (Danielsen et al., 2015; Prince et al., 2008), but they also exist in control patients (Bezerra et al., 2011). Therefore, their role in CRS is uncertain.

Some predisposing factors, such as asthma, are related to CRS (Jarvis et al., 2012). Allergic rhinitis is also more common in patients with CRS, but the role of allergens as an aetiologic factor in CRS is unclear (Fokkens et al., 2020). A meta-analysis revealed that there is significant association between a gastroesophageal reflux disease and CRS (Leason et al., 2017). The prevalence of immunoglobulin deficiencies is higher in patients with CRS than in the general population and can be as much as 23% in difficult-to-treat CRS (Schwitzguébel et al., 2015). Furthermore, active smoking has a strong association with CRS (Hastan et al., 2011).

2.9.4 Pathophysiology

The pathophysiology of CRS is unclear as CRS is a heterogenous disease rather than a specific disease. However, host and environmental interactions at the mucosal surface can lead to complex inflammatory mechanisms (Fokkens et al., 2020). Traditionally, CRS have been divided to chronic rhinosinusitis with nasal polyps or without nasal polyps (CRSsNP). Nowadays, it is understood as a more diverse syndrome with different inflammatory endotypes and clinical phenotypes (Grayson et al., 2019; Steinke & Borish, 2016). Primary CRS is characterised by dominance of

type 2 or non-type 2 endotypes (Fokkens et al., 2020). Secondary CRS is a local manifestation of other diseases. Patients with primary ciliary dyskinesia or cystic fibrosis for example, suffer from CRS frequently. Additionally, patients with autoimmune disorders, such as granulomatous polyangiitis or sarcoidosis, may exhibit CRS symptoms (Fokkens et al., 2020).

2.9.5 Diagnostics

Symptoms

The most common symptoms patients with CRS have are nasal obstruction (95%), facial congestion or pressure (83-85%), nasal discharge (82%) and loss of smell (68%). Other symptoms that are nonspecific include fatigue (84%), headache (83%), ear pain or pressure (68%) and cough (65%) (Bhattacharyya, 2003).

According to the guidelines, symptoms-only based diagnosis is not sufficient due to the high risk of false positives (Fokkens et al., 2020; Orlandi et al., 2021; Rosenfeld et al., 2015). Symptom criteria have a sensitivity of 89% and specificity of 12% compared to CT-findings (Bhattacharyya & Lee, 2010). Similarly, Tahamiler et al. (2007) showed that 70% and 77% of allergic and non-allergic patients fulfilling the symptom-based diagnostic criteria of CRS did not have CT or endoscopic pathology. Individual symptoms have a sensitivity of between 37-73% and specificity of 27-73%, the most sensitive symptom being nasal discharge and the most specific loss of smell (Moore et al., 2017).

In CRS without polyps, symptom-based diagnosis is especially difficult, as many diseases have similar symptoms. For example, different forms of rhinitis (e.g., allergic rhinitis) mimic CRS. In addition, loss of smell might be due to head trauma or viral infection. Tension-type headaches and migraine can cause facial pain or pressure (West & Jones, 2001), and odontogenic infections and sinonasal tumours may produce symptoms that are similar to CRS, although they are usually unilateral.

Clinical examination

Anterior rhinoscopy is easy to perform in an outpatient clinic using a nasal speculum and nasal decongestion. It is a simple way to investigate the presence of oedema,

purulence and polyps. However, it only allows visualisation of the anterior one-third of the nasal cavity.

In comparison to anterior rhinoscopy, nasal endoscopy using a rigid or flexible endoscope allows a thorough visualisation of the whole nasal cavity. It also allows the exclusion of other possible causes of symptoms, such as intranasal neoplasms or foreign bodies. Nasal endoscopy increases diagnostic accuracy from 43% to 69% compared to symptom-based criteria by improving specificity from 12% to 84% while at the same time decreasing sensitivity from 89% to 47% (Bhattacharyya & Lee, 2010). Similar results were found in another study in which endoscopy had a sensitivity of 46% and specificity of 85% compared to CT-findings (Stankiewicz & Chow, 2002). Another case of low sensitivity (36%) but high specificity (95%) was found in a retrospective study (Amine et al., 2013). Endoscopy does not improve diagnostic classification if patients do not fulfil the symptom criteria described in the guideline (Bhattacharyya & Lee, 2010). Inter-rater agreement in endoscopic finding is reliable when evaluating polyps. Fairly good agreement was also present regarding nasal discharge, but not in the case of mucosal oedema (Larsen et al., 2018).

Finally, a meta-analysis concluded that with rigid endoscopy CRS can be diagnosed with high reliability, but negative findings do not rule out the disease (D. H. Kim et al., 2020). Nasal endoscopy is a safe and recommended tool in clinical examination when evaluating patients for CRS (Fokkens et al., 2020; Orlandi et al., 2021). Concerning post-surgical patients, endoscopy is a valuable tool for assessing inflammation and polyposis in operated cavities. In addition, it also allows microbiological samples to be acquired. Cultures from the middle meatus correlate with cultures from the maxillary sinus 75-86% of the time (Araujo et al., 2007; Gold & Tami, 1997; Szaleniec et al., 2021).

Imaging

Performing nasal endoscopy requires expertise and the equipment is often not available in primary care. Compared to CT, endoscopy is cost-effective and no radiation is used. CT is, however, more sensitive. In a meta-analysis comprising 15 studies, endoscopy had a sensitivity of 73% and specificity of 77% when compared to CT in the diagnostics of CRS (D. H. Kim et al., 2020). CT is the gold standard in the diagnostics of CRS and is recommended for use with symptomatic, endoscopy-negative patients or for preoperative planning (Kim et al., 2020; Orlandi et al., 2021).

CBCT is a viable alternative for CRS imaging due to its low radiation dose, low cost and great resolution for bony detail. Moreover, its accuracy is nearly equal to

that of endoscopic findings (Zojaji et al., 2015). The recognition of incidental findings is essential, and CT/ CBCT should not therefore be used in asymptomatic patients to diagnose CRS (Desrosiers et al., 2011; Fokkens et al., 2020). Mucosal thickening of the paranasal sinuses is common in asymptomatic patients (Razi et al., 2021). Furthermore, the symptom severity of CRS cannot be assessed based on CT findings alone (Rathor & Bhattacharjee, 2017; Valtonen et al., 2018). Nowadays, plain radiography has no place in diagnostics (Fokkens et al., 2020).

3 AIMS OF THE STUDY

1. To evaluate whether DMS can discriminate common rhinosinusitis bacteria *in vitro* (I).

2. To examine patients with ARS and to investigate whether DMS can identify bacterial growth in sinus secretions acquired by maxillary sinus puncture and aspiration (II).

3. To investigate whether nasal air can be collected into a collection bag using aspiration and analysed with DMS (III).

4. To evaluate whether DMS can discriminate the nasal air of patients with CRSsNP from those patients with nasal obstruction caused by deviated nasal septum (IV).

4 MATERIALS AND METHODS

4.1 Differential mobility spectrometers

In the studies of this dissertation, three DMS devices were used. In studies I and II, an ENVI-AMC™ device was used. In study III, a DMS prototype was used (Olfactomics Ltd, Finland), and an Ionvision device (Olfactomics Ltd, Tampere, Finland) was used in study IV.

The ENVI-AMC™ device utilises dried and activated charcoal and molecular sieve (5Å)-filtered compressed air as a carrier gas. The device is connected to a personal computer (PC), and the monitor is then used to evaluate dispersion plots. The DMS prototype and the Ionvision device have integrated monitors and there is no need for a separate PC. All the devices were connected to a cloud database (Olfactomics Ltd, Tampere, Finland) for the monitoring and logging of the data.

The measurement time can be freely adjusted, and for the ENVI-AMC™ device it was approximately 3 minutes and approximately 30 seconds for the DMS prototype and Ionvision device.

4.2 Study population

Studies II, III and IV were prospective studies conducted in the department of otorhinolaryngology – head and neck surgery at Tampere University Hospital, Finland.

4.2.1 ARS patients (II)

Patients older than 18 years with ARS symptoms lasting less than four weeks, according to the European guidelines, (Fokkens et al., 2020) were recruited. Exclusion criteria were smoking during the past six months, prior paranasal surgery, severe immunodeficiency or any malignant disease treated in the previous five years. A total of 15 patients met the criteria and were included.

Information on the patients' current rhinosinusitis symptoms and use of antibiotics one month prior to enrolment was obtained. Complete otorhinolaryngologic physical examination was then performed. The patients underwent maxillary puncture and aspiration which were conducted bilaterally unless the patient experienced only unilateral symptoms in which case the affected side only was punctured.

4.2.2 Volunteers (III)

Ten adult volunteers were recruited. Exclusion criteria were pregnancy or lactation, smoking during past month, CRS, prior paranasal surgery, acute upper respiratory infection less than a week ago, any use of nasal sprays during the past week, lower respiratory tract disease, such as COPD or asthma, severe immunodeficiency and any cancer diagnosed less than five years ago.

4.2.3 Patients with CRSsNP and patients with deviated nasal septum (IV)

Eligible patients were consecutively recruited to two groups: the CRSsNP group and the control group. The inclusion criteria for the CRSsNP group were age ≥ 18 years, symptoms meeting European Position Paper on Rhinosinusitis and Nasal Polyps criteria for CRSsNP (Fokkens et al., 2020)and paranasal mucosal changes visible in CBCT or multislice CT. Lund-Mackay (LM) scores were calculated for the CT scans (Lund & Mackay, 1993). Classification was done based on points. For the osteomeatal complex, 0= not occluded or 2=occluded. For each side, the maxillary, frontal and sphenoid sinuses, and the anterior ethmoidal and posterior ethmoidal cells 0= normal, 1= partial opacification, 2= total opacification. Therefore, the maximum score per side was 12. Since asymptomatic patients may have mucosal changes in CT scans (Razi et al., 2021), only those patients with LM scores of four or more were included. Nasal endoscopy was not performed.

Patients were recruited to the control group if they were aged ≥ 18 years, had experienced nasal congestion and deviated nasal septum (DS) was diagnosed based on anterior rhinoscopy, and no evidence of purulence or oedema in the middle meatus or polyps was observed. Again, nasal endoscopy was not performed.

The exclusion criteria for both groups were pregnancy or lactation, smoking during the past six months, nasal polyps, prior paranasal surgery, any use of nasal sprays during the past 24 hours, acute upper respiratory tract infection during the

past two weeks, severe immunodeficiency or malignant disease treated in the previous five years.

All patients were examined and recruited by a single ENT specialist (the author). After giving consent to participate, the patients completed the 22 item Sinonasal Outcome Test (SNOT-22) questionnaire. The test is validated in the Finnish language and has revealed that healthy controls have a mean score of 8.9 (Koskinen et al., 2021). Therefore, patients were included if they had a score of nine or more. No prior preparation, such as fasting and no exercise, were required. Information on the patient's current medication, allergies, height, weight, and any drinking or eating during the past two hours was obtained during an interview.

In total, 58 patients were invited to participate and two declined. Of the two patients who declined to participate, one had CRSsNP and the other had DS. In addition, two patients (one with CRSsNP and one with DS) were excluded because they had used nasal sprays within the past 24 hours. Thus, 54 patients were enrolled in the study.

4.3 Samples

4.3.1 Bacterial plates (I)

Five bacteria species were analysed *in vitro*: *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. aureus* and *Pseudomonas aeruginosa (P. aeruginosa)*. FIMLAB (Tampere, Finland) laboratories provided the culture plates of the bacteria species. Standard biochemical methods and matrix-assisted laser desorption/ionization time-of-flight (VITEK® MS, bio-Mérieux, Marcy-l'Étoile, France) were used to identify the bacteria. Since *H. influenzae* only grows on chocolate agar, it was used as the growth medium for all the bacterial species to avoid a confounding effect from the medium.

The plates were inserted to a specific chamber that was connected to an ENVI-AMC™ device (Figure 3). The measurement sessions were initiated with a baseline measurement with plates containing tap water. Each bacterial plate was measured twice, producing two measurements per sample. After each culture plate, the device was rinsed with measuring tap water to control for carry-over contamination. Previous experience with the device has shown that two water measurements are usually enough for rinsing. In addition, the dispersion plot was visually assessed for contamination. If significant contamination was noted, water measurements were

repeated to rinse the system with water vapours until the dispersion plot had returned to baseline. The measurement cycle with two water measurements takes approximately 15 minutes.

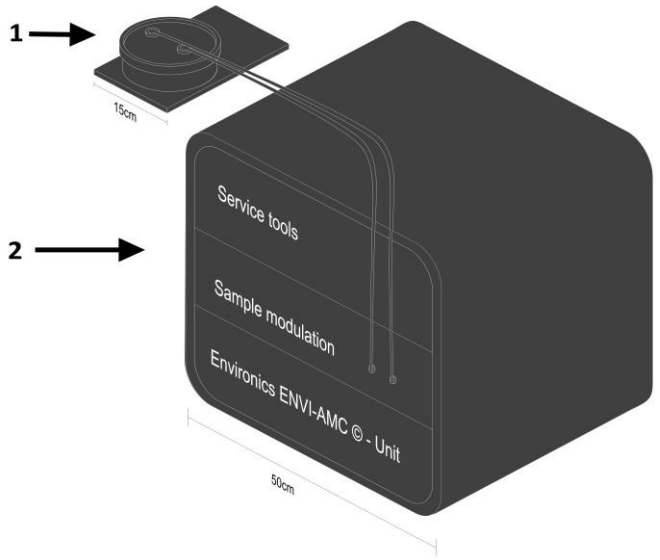

Figure 3. Illustration of the DMS device. The bacterial plates were placed into the measurement chamber (1) from which the evaporated molecules were channelled to the analyser (2). (I)

A total of 107 bacterial plates comprising 24 samples of *S. aureus*, 22 samples of *P. aeruginosa*, 26 samples of *S. pneumoniae*, 21 samples of *H. influenzae*, and 14 samples of *M. catarrhalis* were measured. These resulted in 222 measurements (49 of *S. aureus*, 45 of *P. aeruginosa*, 52 of *S. pneumoniae*, 46 of *H. influenzae*, and 30 of *M. catarrhalis*). One sample of *S. aureus* was measured only once, and two samples were measured three times. One sample of *P. aeruginosa* was measured three times. Two samples of *H. influenzae* and one sample of *M. catarrhalis* were measured four times.

4.3.2 Samples from the maxillary sinuses (II)

Samples from the maxillary sinuses were collected using maxillary sinus puncture and aspiration, which were conducted under local anaesthesia. An anaesthetic cream (Tapin cream, Orifarm Generics, Odense, Denmark) containing lidocaine 25 mg/g and prilocaine 25mg/g was applied into the inferior meatus. For vasoconstriction

and mucosal decongestion, a cotton-tipped aluminium swab containing adrenalin/epinephrine was placed into the inferior meatus for a few minutes. The puncture was performed through the inferior meatus with a 1.6 G needle, the tip of which was introduced close to the posterior wall of the maxillary sinus. Then, the patient was placed horizontally and, after 30 seconds, the maxillary sinus contents were aspirated with a 5 ml syringe. If no aspirates were obtained, two millilitres of 0.9% sterile sodium chloride solution were applied into the maxillary sinus and aspiration was repeated. If no pus was found in the aspirate, the syringe was discarded.

Approximately 0.5 to 1 ml of aspirate was then injected into an M40 Amies Agar Gel Transystem tube for bacterial culture. The remaining contents of the syringe were sealed with a cap, put in a Minigrip bag and stored in a fridge for later DMS analysis. Maximum storing time was set for 48 hours, but median storing time was 4 hours. The syringes were transported for DMS analysis after storing. The time between transportation and analysis was between 15 and 40 minutes. The contents of the syringe were injected onto an empty agar plate and the plate placed into the specific chamber which was connected to the ENVI-AMC™ device (Figure 3). The measuring cycle was identical to that in study I. Each plate was measured twice. However, only one measurement was used in the data analysis.

The bacterial culture was performed on blood agar, chocolate agar and fastidious anaerobic agar plates in aerobic and anaerobic conditions for 48 hours. Bacterial identification was performed according to standard procedures at the FIMLAB laboratories. The results of the bacterial cultures were reported semi-quantitatively (slight growth, moderate growth, and heavy growth).

4.3.3 Nasal air (III, IV)

Nasal air was aspirated using the suction pump SP 625 EC-LC-DU (Spiggle & Theis Medizintechnik GmbH, Overath, Germany) powered by AA-batteries. A metal Politzer nasal olive was inserted into the patient's nostril and connected to the pump with a Teflon tube. Another Teflon tube was used to connect the pump to a 750 ml GaSampler Single-Patient Collection Bag (Quintron instrument Company Inc., Milwaukee, WI, USA), which is a metallised polyester bag. Small pieces of silicone were used to connect the tubes to the pump.

To prevent contamination of the air from the pharynx, the soft palate must be closed. This can be achieved by the patient blowing against a resistance of at least 10

cm water as instructed by the ATS/ERS (American Thoracic Society; & European Respiratory Society, 2005). We chose a pressure of 15 centimetres water.

When the pump is started, ambient air is entrained through the patient's open nostril and through the nasal cavity to the contralateral nostril connected with the nasal olive. The seated patient inhales to total lung capacity and then begins to blow against resistance. At this point, the air in the nasal cavity and the Teflon tubes still contains air from the pharynx. The total length of the Teflon tubes is approximately 500 mm with inner and outer diameters of 6 mm and 8 mm, respectively. Thus, the total volume of the tubes is 14 ml. It has been estimated that the volume of each nasal cavity is approximately 16 ml (Valtonen et al., 2020), resulting in a total volume in the nasal cavities and Teflon tubes of approximately 46 ml. The pump can induce a flow of 192 ml/s (11.5 l/min). Therefore, to clear contamination, the suction continues for 1 to 2 seconds while the soft palate is closed. Thereafter, the valve to the bag is opened.

Less than 10 seconds is required to fill the bag. Then, the valve is closed; the patient stops blowing, and the pump is shut down. Each patient used two Teflon tubes which were disposed of after the samples were taken.

In study III, each volunteer gave one nasal air sample on two separate days within a period of one week, resulting in 20 nasal air samples. In study IV, only one bag per patient was collected. No adverse effects were noted.

On each day, we first collected a bag of room air for background VOC comparison. The pump was cleaned between patients by aspirating with room air for two minutes. Each bag was analysed with a DMS device within six hours. According to the manufacturer of the bags, this is the maximum time the bags can be stored.

The collection bag was attached to the DMS device with Teflon tubes with small pieces of silicone at the ends. A pneumatic ejector VR 05 (Schmalz, Glatten, Germany) produced a vacuum for sampling from the bag. Air flow from the sample bag was adjusted to 400 ml per minute with the Gilibrator-2 system (Sensidyne, St. Petersburg, FL, USA). Cleaned, pressurized air was also used, and it diluted the sample air to a ratio of 10:1. Thus, total volumetric flow was 4.4 litres per minute. The DMS device can handle an air flow of 3 litres per minute, so approximately 1.4 litres per minute were lost. The set-up for the collection of nasal air (without the plastic bottle) and attachment of the full bag to the DMS device is shown in Figure 4.

Figure 4. Above: the pump used to aspirate nasal air. A collection bag at the end of Teflon tube and a nasal olive at the end of another Teflon tube. Below: the DMS device attached to the full collection bag and ready for a measurement. (IV)

Each measurement lasted about 30 seconds, and each collection bag was measured three times while connected to the device. Thus, the analysis of one bag lasted approximately 1.5 minutes. However, as the volume of the bags were 750 ml and the flow rate was 400 ml per minute, the analysis cycle would require a volume of 1.2 litres. The flow from the sample was not, however, a constant 400 ml per minute because of the potential resistance in the bags when the volume of air was diminishing. Between measurements of the bags, we measured the room air aspirated through the DMS device to evaluate possible contamination.

In study III, we took 60 measurements of nasal air samples and 43 measurements of room air aspirated through the DMS device (termed: reference air). We also took 15 measurements of five bags of room air (termed: room air). However, one measurement was accidentally deleted from the device history leaving 14 measurements. In study IV, 54 patients were included, providing 54 samples of nasal air.

4.4 Ethical aspects

All patients and volunteers provided written informed consent. The study was approved by the Ethics Committee of Tampere University Hospital (R16103) and conducted according to medical device trial regulations and approved by the National Supervisory Authority for Welfare and Health. All procedures performed in the study were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

The patients with ARS (II) were exposed to mild and transient discomfort due to the maxillary puncture and aspiration. A local anaesthetic was used to alleviate pain before the puncture. The primary aim of the maxillary puncture was to alleviate symptoms and obtain a bacterial sample. All the punctures were performed by ear, nose and throat specialists or residents who are familiar with the procedure. Furthermore, the Finnish guidelines consider the procedure as a treatment option if the patent has severe symptoms or other treatment fails. Therefore, the procedure is considered to be ethical.

The aspiration of nasal air (III, IV) was achieved by using a pump that was operated by AA-batteries. The aspiration did not cause pain or discomfort as it only produces transnasal airflow. A Politzer nasal olive was used to seal the nostril and prevent tissues being sucked into the tubes, which could have potentially caused pain or epistaxis. The risks for using the pump were, therefore, considered to be rare and minor. Furthermore, as the aspiration was well-tolerated, the procedure was considered ethical.

4.5 Analysis of DMS data

4.5.1 Software

The DMS data analysis was done with MATLAB (The MathWorks, Natick, MA, USA) in studies I, II and IV. In study III, the statistical software R in the RStudio environment (Boston, MA, USA) was used.

4.5.2 Pre-processing (III, IV)

In DMS, pre-processing may include row-wise normalisation which is used to emphasize the signals in the high-separation areas on the dispersion plot (Figure 5). Each row of the dispersion plot was scaled between 0 and 1, using the minimum and maximum value of the row. Noise threshold was defined by an analysis of a histogram of all the intensity data in which a gaussian-shaped peak can be observed at the smallest end, which is normally distributed background noise. The values below this were substituted with the global minimum of the spectra before the row-wise normalisation.

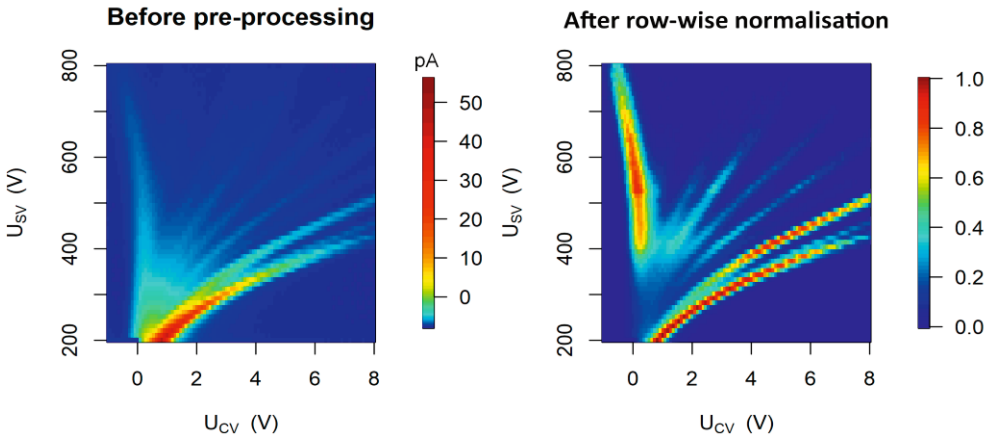

Figure 5. Averaged nasal measurements (negative side) before and after pre-processing and normalisation. In the row-normalisation, 'fragment-like' peaks on the high separation voltage (U_{SV}) values are the result of the normalisation technique. pA: picoamperes; U_{CV}: compensation voltage; V: volt. (III) © IOP Publishing. Reproduced with permission. All rights reserved.

4.5.3 Repeatability verification (III, IV)

To make reliable inference from the DMS measurements, the measurements must be repeatable. Thus, we need a method to compare the similarities and differences between the measurements. DMS produces high-dimensional multivariate data in which the combined dimensionality of positive and negative ions is d = 12 000. As a result, the comparison of the measurements is not simple, and traditional univariate testing approaches cannot be used. Therefore, to estimate the repeatability and inter-

class similarity of the DMS measurements, several difference and similarity measures were used.

The resemblance of the measurements can be measured with distance metrics, similarity metrics or dissimilarity measures. The distance between two identical measurements is 0, and this distance increases as the measurements are further away from each other in their feature space. The distance metric used in studies III and IV was Euclidean distance, which is the distance between two p-dimensional data vectors x and y defined as follows:

$$d(x, y) = \sqrt{\sum_{i=1}^{p}(x_i - y_i)^2} \quad (2)$$

In theory, the upper limit for the distance does not exist. In contrast, similarity between two observations is 1 for identical observations and 0 for completely different observations. To have a comparable "similarity" metric for distance, a concept of dissimilarity (1-similarity) can be used. In study III, dissimilarity versions of cosine similarity as well as Pearson's and Spearman's rank correlation were used.

4.5.4 Spectral archetypes of samples (III, IV)

Spectral archetypes were created by averaging each measurement of the same sample type into a single spectrum. The effectiveness of this approach was tested by comparing the within-group distances and dissimilarities to the between-group dissimilarities. In study III, the nasal air sample data were used to form the archetypes. To avoid bias, a separate archetype was calculated for the nasal air sample data of each measurement day. The distance was then calculated between the archetypes and each individual measurement from the other days. The distributions of the within-group and the between-group distances and dissimilarities were then compared.

In study III, the statistical significance of the findings was tested with Kolmogorov-Smirnov test, which is a general non-parametric statistical test without any distribution assumptions. In study IV, the same test was used, but the statistical significance was determined by the Bonferroni-corrected *p*-value for each feature ($p < 0.05/6000$).

4.5.5 Classification

LDA

LDA is a commonly used supervised pattern recognition method for dimensionality reduction (Berrueta et al., 2007). The method attempts to find vectors that maximise the distance between samples from different classes and, at the same time, minimise the distance of samples of the same classes. As the DMS data have high dimensionality (d = 12 000), regularisation is required. This can be performed using a shrinkage LDA (sLDA), which has previously been successfully applied in the classification of DMS data (Kontunen et al., 2018; Sutinen et al., 2019).

kNN

kNN is a widely used supervised method for performing patter recognition (Berrueta et al., 2007). The method compares a given test sample with similar training samples. The samples are stored in *n*-dimensional space. When an unknown sample (e.g., the test sample) is given, the classifier searches the *k* sample or samples that are closest to it. The test sample is assigned to the most common class of neighbours. The integer *k* can have any positive value. For example, when k=1, the test sample is classified as the training sample that is closest to it. When k=3, the three closest neighbours are searched for. If the closest are two A's and one B, the test sample is then classified as A. Usually, the choice of the value of the integer *k* is empirical and small, such as 3 to 5 (Berrueta et al., 2007). When *k* becomes larger, the computational time becomes longer (Han et al., 2011).

PCA

PCA is an unsupervised pattern recognition method. Both LDA and PCA are dimensionality reduction methods, but in PCA the data are linearly transformed into a feature space that maximises the variance observed in the data, whereas LDA finds a vector that gives maximum separation between classes (Berrueta et al., 2007).

Validation

To estimate the classifier's ability to classify unknown samples, internal validation was performed with different CV methods, such as LOOCV, leave-one-day-out CV or 10-fold CV, depending on the data (II, III, IV). Furthermore, external validation with training and test set was performed in study I.

Reporting the performance of DMS

The diagnostic accuracy of DMS was evaluated by calculating the correct classification rate of the sample types in all the studies. Furthermore, in tests where the result was a binary outcome (positive or negative), the diagnostic parameters used were sensitivity, specificity, PPV and NPV (Table 3). Here, sensitivity means the proportion of true positives in the group of patients with the disease. Specificity is the proportion of true negatives in the group of patients without the disease. PPV is calculated by dividing the number of true positives by the total number of patients with positive results. It indicates the probability of having the disease if the test is positive. NPV is calculated by dividing the number of true negatives with the total number of patients with negative results. This indicates the probability that the patient does not have the disease if the test result is negative. The Wilson score interval method was used to calculate 95% confidence intervals (*CI*).

Table 3. Interpretation of the confusion matrix with binary outcomes.

		Predicted class	
		+	-
True class	+	True positives (TP)	False negatives (FN)
	-	False positives (FP)	True negatives (TN)

Sensitivity = TP/(TP+FN); specificity = TN/(TN+FP); positive predictive value (PPV) = TP/(TP+FP); negative predictive value (NPV) = TN/(TN+FN).

4.6 Comparison of baseline characteristics of the patents (IV)

IBM® SPSS® Statistics for Windows version 27 (Armonk, NY, USA) was used to compare the baseline characteristics of the patents (age, gender, body mass index (BMI), SNOT-22 scores) to evaluate whether there were any statistical differences between the two groups (Table 4). The normal distribution of the data was evaluated by visual inspection of the histograms and by Shapiro-Wilk test. For normally distributed variables (age), the comparison was made with independent samples *t* test and for non-normally distributed variables (BMI, SNOT-22) with Mann-Whitney *U* test. Gender was compared with Fisher's exact test.

Table 4. Demographics of the patients and comparison of the groups. (IV)

	CRSsNP (*n* = 27)	DS (*n* = 27)	*p*-values
Gender male/female	13/14	22/5	0.021*
Age, median (range)	51 (24-71)	43 (23-69)	0.140**
BMI, median (range)	28.4 (22.0-39.1)	27.1 (19-39.8)	0.169***
SNOT-22 score, median (range)	38 (9-57)	29 (9-67)	0.345***
Lund-Mackay score, median (range)	8 (4-19)		

BMI: body mass index; CRSsNP: chronic rhinosinusitis without nasal polyps; DS: deviated septum; SNOT-22: Sino-Nasal Outcome Test

* Fisher's exact test

** Independent samples *t* test

*** Mann-Whitney *U* test

5 RESULTS

5.1 Detection of bacterial plates containing rhinosinusitis bacteria (I)

Examples of the dispersion plots of the bacterial samples are shown in Figure 6. After LOOCV, LDA classified 77% of the bacteria correctly, whereas respective results for kNN, when k=1, were 84% (Table 5). When k was 2 and 3, the correct classification rate was 93% and 76%, respectively. After the train-test sets, classification with LDA and kNN (k=1) were 77% and 79%, respectively.

Table 5. Confusion matrix of all the bacterial samples using *k*-nearest neighbour when *k*=1. (I)

	Predicted class				
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Streptococcus pneumoniae*	*Haemophilus influenzae*	*Moraxella catarrhalis*
True class					
Staphylococcus aureus	49	0	0	0	0
Pseudomonas aeruginosa	1	33	0	1	10
Streptococcus pneumoniae	2	0	43	5	2
Haemophilus influenzae	0	0	3	43	0
Moraxella catarrhalis	0	8	3	0	19

To evaluate the performance of the eNose to discriminate only ARS bacteria, *P. aeruginosa* was excluded. After LOOCV, LDA and kNN (k=1) had an accuracy of 84% and 92%, respectively. In the train-test sets, the accuracies with LDA and kNN (k=1) were 83% and 85%, respectively.

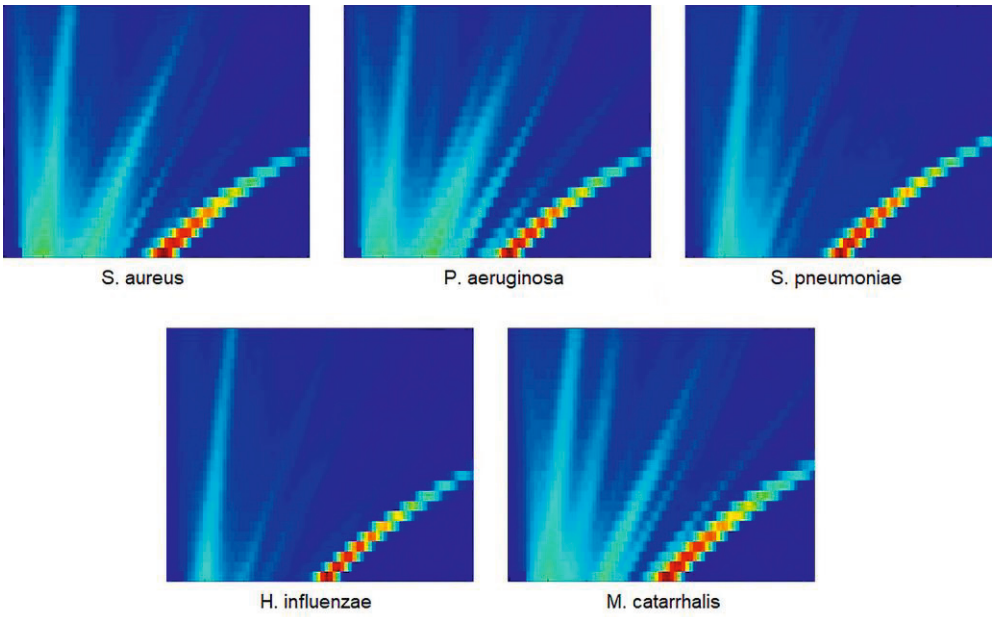

Figure 6. Examples of dispersion plots for positive ion spectrums. (I) © Springer. Reproduced with permission. All rights reserved.

5.2 Detection of bacterial positive and negative samples acquired from ARS patients (II)

A total of 26 samples from 15 patients were obtained. Nine (9/26, 35%) of the samples were culture positive and 17 (17/26, 65%) were culture negative. Positive cultures demonstrated five *S. pneumoniae*, one *H. influenzae*, one *Streptococcus milleri*, one *Citrobacter koseri* and one *Aggregatibacter aphrophilus*. All the samples except one had heavy bacterial growth. In one sample, the bacterial growth was slight (*S. pneumoniae*). This patient had the same bacteria with heavy growth in the contralateral sinus, and the slight bacterial growth was also considered positive.

Prior antibiotics use was noted in nine patients (9/15, 60%). Four of these patients had positive bacterial culture in at least one sinus. Six patients had not used antibiotics and three of them (50%) had positive bacterial culture.

After LOOCV, the accuracy of kNN (k=2) was 85% (*CI*, 66-94%) sensitivity of 67% (35-88%), specificity of 94% (73-99%), positive predictive value of 86% (49-97%) and negative predictive value of 84% (62-94%). The confusion matrix is presented in Table 6. LDA produced accuracy of 73% (54-86%), sensitivity of 56% (27-81%), specificity of 82% (59-94%), positive predictive value of 63% (31-86%) and negative predictive value of 78% (55-91%).

Table 6. Confusion matrix presenting the results of k-nearest neighbour when k=2.

	Predicted class	
	Bacteria +	Bacteria -
Bacterial culture +	6 (TP)	3 (FN)
Bacterial culture -	1 (FP)	16 (TN)

FN: false negative; FP: false positive; TN: true negative; TP: true positive. (II)

5.3 Analysis of nasal air from healthy volunteers (III)

PCA decomposition demonstrates the inherent clustering of the data (Figure 7). The nasal air measurements are distinguishable from the reference and room air measurements, while the measurement day also affects the measurements (Figures 7a and 7b). The three nasal air measurements from the same bag are usually observed close together, but no participant-wise clustering is observed if the measurements of both bags from the same participant are studied (Figure 7c).

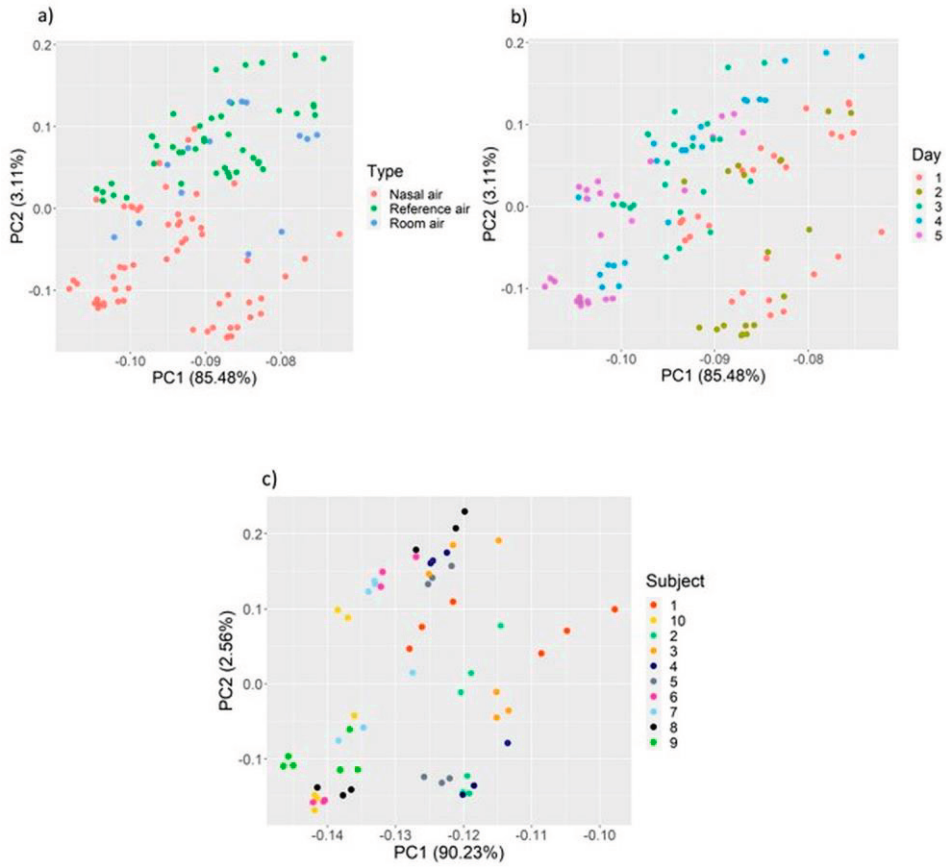

Figure 7. The two first principal components (PC) of (a) the entire dataset grouped by measurement type, (b) the entire dataset grouped by measurement day and (c) the nasal air samples grouped by participants. (III) © IOP Publishing. Reproduced with permission. All rights reserved.

The sample types were 100% correctly classified with the sLDA after LOOCV, demonstrating perfect linear separability. The discrimination rate between ten study participants was 13.3% after 2-fold CV and, as such, does not significantly differ from the guess level of 10%. No significant clustering by participant is present (Figure 7c).

Figure 8 shows the boxplots of the distance and the dissimilarity metrics between the data groups and the nasal air sample archetype. In all cases, the within-group distances and dissimilarities were notably lower than the corresponding between-group metrics. The nasal air sample archetype demonstrated statistically significance on a 95% significance level compared to the differences between the distributions

of the other types. Furthermore, comparison of all sample types showed statistically significance ($p < 0.05$) in Kolmogorov-Smirnov test.

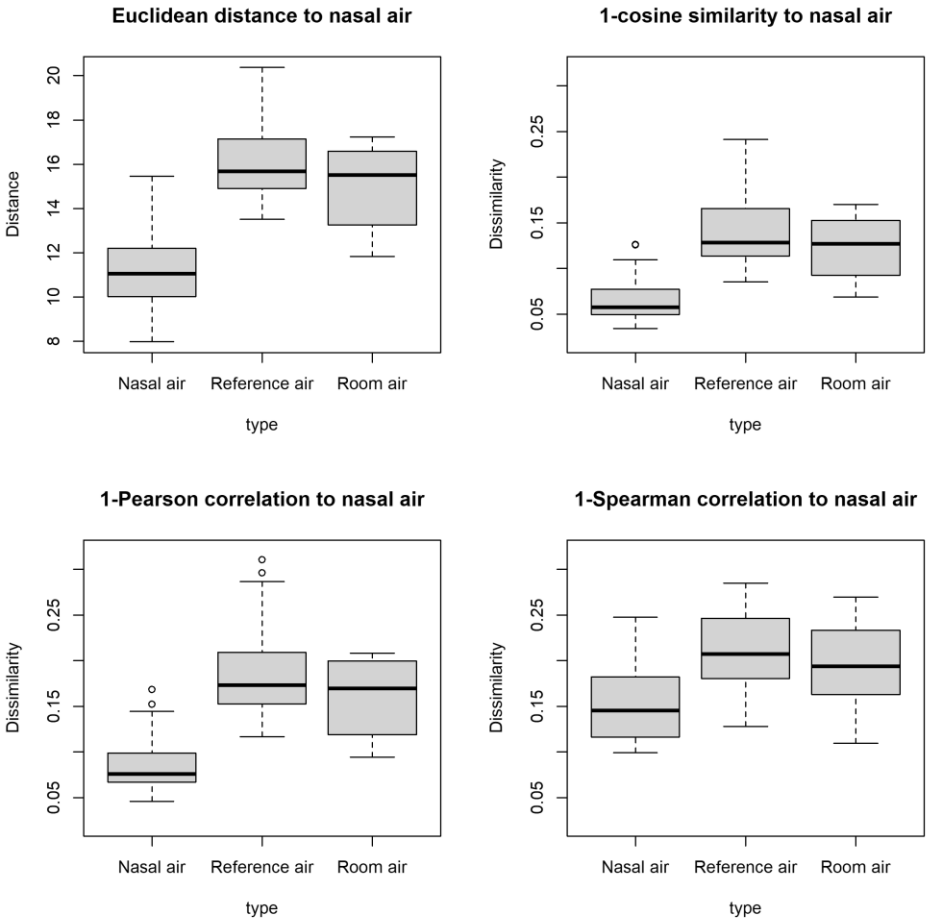

Figure 8. The boxplots of the cross-validated distance and dissimilarity metrics between the data groups and the nasal air sample archetype. The dissimilarity metrics all have the same scale on the vertical axis, whereas the Euclidean distance has its own, non-comparable scale. (III)

Figure 9 shows the absolute humidity of the diluted sample types. Nasal air is more humid than reference and room air, but the humidity also varies greatly between measurement days. Furthermore, the averaged dispersion plots of each sample type demonstrate differences in the reactant ion peak that resembles water (Figure 10).

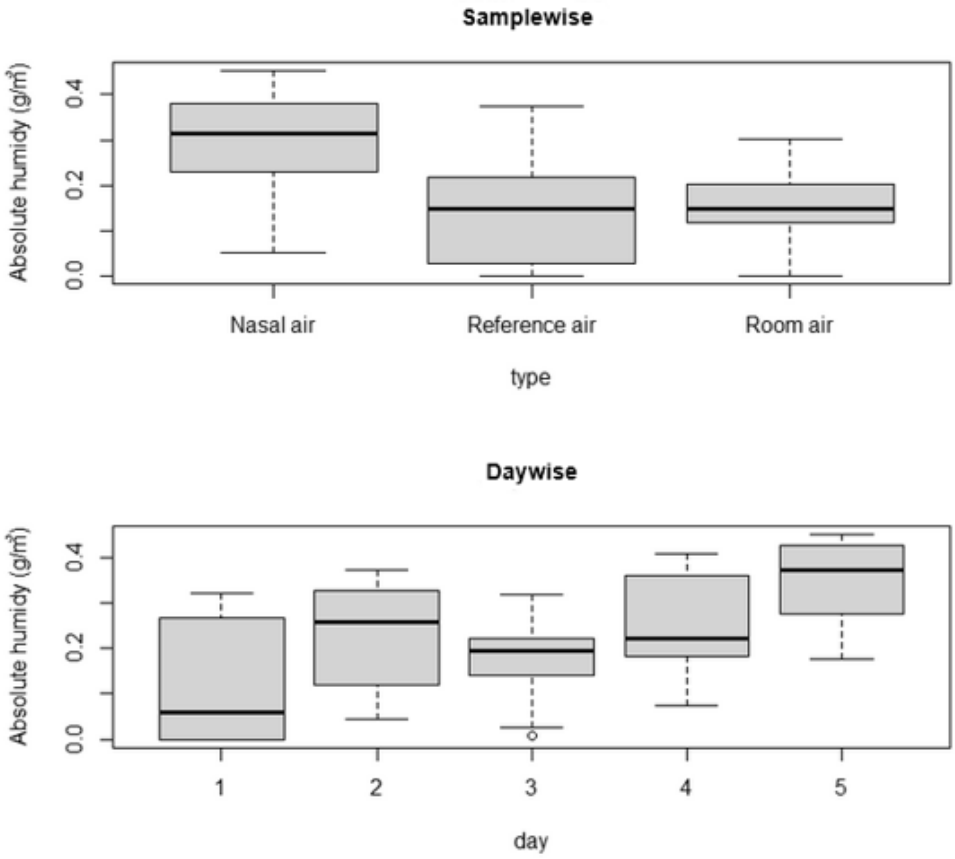

Figure 9. Absolute humidity in diluted air showing variance between samples and measurement days. (III) © IOP Publishing. Reproduced with permission. All rights reserved.

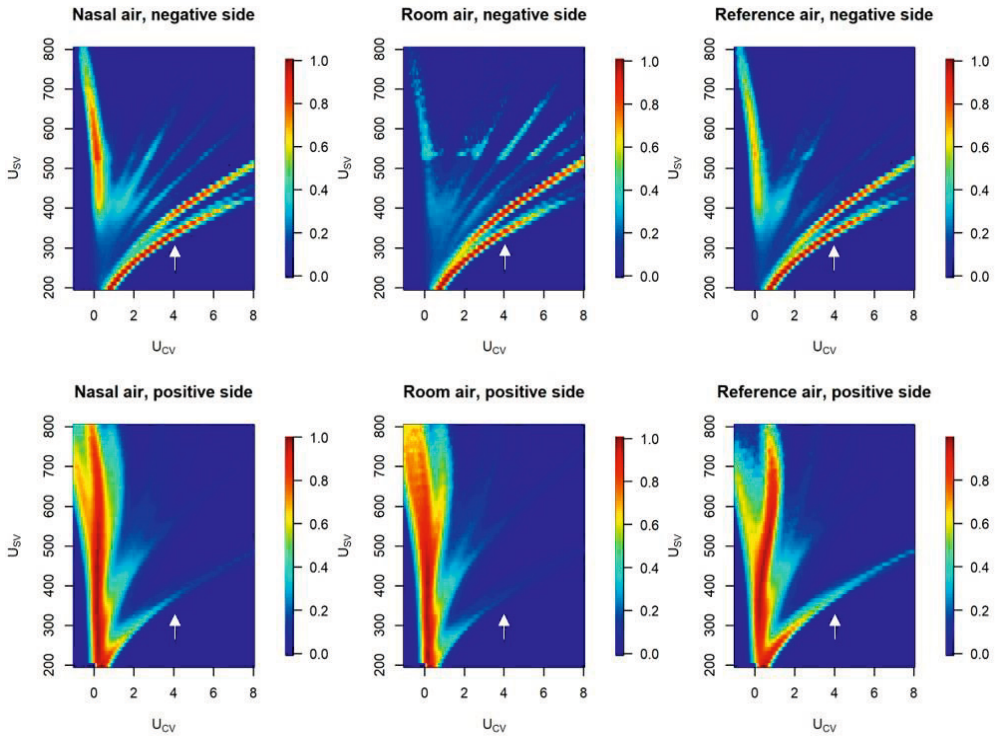

Figure 10. The averaged dispersion plots of each sample type after pre-processing with row-normalisation technique. Reactant ion peak (a water peak) that resembles water in the spectrum is shown with a white arrow. U_{SV}: separation voltage; U_{CV}: compensation voltage; V: volt. (III) © IOP Publishing. Reproduced with permission. All rights reserved.

5.4 Distinguishing patients with CRSsNP from patients with DS (IV)

Both groups consisted of 27 patients. The CRSsNP group consisted of 13 males and 14 females, and the DS group consisted of 22 males and five females. Only gender was statistically significantly different ($p = 0.021$) between groups, but other variables (age, BMI and SNOT-22 score) were not ($p > 0.05$) (Table 4). The methodology revealed no problems, since the nasal air samples were differentiated from the room air samples with a classification accuracy of 94% (95% *CI*, 91-96).

After row-normalisation and 10-fold CV, DMS distinguished CRSsNP from DS patients with an accuracy of 69% (95% *CI*, 55-79), a sensitivity of 67% (48-81%) and specificity of 70% (52-84%). The confusion matrix is presented in Table 7.

Table 7. A confusion matrix presenting the classification results of differential mobility spectrometry between chronic rhinosinusitis without nasal polyps (CRSsNP) and deviated septum (DS). (IV)

	Predicted class	
	CRSsNP	DS
True class		
CRSsNP	18	9
DS	8	19

The archetype spectra for CRSsNP and DS are shown in Figure 11. The visual comparison of these reveal differences, especially with negative ions. Furthermore, the differences are also visible with Bonferroni-corrected statistical differences in the spectra (Figure 12).

Subgroup analysis was performed between patients with CRSsNP who had an LM score ≥ 10 ($n = 11$) and randomly chosen patients with DS ($n = 11$). The accuracy, sensitivity and specificity of DMS were 82% (52-95%), 82% (52-95%) and 82% (52-95%), respectively.

As the groups only differed significantly ($p < 0.05$) by gender (Table 4), we performed a classification between genders. The accuracy of the DMS was 46%, showing that classification is not biased due to gender.

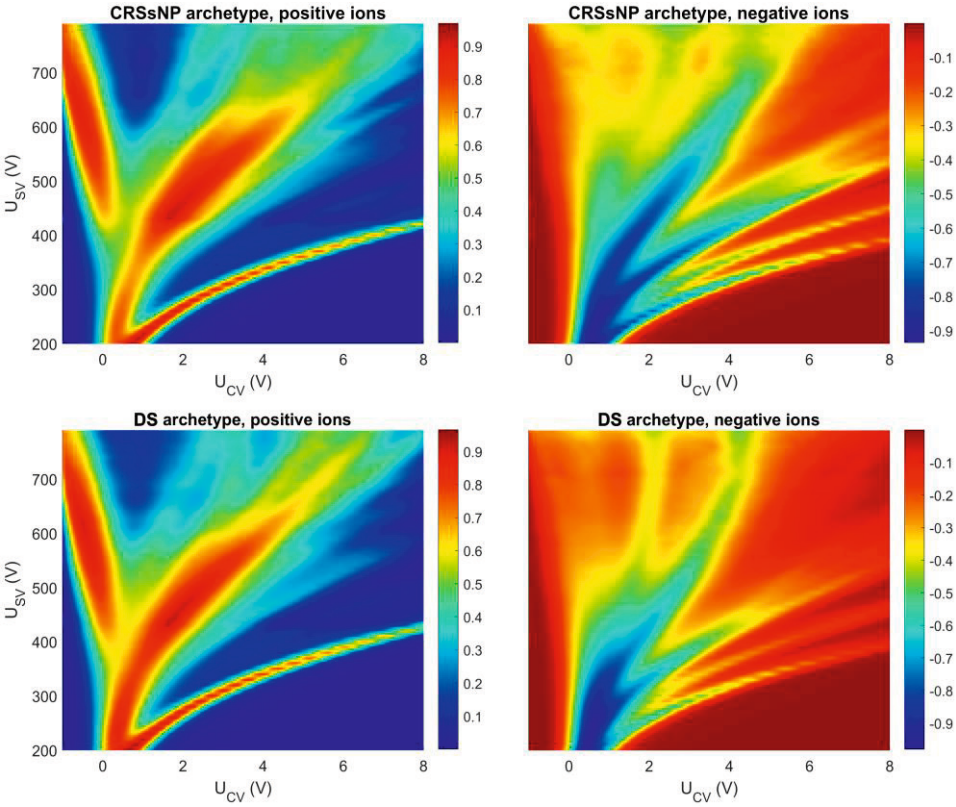

Figure 11. The row-wise normalised archetype spectra for chronic rhinosinusitis without nasal polyps (CRSsNP) and deviated septum (DS). U_{SV}: separation voltage; U_{CV}: compensation voltage; V: volt. (IV)

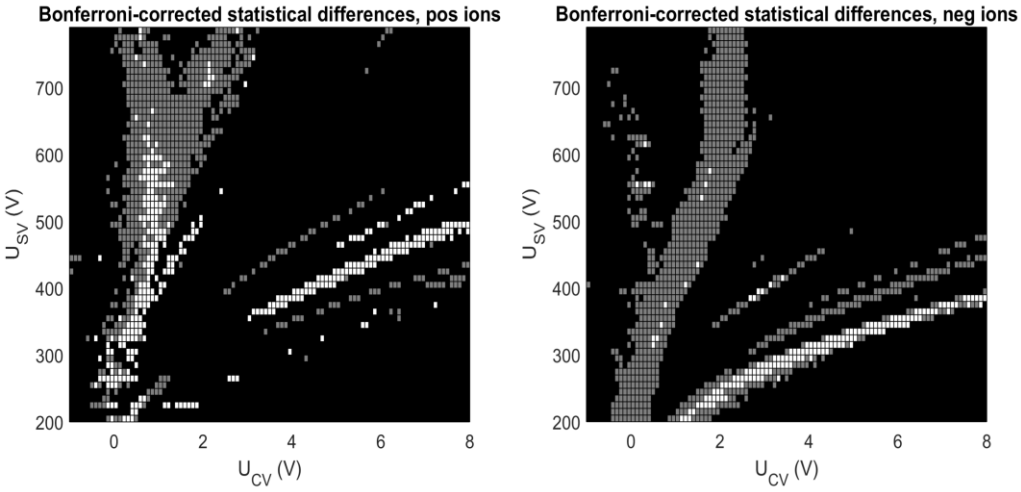

Figure 12. Features with Bonferroni-corrected statistical differences between the classes highlighted with grey for the complete dataset (54 patients) and with white for the subset of patients with a Lund–Mackay score of more than 10. Neg: negative; Pos: positive; U_{SV}: separation voltage; U_{CV}: compensation voltage; V: volt.

6 DISCUSSION

The diagnosis of ABRS is a challenging task. The bacterial prevalence in ARS is estimated to be around 16-61% (Autio, Tapiainen, et al., 2015; Ebell et al., 2019; S. Smith et al., 2015; Thunberg et al., 2013). The definite diagnosis of ABRS requires positive bacterial culture either acquired endoscopically from the middle meatus or by maxillary sinus puncture and aspiration. However, endoscopes are not available in primary care and maxillary sinus puncture is a rarely performed procedure. As a result, antibiotics are prescribed in over 80% of ARS (Benninger et al., 2016; Pouwels et al., 2018; Sharma et al., 2017) which predisposes to the side effects of antibiotics and increases antibiotic resistance.

The symptom-based diagnostics of CRS has high sensitivity (89%) but very low specificity (12%) (Bhattacharyya & Lee, 2010). CBCT or CT can confirm the diagnosis of CRS in patients, which fulfils the symptom criteria, but the lack of availability and exposure to ionizing radiation limits their use. Furthermore, the imaging findings do not correlate with the severity of the patient's symptoms (Gregurić et al., 2017).

The results of the studies of this dissertation clearly demonstrate the feasibility and potential of DMS for use in the diagnostics of ARS and CRS. First, DMS could distinguish common rhinosinusitis bacteria *in vitro* with good accuracy. Moreover, the accuracy improved further when only the most common ARS bacteria were examined. Second, DMS distinguished bacterial positive and negative pus samples acquired by maxillary puncture and aspiration from patients suffering from ARS with a sensitivity of 67% and specificity of 94%. This supports the hypothesis that VOCs released from the pus could be potentially diagnosed using methods other than traditional ones, such as collecting breath air. Third, the collection of nasal air by aspiration into a breath collecting bag and a subsequent analysis with DMS works faultlessly. Nasal air was distinguished from room air perfectly. Fourth, patients with CRSsNP were distinguished from patients with DS by an analysis of aspirated nasal air with a sensitivity of 67% and specificity of 70%. Furthermore, the rates improved when patients with CRSsNP who had more severe inflammatory disease were compared to randomly chosen patients with DS.

6.1 Identifying bacteria *in vitro* with DMS (I)

In previous *in vitro* studies, eNoses have detected bacterial species. Studies with the Cyranose 320 have shown that single and polymicrobial species of swab samples taken from diabetic foot wounds can be distinguished from each other, common upper respiratory pathogens, such as *S. aureus*, *S. pneumoniae* and *H. influenzae*, can be discriminated and biofilm versus non-biofilm-producing bacteria can be identified (Lai et al., 2002; Thaler et al., 2008; Yusuf et al., 2015). The IMS prototype has been shown to detect common wound infection pathogens and to distinguish methicillin-sensitive *S. aureu*s and methicillin-resistant *S. aureus* from each other (Saviauk et al., 2018). Our results (I) are in line with previous studies, as bacterial samples were distinguished from each other with an accuracy of between 77% and 85%. However, almost all the samples were measured twice, which may have affected the validation and given overoptimistic results. Table 5 shows that there are false estimates between *P. aeruginosa* and *M. catarrhalis* and between *H. influenzae* and *S. pneumoniae*, which is also seen in dispersion plots (Figure 5). This demonstrates that potentially there are similarities in the gas-phase of the bacteria. A larger sample size would probably increase the discrimination power. However, to reveal the specific compounds of the VOCs released by the bacteria, GC-MS should be employed.

6.2 The diagnostics of ARS with DMS (II)

The discrimination of pathogens becomes more cumbersome when the samples are acquired *ex vivo,* as the immunological reaction to infection most likely affects the VOCs emitted from the sample. Furthermore, exogenous VOCs may also play a role. Study II demonstrated that bacterial positive and negative pus samples were identified with an accuracy of 85%, a sensitivity of 67% and specificity of 94% with kNN (k=2) after LOOCV. Only one previous study has examined the diagnostics of ABRS with an eNose. In that study, the study population consisted of patients who were suspected of having ABRS based on clinical criteria. Exhaled breath was collected via nasal continuous positive airway pressure mask and analysed with the Cyranose 320. The eNose could distinguish patient with ABRS from healthy controls with an accuracy of 72% after LOOCV, which is in line with our results (Thaler & Hanson, 2006). However, our study consisted of only ill patients, which is a similar situation to daily outpatient practice, where there is a need to identify ABRS to avoid unnecessary antibiotic treatment. The results of study II are limited by the sample

size, especially the bacterial positive samples. Therefore, the dispersion plots of specific bacteria species could not be assessed. Furthermore, only monomicrobial findings were noted. The polymicrobial setting could alter the VOC profile, although in an *in vitro* setting this was not the case (Yusuf et al., 2015).

It would also have been valuable to compare the measurement signature of the pus samples containing the same bacteria species to those of the bacterial isolates measured in study I. This would have given better insight as to whether the VOCs that originated from the pus would have altered the measurement signature of specific bacteria. Unfortunately, there were not enough samples to do this.

6.3 Utilisation of nasal air with DMS (III, IV)

Although exhaled breath is commonly used as a sample material, only a few studies have used nasal air (E. I. Mohamed et al., 2003; Steppert, Steppert, Bollinger, et al., 2021; Steppert, Steppert, Sterlacci, et al., 2021; Thaler & Hanson, 2006) In the study by Mohamed et al. (2003), samples were collected from patients with CRS and controls. Nasal out-breath was collected into sterile plastic sacks by exhalation through the nose. Before collection of the breath, the patients had a tampon of sterile cotton held in their nasal middle meatus to stimulate nasal secretions. Then, the tampon was introduced to the plastic sack. Thus, the analysed air was not pure nasal air. After LOOCV, the accuracy of the eNose was 60%. Steppert et al. (2021a) aspirated nasal air for 10 seconds during normal respiration from patients with confirmed influenza-A infection. The results were compared to nasal air samples from persons with negative test results and to healthy volunteers. The aspiration was performed online in such a way that the patient's nostril was connected to the IMS coupled with a multicapillary column via foam cuffed oxygen catheter, filter and perfusor line. The device could detect influenza-A-infected patients with 100% sensitivity and specificity. In their other study, the exhaled air of patients with SARS-CoV-2 infection was examined using the same device, and the samples were distinguished from patients with influenza-A infection and healthy controls with an accuracy of 97% after cross-validation (Steppert, Steppert, Sterlacci, et al., 2021).

In studies III and IV, aspirated nasal air was collected with a method similar to the validated method for the measurement of nasal NO (American Thoracic Society; & European Respiratory Society, 2005). Blowing against resistance during aspiration allows the soft palate to be closed. This prevents air flow from the oral cavity which in turn, could affect the VOC profile. Ammonia, ethanol and hydrogen cyanide levels

are significantly less in nose-exhaled breath than in mouth-exhaled breath (D. Smith et al., 2008; T. Wang et al., 2008). These compounds could alter the measurement signature, as IMS is sensitive to them (Myles et al., 2006; Seto et al., 2019; Sielemann et al., 2001). Thus, the analysis of nasal air potentially reduces these confounding factors. Furthermore, aspiration of nasal air is not influenced by the portion of breath sampling. For instance, alveolar and mixed expiratory samples produce different VOCs and expiratory flow rate, and breath hold influences the eNose pattern (Bikov et al., 2014; Miekisch et al., 2008).

The nasal air was collected with a system built for the study purpose. Even though the aspiration was painless and no complications such as epistaxis were noted, the method is susceptible to confounding effects. Room air consists of exogenous VOCs. To reduce their impact, an inspiratory VOC filter and rinsing of the patient's lungs with filtered air for 2 to 5 minutes is possible (Dragonieri et al., 2012; van de Goor et al., 2020). With the method we used, this would have demanded the use of a filter attached to the patient's nostril. Moreover, it is not known how long it would take to rinse a patient's nasal cavity. The volume in the nasal cavity is estimated to be 32 ml (Valtonen et al., 2020). Therefore, a few seconds aspiration with our pump would be enough. However, the clean air supply can affect the measurement signature, as it might reduce the concentrations of likely endogenous VOCs and increase exogenous VOCs (Gilio et al., 2020). In our method, the pump and the tubes can release contaminant VOCs. To diminish the impact of this, Teflon tubes were used. Teflon is inert as a material and has been shown to be suitable for use with the eNose (Karjalainen et al., 2020). The pump worked with batteries, and a reduction in the flow of the air was observed as they were running out. This could affect the measurement signature of the eNose in a similar way as the values of NO are affected (American Thoracic Society; & European Respiratory Society, 2005). Furthermore, the closure of the soft palate was not verified by measuring nasal carbon dioxide. Nevertheless, blowing against resistance of a minimum of 10 water centimetres is adequate (American Thoracic Society; & European Respiratory Society, 2005).

In studies III and IV, the system for aspiration of the nasal air and subsequent analysis with DMS was evaluated. Nasal air was discriminated from room air with 100% (III) and 94% (IV) accuracy. The results demonstrate that the measurement protocol works and the day-to-day variation or baseline drift of DMS is not evident to a significant degree. Nasal air contains endogenous VOCs and the concentration of some VOCs in the room air might change during the air flow through the nasal cavity, which might explain the separability of nasal air and other sample types. The

collection of the nasal air and room air took place in a hospital environment, but the DMS device was located elsewhere, which might explain why the reference was different. Most importantly, the air is saturated to between 90 and 100% in the nasal cavity (Lindemann et al., 2003; Rouadi et al., 1999). Thus, the nasal air is more humid than other samples, but the humidity varies between measurement days (Figure 8). The reaction ion peak that resembles water in the spectrum shows differences between sample types. However, there is significant variation caused by other compounds in other areas of the spectrum as well (Figure 9).

6.4 The diagnostics of CRSsNP with DMS (IV)

The results of study IV are comparable to previous studies (Broza et al., 2018; E. I. Mohamed et al., 2003). Mohamed et al. (2003) used an eNose developed for research purposes and examined five chronic rhinosinusitis patients and five healthy controls. The accuracy of the eNose was 60% after LOOCV. In a recent study, an eNose based on an array of nanomaterials-based sensors was used to analyse the exhaled breath of CRS patients and healthy controls. The results were externally validated and revealed that patients with CRSsNP were distinguished from controls with a sensitivity of 80% and specificity of 89% (Broza et al., 2018).

In these previous studies (Broza et al., 2018; E. I. Mohamed et al., 2003), in contrast to study IV, patients with CRS were compared to healthy controls, which might have overestimated the diagnostic accuracy (Whiting et al., 2013). The control group in study IV consisted of patients with nasal symptoms due to anatomical factors rather than inflammatory ones, even though DS may predispose to chronic mucosal inflammation (Kumar et al., 2017) which could affect the measurement signature of DMS and influence the accuracy. Another confounding effect is the lack of CT examination in the patients with DS. Therefore, some of the patients with DS could have had mucosal inflammation in the paranasal sinuses. Anterior rhinoscopy was used to exclude nasal polyps in the patients with DS which, in some cases, can result in false negative findings. Furthermore, other paranasal diseases can alter the measurement signature. Allergic rhinitis, for instance, can affect the VOC profile in eNose analysis compared to healthy volunteers (Saidi et al., 2015). In addition, only CRSsNP was included in study IV. Different endotypes and phenotypes of CRS could demonstrate a different VOC profile.

Interestingly, subgroup analysis revealed that LM scores of ten or more were more effectively distinguished from patients with DS. A more severe inflammatory

disease may lead to a more prominent measurement signature. The sample size was, however, limited. Another method to measure disease severity is the symptoms-based questionnaire SNOT-22. SNOT-22 scores revealed differences, although not statistically significant, between the CRSsNP and DS groups, as median values were 38 and 29, respectively. However, both values fall into the category of moderate disease (Toma & Hopkins, 2016). As the SNOT-22 scores do not correlate with LM scores (Gregurić et al., 2017; Hopkins et al., 2007; Valtonen et al., 2018), it remains unclear whether symptom severity affects the VOC profile.

6.5 Data-analysis (I-IV)

Pre-processing was made with the row-wise normalisation (III, IV) which highlights the higher parts of the DMS dispersion matrices, where the peak separation is the highest, but the signal is the weakest. This method seems to improve the classification accuracy.

Different supervised and unsupervised machine learning methods, such as LDA, kNN and PCA, were used. The methods are simple to understand and use and they are usually computationally inexpensive. Further, the distance metrics used (III, IV) provided visual information on the separability of the classes (Figure 7). As previously shown, there is a significant number of techniques for dimensionality reduction and classification, but none of the techniques are superior in breath analysis to eNose technology (Leopold et al., 2015).

To assess the true performance of the classifier, validation should be performed. In all the studies, CV was performed to reduce overoptimistic results. However, the generalisability of the model should be evaluated by external validation (Marco, 2014). Unfortunately, due to the limited sample size, external validation was only performed in study I. This has been the case in many other studies too, as external validation is performed in only 10-15% of them (Farraia et al., 2019; Leopold et al., 2015).

6.6 Advantages and disadvantages of DMS

The optimal eNose would perform at room temperature, be sensitive, portable, have a short response and recovery time, and be reliable (minimal baseline drift) and robust against environmental effects such as humidity. It should also require only

minimum servicing. In addition, it should not be dependent on pressurised air, as the quality of the air might vary and affect the standardisation. The possibility of online sampling would be preferable, as there would not be a need for sampling bags. Compared to GC-MS, eNoses do not require complex sample preparation and trained personnel to operate them.

Many eNoses are based on different sensor types, each having their own strengths and weaknesses. The most commonly used sensors are MOS sensors and (Baldini et al., 2020; Farraia et al., 2019). The former is relatively sensitive and are cheap and simple to produce. However, they usually operate at high temperatures, leading to high power consumption. Furthermore, MOS sensors suffer from poor long-term stability. CP sensors have higher sensitivity but have a short lifetime and suffer from baseline drift and sensitivity to humidity (Arshak et al., 2004; Nazemi et al., 2019; Zohora et al., 2013).

In contrast, DMS provides real-time analysis at room temperature. Further, it has much higher sensitivity (< particles per billion) than MOS sensors or CP sensors. In the studies of this dissertation, offline measurements were used. However, online measurements are also possible. In studies I and II, water vapour was measured to rinse the device to reduce contamination after each sample measurement. During the study period, no significant baseline drift was noted within the devices, proving the reliability of DMS. A disadvantage of DMS is sensitivity to humidity. Thus, environmental factors such as different periods of the year could affect the measurement of the same compound. Sampling nasal air, however, saturates the sample to 90-100% (Lindemann et al., 2003; Rouadi et al., 1999) which, most likely, reduces the impact of environmental humidity. Another disadvantage of DMS is that the DMS devices used in the studies of this dissertation were not portable, although they were compact in size.

6.7 Challenges with the analysis of VOCs

Although eNoses can analyse the VOCs emitted from different sample types (e.g., blood, urine, faeces and pus), breath is probably the most studied sample type (Farraia et al., 2019). However, many issues are related to "breathprinting" and are discussed thoroughly in several articles (Hanna et al., 2019; Issitt et al., 2022; Jia et al., 2019; Lourenço & Turner, 2014; Miekisch et al., 2012; C. Turner, 2016).

The sampling method has an impact on the VOC profile. As previously discussed in chapter 6.3, room air, clean air supply, sampling device and nasal/oral breath

sampling may interfere with the VOC profile. Sample storage is another potential issue. In studies III and IV, the nasal air samples were stored in breath collecting bags made of metallized polyester. The advantages of bags are that they are cheap in price and moderate in durability. However, VOCs adsorb from the bags over time, which affects the storing time (Mochalski et al., 2009; Pet'Ka et al., 2000). The bags in studies III and IV were analysed within 6 hours as instructed by the manufacturer. The re-use of the collection bags is possible with cleaning protocols, which would reduce costs (Beauchamp et al., 2008; Mochalski et al., 2009). However, cleaning may still fail to remove some compounds (McGarvey & Shorten, 2000) and re-use of the bags may lead to micro-damage affecting their inertness. Online sampling would bypass these potential problems with sample collection, and it is also technically possible with DMS.

The patient's physiologic condition has an effect on VOCs. For example, pregnancy can change the VOC profiles in eNose analysis (Bikov et al., 2011). Furthermore, a patient's diet may influence VOCs (Krilaviciute et al., 2019). Sometimes, fasting before sample collection is advocated (Dragonieri et al., 2016; Fens et al., 2011; Gruber et al., 2014), but the role of fasting or diet on VOCs is unclear (Jia et al., 2019). Other possible covariates include age, gender, smoking status and comorbid diseases. It is unclear, however, which of them should be adjusted for breath analysis (Broza et al., 2018; X. Chen et al., 2017; Dragonieri et al., 2016; Filipiak et al., 2012; Kischkel et al., 2010; Tiele et al., 2019). In studies III and IV, potential confounders, such as smoking, a recent acute upper respiratory tract infection and malignant diseases, were excluded. As medication could affect the VOC profile, patients were advised to refrain from using their nasal sprays for a week prior to sample collection in study III. After completing study III, we estimated that a shorter period would probably be sufficient, and 24 hours was chosen for study IV. The impact of nasal sprays on the VOC profile is unknown, but nasal decongestants and corticosteroid sprays have been shown to decrease nasal NO (Chatkin et al., 1999; Dillon et al., 1996; Vural & Gungor, 2003). Furthermore, corticosteroid sprays have a prolonged effect on inflammation, and the time the sprays take to wear-off is unknown.

6.8 Strengths and limitations

The main strength of the studies in this dissertation is the adding of new information to the current literature concerning the diagnostics of ARS and CRS with gas-phase

mixture analysis. Moreover, these are prospective studies and the first ones concerning otorhinolaryngologic diseases and DMS technology. As there is some lack of standardisation in previous studies concerning breath air, further emphasis was placed on evaluating the analysis of nasal air using a method that resembles the validated method for the measurement of nasal NO (American Thoracic Society; & European Respiratory Society, 2005). An additional strength of the studies is the promoting of interdisciplinarity including medical, data and engineering science.

The findings of these studies are limited due to the relatively small sample size which prevented external validation, with the exception of study I. External validation would have alleviated overfitting and demonstrated the generalisability of the results. As three different DMS devices were used, the data matrixes analysed with one DMS device cannot be compared with the data matrixes analysed with another.

6.9 Future aspects

The diagnostics of ARS and CRS is a challenging task using current methods. The implementation of an eNose to point-of-care use is intriguing, as it could provide a rapid, easy to perform and non-invasive way to diagnose diseases or perhaps, monitor therapeutic responses. The analysis of gas-phase mixtures is, however, highly complex. The literature covering eNose studies is heterogeneous with the use of different sensor technologies, diverse sampling procedures, and a wide scale of data analysis methods. There is, therefore, an urgent need for standardised methods. This is already being attempted, as instructions have been provided by the European Respiratory Society (Horváth et al., 2017). Databases, such as the Human Breathomics Database, provide information on the VOCs in breath and serve as resources for complimentary investigations (Kuo et al., 2020).

Many eNose studies evaluate the performance of the eNose in a specific task with a specific population. Further validation studies testing the built classifier are rarely performed. Ideally, large cohort studies should be undertaken and the results of the eNose compared to a gold standard diagnostic test and externally validated in a different patient cohort. Only then could eNose technology be brought to a real-life clinical setting.

DMS demonstrated its ability to analyse different sample types and to be used in further studies. Because the analysis of aspirated nasal air with an eNose is rarely studied, further emphasis on the use of the method in research studies concerning

rhinologic diseases is encouraged, as nasal air is simple to collect, non-invasive and may reduce confounding factors compared to exhaled breath. For example, different endotypes and phenotypes of CRS may emit a distinct VOC profile that could be recognized with DMS.

7 CONCLUSIONS

1. DMS can discriminate common rhinosinusitis bacteria *in vitro* with very good accuracy.

2. DMS was found to have very good accuracy to distinguish bacterial positive and negative pus samples acquired from ARS patients by maxillary sinus puncture and aspiration.

3. The method for aspirating nasal air into a collection bag and subsequent analysis with DMS was found reliable.

4. DMS can identify patients with CRSsNP from those patients with DS by analysing aspirated nasal air. The classification accuracy improved when inflammatory findings were more severe in patients with CRSsNP.

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PUBLICATION I

In vitro detection of common rhinosinusitis bacteria by the eNose utilizing differential mobility spectrometry

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European Archives of Otorhinolaryngology. 2018;275(9):2273-79
doi: 10.1007/s00405-018-5055-8

In vitro detection of common rhinosinusitis bacteria by the eNose utilising differential mobility spectrometry

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Acknowledgements

Compliance with Ethical Standards

Conflicts of Interest

Niku Oksala, Markus Karjalainen, Anton Kontunen, and Antti Roine are shareholders of Olfactomics Ltd, which is about to commercialise proprietary technology for the detection of diseases by ion mobility spectrometry.

The rest of the authors declare that they have no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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Abstract

Acute rhinosinusitis (ARS) is a sudden, symptomatic inflammation of the nasal and paranasal mucosa. It is usually caused by respiratory virus infection, but bacteria complicate for a small number of ARS patients. The differential diagnostics between viral and bacterial pathogens is difficult and currently no rapid methodology exists, so antibiotics are overprescribed. The electronic nose (eNose) has shown the ability to detect diseases from gas mixtures. Differential mobility spectrometry (DMS) is a next-generation device that can separate ions based on their different mobility in high and low electric fields. Five common rhinosinusitis bacteria (*S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *S. aureus*, and *P. aeruginosa*) were analysed *in vitro* with DMS. Classification was done using linear discriminant analysis (LDA) and k-nearest neighbour (KNN). The results were validated using leave-one-out cross-validation and separate train and test sets. With the latter, 77% of the bacteria were classified correctly with LDA. The comparative figure with KNN was 79%. In one train-test set, *P. aeruginosa* was excluded and the four most common acute rhinosinusitis bacteria were analysed with LDA and KNN; the correct classification rate was 83% and 85%, respectively. DMS has shown its potential in detecting rhinosinusitis bacteria *in vitro.* The applicability of DMS needs to be studied with rhinosinusitis patients.

Keywords

Electronic nose, eNose, differential mobility spectrometry, acute rhinosinusitis

Introduction

Acute rhinosinusitis (ARS) is a sudden, symptomatic inflammation of the nasal and paranasal mucosa. It is usually caused by respiratory virus infection, but bacterial prevalence remains poorly defined. In two often-cited studies [1, 2], the bacterial prevalence was 0.5–2%, but according to a recent meta-analysis [3], the prevalence is likely higher. Autio et al. (2015) have found bacterial causes in 40% of cases [4]. In Finland (2016), 1.3% of all patient visits to daily outpatient practices are due to ARS [5]. In a study conducted in Asia, it was estimated that 6–10% of appointments with a doctor are due to ARS [6].

Antibiotics are usually prescribed, even though their effect is limited in clinically diagnosed, uncomplicated ARS [7]. The most common bacteria in ARS are *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Staphylococcus aureus* [8]. One explanation for the poor antibiotic response is that only 40% of ARS are bacterial culture-positive [4]. Nasal endoscopy provides the possibility to take microbiological samples from the middle meatus, but equipment is only available to ear, nose, and throat (ENT) specialists. A maxillary sinus puncture and aspiration can be considered the gold standard in diagnosing bacterial ARS, but it seems that endoscopically directed middle meatal cultures correlate well with aspiration [9].

Since the early days of medicine, doctors have linked certain diseases to specific smells. During the last decades dogs' ability to detect certain diseases by smelling have been studied [10]. Dogs use in clinical practice has pitfalls due to extensive training and their limited life-span. The odour emitted from our bodies consists of volatile organic compounds (VOCs) [11–13]. Endogenous VOCs are released as by-products of normal cell metabolism, but also directly by microbes or due to an inflammatory response to infection [11]. Certain VOCs can be specific biomarkers of diseases [13]. Most commonly, however, patterns of multiple VOCs perform better than isolated biomarkers [14]. The electronic nose (eNose) is a device that attempts to mimic mammalian olfactory sense. It comprises an array of nonselective chemical sensors, pre-processing electronics, and a computer that interprets sensor signals [15]. Different kinds of sensor arrays exist – for example, metal–oxide–semiconducting field-effect transistors (MOSFET), conducting polymer sensors, and optical sensors [15]. The eNose can also be constructed with more than one type of sensor. VOCs interact with sensors and produce a so-called smell print. The chemical characteristics of VOCs determine the response pattern of the eNose, which can be measured. The eNose does not differentiate specific components of the smell print [16], and it therefore performs a qualitative rather than a quantitative analysis.

Differential mobility spectrometry (DMS), although not an eNose in the strictest sense, provides analogous information from gas mixtures. The DMS is highly more sensitive and selective device compared to the classic eNose. Devices with voltage adjustable ion filters have an enhanced ability to analyse complex samples without precipitation. The principle of DMS is explained thoroughly in other publications [17, 18]. Analogously to commonly used electrophoresis, the device separates the molecular mixture to its components, creating a data matrix also known as a dispersion plot or a "chemical fingerprint" of the sample. The dispersion plot is usually represented as a heat map color-scheme. The working principle of the method is illustrated in **Figure 1**.

Several studies have shown the ability of the eNose to detect diseases from gaseous headspace – for example, differentiating prostatic cancer from benign prostatic hyperplasia [19] or diagnosing head and neck cancer [20], tuberculosis [21], and ventilator acquired pneumonia [22]. In a study by Thaler and Hanson (2006), the eNose was able to diagnose acute bacterial rhinosinusitis with a 72% probability [23].

There is a need for a new, easy-to-use tool for diagnosing rhinosinusitis bacteria, and the eNose has strong potential in this application. In addition, DMS provides next-generation technology to the eNose field. Our aim was to study whether DMS could identify common acute (*S. pneumoniae*, *H. influenzae*, *M. catarrhalis*) and chronic (*S. aureus*, *P. aeruginosa*) rhinosinusitis bacteria *in vitro*.

Materials and methods

FIMLAB (Tampere, Finland) laboratories provided the cultures of all five bacteria species. The bacteria were identified using standard biochemical methods and matrix-assisted laser desorption/ionisation time-of-flight (VITEK® MS, bioMérieux, Marcy-l'Étoile, France). Since *H. influenzae* grows only on chocolate agar, it was used as the growth medium for all the bacterial species to avoid a confounding effect from the medium.

The device, **Figure 2**, used in this study was an Envi-AMC (Environics Ltd, Mikkeli, Finland). It is based on DMS technology, utilising Americium ^{241}Am as an ionisation source. Dried and activated charcoal- and molecular sieve (5Å)-filtered compressed air was used as a carrier gas. The device was connected to a cloud database (Olfactomics Ltd, Tampere, Finland) for the monitoring and logging of the data. Two individual devices were used in the study due to the sensor malfunction of the first device.

The measurement sessions were initiated with a baseline measurement with tap water. Each bacterial plate was measured twice, producing two measurements per sample. The device must be rinsed with water after every culture plate to control for carry-over. Based on our previous studies with the device, two water measurements are usually enough for rinsing. Each measurement produces a dispersion plot which is presented in the graphical user interface of the device. The dispersion plot was visually assessed for contamination. If significant contamination was noted, water measurements were repeated to rinse the system with water vapours until the dispersion plot had returned to baseline. The measurement cycle with two water measurements takes approximately 15 minutes. The bacteria were measured in random order with each bacteria spread over multiple sessions in order to avoid bias from sensor drift.

The analysis was made with Matlab (The MathWorks, Natick, MA, USA). Linear discriminant analysis (LDA) and k-nearest neighbour (KNN) methods were used to classify samples. To avoid overfitting, the results were cross-validated by using leave-one-out cross-validation (LOOCV) and separate train and test sets. In LOOCV, each sample was removed from the sample set and then classified with the data of the remaining samples. When using train and test sets, a randomly selected 30% of the samples (test set) was removed from the sample set and then classified with the data of the remaining 70% (train set). This was repeated five times because of the randomisation element, and the mean classification rate of the five drives was used in the final results.

A total of 107 samples were analysed. Of these samples, there were 24 samples of *S. aureus*, 22 samples of *P. aeruginosa*, 26 samples of *S. pneumoniae*, 21 samples of *H. influenzae*, and 14 samples of *M. catarrhalis*. A total of 222 individual measurements were made (49 of *S. aureus*, 45 of *P. aeruginosa*, 52 of *S. pneumoniae*, 46 of *H. influenzae*, and 30 of *M. catarrhalis*). One sample of *S. aureus* was measured only once and two samples were measured three times. One sample of *P. aeruginosa* was measured three times. Two samples of *H. influenzae* were measured four times, as was one sample of *M. catarrhalis*.

Results

Using LOOCV, 77% of the bacteria were classified correctly with LDA. With KNN, when k=1, the classifications were 84% correct. When *k* was 2 and 3, the correct classification was 93% and 76%, respectively.

Using separate train and test sets, the mean correct classification rate with LDA was 77%. With KNN, when k=1, the classification rate was 79.0%.

We also wanted to see how well the eNose discriminates only the ARS bacteria, so *P. aeruginosa* samples were excluded from the data. In this scenario, with LOOCV, LDA classified 84% of the samples correctly, while KNN, when k=1, classified 92% of the samples correctly. With the train and test sets, LDA had a mean classification rate of 83%, and with KNN, when k=1, the classification rate was 85%.

The performance of the KNN classifiers are presented as a so-called confusion matrix in **Tables 1** and **2**. Examples of the dispersion plots of the bacterial samples are shown in **Figure 3**.

Discussion

Our results show that DMS is able to discriminate five different rhinosinusitis bacteria and the four most common ARS bacteria with a reasonably high accuracy. Since the microbiological cause of ARS is challenging and time-consuming to diagnose, these results lay the foundations for a new diagnostic approach.

To our knowledge, there are few *in vitro* studies concerning the eNose and ENT bacteria [24, 25]. Lai et al. (2002) used Cyranose 320 (Cyrano Technologies, Pasadena, CA, USA) in their *in vitro* analysis of ten common upper respiratory bacteria, which included those tested in our study, but also others, such as *Streptococcus* Group A, *Klebsiella* species, and *Proteus mirabilis.* The eNose was able to distinguish three tested bacteria from control swabs. Lai et al. also tested discrimination ability across a variety of bacterial species, but in quite many cases, the eNose gave false results. Moreover, cross-validation was not used, giving overoptimistic results for the performance of the eNose [24].

Thaler et al. (2008) examined the ability of the Cyranose 320 to distinguish biofilm-producing *Pseudomonas* and *Staphylococcus* from non-biofilm-producing strains of the same species [25]. The samples were tested over 24 days along with control data saline to check the consistency of the sensor responses. Consistent responses were received for 22 days. Internal validation was performed using the leave-one-day-out method. Furthermore, they also divided the data into training and testing sets with success rates ranging from 72% to 100%, which are quite similar to ours. Although Thaler et al.'s sample size was larger (198 samples of each bacteria), our study tested five different bacteria species. The technology in Cyranose 320 relies on conducting-polymer sensors, which have lower

sensitivity, specificity, and accuracy than DMS [26]. In addition, the conducting-polymer sensors suffer from irreversible contamination.

Our analysis also used bacteria *in vitro*. There are a few limitations regarding the study method. First, we acquired bacterial plates from a laboratory, and there was variance in the duration of plate storage in the refrigerator before measurements. Some were measured on the same day and some after a few days. Analysing all species over multiple sessions minimised bias from this variation. It is not known if some or all of the bacteria on the plate had died before measurement. This could lead to a weaker smell print but, on the other hand, it is also possible that bacteriolysis releases molecules that actually enhance detection. In addition, there was unavoidable variance in the confluence of the bacteria on the agar plate, which could affect DMS performance. As seen from the confusion matrix presented in Table 1, the device distinguishes all *S. aureus* correctly, but there are false estimates between *P. aerigunosa* and *M. catarrhalis* and also between *H. influenzae* and *S. pneumoniae*, demonstrating that there are probably similarities in the gaseous headspace of these bacteria. We must also emphasise that the specific molecules detected by the eNose are unknown. Thus, the smell print might change when the sample is acquired from a patient because there are also other molecules affecting it, such as those produced by the host inflammatory response. Two preliminary clinical trials have been conducted concerning rhinosinusitis diagnostics with the eNose [23, 27]. Nasal out-breath was sampled in both studies. The correct results varied between 60% and 72% with a modest sample size and limited cross-validation.

There are many types of eNose sensor, and they all have their strengths and weaknesses. For example, surface acoustic wave sensors benefit from their high sensitivity to a broad spectrum of VOCs and relatively low costs in biomedical applications, but they suffer from reproducibility issues [14]. Metal oxide-semiconductor sensors, on the other hand, are easier to reproduce but suffer from poor long-term stability.

The eNose in this study is based on DMS technology [17], which has been studied in various medical applications [18], but to our knowledge there are no publications regarding bacteria affecting the ENT area. The advantages of DMS include its high sensitivity and its ability to work at room temperature. Device-to-device variation is also limited, as we demonstrated in our study by changing the sensor during the study period. Its disadvantage is its susceptibility to contamination, which requires the sensors to be rinsed with water vapours between measurements.

The eNose produces multidimensional data, which requires pre-processing and classification to build a model. A variety of techniques for data analysis exists, but there is no consensus as to which one is

best [28]. Therefore, depending on the sensor and application, different methods should be tested. A recent review [28] aimed to give an overview of the currently used dimension reduction, classification, and validation methods. Although cross-validation is considered crucial [19], a literature review regarding the investigation of exhaled breath and eNoses yielded 46 studies, with internal validation performed in 29 (63%) studies and external validation performed only in 7 (15%) studies. In our study, the optimal data analysis method gave correct results in 93% of cases. To minimise the risk of overfitting, we utilised the leave-one-out method for internal validation in all measurements. External validation with the train-test set, which is considered the most robust method of cross-validation [29], yielded a correct classification rate of 79%, which is still an excellent result considering the modest sample size. We therefore consider our methods of cross-validation adequate.

At the moment, Gram staining and a bacterial culture are the gold standard for microbial identification from samples. They are cheap and easy to do but requires laboratory setting and staff. For all the bacteria tested in our study, it usually takes one day to get the results in culture and approximately a day more for information about antibiotic sensitivity.

DMS can analyse complex samples in real-time without prior sample preparation. For example, it can differentiate *Clostridium difficile*- positive stool samples from negative ones with high accuracy [30]. DMS has also shown ability to differentiate methicillin-resistant *S. aureus* from methicillin-sensitive *S. aureus* [31]. Its high sensitivity allows detection of compounds even in low concentration. Our device is easy to use and the actual measurements can be done by almost anyone after brief instructions.

Although in this study we analysed bacterial plates, the same methodology can be applied to a clinical setting. Samples acquired from rhinosinusitis patients can be analysed with DMS easily. An empty agar plate can be covered with pus collected from the maxillary sinus or middle meatus and analysed in the same manner as we did with the bacterial plates. The results can be compared with the results presented in this study. Our device does not have data-processing software within itself. Therefore, it does not give an instant result which bacteria grows in a sample. Thus, it is not yet possible to use the device in a clinical practice. Technically, there is only need for an analysis software to be integrated to the device to have an instant result after measurement. The device has a clear potential for point-of-care use since it is relatively compact in size, works in room temperature, utilizes ambient air as carrier gas and tap water for rinsing. Since DMS analyses bacterial headspace, it is also relatively easy to develop an analytical method for the analysis of exhaled nasal air, utilising, for example,

existing methods for nasal nitric oxide measurement, analogous to the sampling used in exhaled breath studies [32].

Conclusion

The diagnostics of bacterial ARS are uncertain, so there is a need for a new diagnostic tool. DMS has proven its potential in detecting rhinosinusitis bacteria *in vitro*. The positive results remained after rigorous cross-validation, indicating that the performance of DMS is realistic. Therefore, the device is most likely able to distinguish between unknown samples. More studies are needed on the clinical applications of DMS in rhinosinusitis patients.

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Legend to the Figures:

Figure 1. The working principle of DMS. DMS is analogous to **electrophoresis (A)** in which molecules are driven through the medium by an electrical current. The molecules in the medium cause drag that depends on the size and shape of the molecule. The electrical charge and mass also affect the speed of the molecule. Due to these factors, different molecules travel at different speeds. In **DMS (B)** molecules that evaporate from the sample are driven with a flow of air. They are given an electric charge by **ionization**, allowing discrimination of molecules according to their charge. The molecules then enter **separation** phase where they are exposed to intermittent high- and low electric fields. Ionized molecules move towards the oppositely charged plate at different speed in low and high fields, thus resulting in another dimension for separation. Analogously to electrophoresis, the collisions with medium also discriminate the molecules according to their size and shape. After separation, molecules collide with the **detector**, resulting in a signal.

Figure 2. Illustration of the DMS device. The bacterial plates were placed into the measurement chamber (no 1) from which the evaporated molecules were channelled to the analyzer (no 2).

Figure 3. Examples of dispersion plots for positive ion spectrums.

PUBLICATION II

The Detection of Bacteria in the Maxillary Sinus Secretion of Patients with Acute Rhinosinusitis Using an Electronic Nose—A Pilot Study

Virtanen J, Roine A, Kontunen A, Karjalainen M, Numminen J, Oksala N, Rautiainen M, Kivekäs I

Submitted

PUBLICATION III

Differentiation of aspirated nasal air from room air using analysis with a differential mobility spectrometry-based electronic nose: a proof-of-concept study

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Journal of Breath Research. 2021;16(1):016004

Differentiation of aspirated nasal air from room air using analysis with a differential mobility spectrometry-based electronic nose: a proof-of-concept study

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Abstract

Over the last few decades, breath analysis using electronic nose technology has become a topic of intense research, as it is both non-invasive and painless, and is suitable for point-of-care use. To date, however, only a few studies have examined nasal air. As the air in the oral cavity and the lungs differs from the air in the nasal cavity, it is unknown whether aspirated nasal air could be exploited with electronic nose technology. Compared to traditional electronic noses, differential mobility spectrometry uses an alternating electrical field to discriminate the different molecules of gas mixtures, providing analogous information. This study reports the collection of nasal air by aspiration and the subsequent analysis of the collected air using a differential mobility spectrometer. We collected nasal air from ten volunteers into breath collecting bags and compared them to bags of room air and the air aspirated through the device. Distance and dissimilarity metrics between the sample types were calculated and statistical significance evaluated with Kolmogorov-Smirnov test. After leave-one-day-out cross-validation, a shrinkage linear discriminant classifier was able to correctly classify 100% of the samples. The nasal air differed ($p < 0.05$) from the other sample types. The results show the feasibility of collecting nasal air by aspiration and subsequent analysis using differential mobility spectrometry, and thus increases the potential of the method to be used in disease detection studies.

1. Introduction

Volatile organic compounds (VOC) are potential biomarkers of diseases, and their use in disease diagnostics has become a fast-growing field of research [1, 2]. A sample of exhaled human breath usually contains between 200 and 300 VOCs [3, 4]. Moreover, the sampling of exhaled breath is a non-invasive, painless technique that has potential for point-of-care use.

The gold standard method for VOC analysis is gas chromatography-mass spectrometry (GC-MS), which can identify individual compounds in breath samples. However, the method is expensive and requires experienced personnel to operate it. An additional drawback of mass spectrometry is that it often requires selective sampling, which limits the breadth of the molecules analyzed. In contrast to the GC-MS sampling method, the electronic nose (eNose) analyzes VOCs qualitatively, typically using an array of sensors that deliver a measurement signature, which could represent the VOC pattern of a certain disease [2]. A pattern recognition algorithm is then taught to discriminate different VOC patterns, and thus potentially discriminate diseased patients from healthy ones. eNose devices are usually relatively compact and they can perform a sample analysis in minutes. Indeed, eNose technology has even been shown to outperform mass spectrometry.

Differential ion mobility spectrometry (DMS) - also known as field asymmetric ion mobility spectrometry (FAIMS) - is a technique that uses an alternating electrical field to discriminate the different molecules of a sample. Although DMS is not based on sensor arrays, as is the case with traditional eNoses, it provides analogous information on gas-phase molecules. The operating principle of DMS makes it less prone to drifting and the batch-to-batch variation that has plagued many semiconductor sensor-based eNoses [5]. In DMS, however, the molecules of the sample need to be ionized. Although different ionization methods exist, not all molecules can be ionized using one specific method. Therefore, DMS is selective to a

certain range of VOCs. Theoretical advantages of DMS over microscale FAIMS are the longer residence time and the higher number of oscillations, which improves the separation capacity of the system. A further theoretical advantage of DMS over drift and travelling waves is the ability to perform continuous analysis compared to the pulsed measurements of the other methods. The additional advantage of DMS and FAIMS is that the method provides information on the behavior of molecules in high and low fields [6].

Numerous studies have examined the application of eNose technology in disease diagnostics with encouraging results. Classic asthma, for example, has been distinguished from chronic obstructive pulmonary disease with a sensitivity of 91% and a specificity of 90% [7]. Further, an ion mobility spectrometry-based eNose was able to diagnose prostate cancer from urine with a sensitivity of 78% and a specificity of 67% [8]. Using DMS analysis of urine samples, malignant ovarian tumors were differentiated from healthy controls with a sensitivity of 91% and a specificity of 63% [9]. As reported in a review by Farraia *et al.* [10], many published studies use exhaled breath as a sample material. We are, however, aware of only a few studies that have investigated the use of nasal air with an eNose. In these studies, patients exhaled through the nose, or the air was aspirated during normal respiration either into a breath-collecting bag or into an eNose [11-14]. Thus, air from the lungs and the pharynx could have affected the results since it is known that expiratory flow rate, breath hold and the fraction of breath analyzed can alter the measurement signature of the eNose [15, 16] and have a subsequent impact on the reproducibility of the measurements.

To only examine nasal air, the sample should be collected using aspiration as described by the American Thoracic Society and the European Respiratory Society (ATS/ERS) in their guidelines for the measurement of nitric oxide (NO) [17]. The aim of this study is therefore to report the collection and subsequent analysis of nasal air using an electronic nose based on DMS technology.

2. Materials and methods

2.1 System for the aspiration of nasal air

The device used for the aspiration of nasal air was a suction pump SP 625 EC-LC-DU (Spiggle & Theis Medizintechnik GmbH, Germany) operated with AA-batteries. A metal Politzer nasal olive was inserted to the patient's nostril and connected to the pump with a Teflon tube. Another Teflon tube was used to connect the pump to a 750 ml GaSampler Single-Patient Collection Bag (Quintron Instrument Company Inc. USA), which is a metalized polyester bag. Small pieces of silicone were then used to connect the tubes to the pump.

To prevent contamination of the air from the pharynx, the soft palate must be closed. Closure of the soft palate can be achieved by the patient blowing against a resistance of at least 10 cm H_2O as instructed by ATS/ERS [17]. In our study, a pressure of 15 cm H_2O was chosen. This was simply performed by measuring 15 centimeters of tap water into a plastic bottle and then asking the patient to blow bubbles in the water. During the aspiration of nasal air, the blowing was supervised by a nurse.

When the pump is started, ambient air is entrained through the patient's open nostril and through the nasal cavity to the contralateral nostril connected with the nasal olive. The seated patient inhales to total lung capacity and then begins to blow against a resistance. At this point, the air in the nasal cavity and the Teflon tubes still contains air from the pharynx. The total length of the Teflon tubes is approximately 500 mm with inner and outer diameters of 6 mm and 8 mm, respectively. Thus, the total volume of the tubes is 14 ml. It has been estimated that the volume of each nasal cavity is approximately 16 ml [18], resulting in a total volume in the nasal cavities and Teflon tubes of approximately 46 ml. The pump can induce a flow of 192 ml/s (11.52 l/min). Therefore, to clear contamination, the suction continues for 1 to 2 seconds while the soft palate is closed before the valve to the bag is opened.

It takes less than 10 seconds to fill the bag. Then, the valve is closed; the patient stops blowing, and the pump is shut down. Each participant used two Teflon tubes which were disposed of after taking the samples.

2.2 DMS device

The DMS device used in this study was the differential ion mobility spectrometer prototype Ionvision (Olfactomics Ltd, Finland). The DMS electrode was 20 mm in length, 8 mm in width, and the analytical gap was 0.25 mm. In DMS, the gas phase molecules are ionized by 4.9 kV soft x-ray. The ions travel in buffer gas in a channel formed by two electrodes, which create an oscillating electric field U_{SV} perpendicular to the motion of the ions. At the end of the channel is a detector, which consists of a Faraday plate connected to a transimpedance amplifier. The electric field has high- and low-voltage phases that cause the ions to travel in a zig-zag motion. If the ions hit the electrodes, they lose their charge before reaching the ion detector. To counter this effect, a compensation voltage U_{CV} is applied. At a certain electric field and compensation voltage value, certain ions reach the detector and generate a pA-range current signal that is detected. Scanning different electric fields and compensation voltages creates a measurement signature that can be presented as a dispersion matrix.

In this study, the samples were scanned with 60 evenly spaced U_{SV} values, ranging from 200 V to 800 V, and 100 evenly spaced U_{CV} values ranging from -1 V to 8 V. Thus, the resultant dispersion field was 800 V/mm – 3.2 kV/mm. The measurement was done simultaneously in positive and negative ion channels, resulting in data vectors of 12 000 dimensions per each measurement in total. The data matrices along with the measurement parameters are then saved as .json files by the DMS device.

2.3 Test participants

We recruited ten adult volunteers to the study. Exclusion criteria were as follows: pregnancy or lactation, smoking during past month, chronic rhinosinusitis, prior

paranasal surgery, acute upper respiratory infection less than a week ago, any use of nasal sprays during the past week, lower respiratory tract disease, such as COPD or asthma, severe immunodeficiency, and any cancer diagnosed within the past five years.

Of the ten participants, four were women and six were men. Mean age was 45 years (range 33 to 64). All participants were able to provide a sample after one attempt. No adverse effects were observed.

2.4 Collection and analysis of the samples

The samples were collected in the same room in the University Hospital to avoid any variation from environmental factors. Each participant provided one nasal air sample on two separate days, resulting in 20 nasal air samples. The collection of samples was completed in five days. Every day, we collected a bag of room air for background VOC comparison, resulting in five room air samples. The bags were connected to the pump with a Teflon tube and a silicone connector in the same manner as the nasal air sampling. However, the pump aspirated room air without having a nasal olive and Teflon tube attached to the inflow port. To remove any VOCs left by the previous participant, the pump was used to aspirate the room air for two minutes between subjects. Each bag was then transported to a separate location and analyzed with the DMS device within six hours, which is the maximum storing time according to the manufacturer of the collection bags.

The collection bag was attached to the DMS device with Teflon tubes. Small pieces of silicone were used between the connections of the tubes. We used a pneumatic ejector VR 05 (Schmalz, Germany) to produce a vacuum for sampling from the bag. Air flow from the sample bag was adjusted to 400 ml per minute with the Gilibrator-2 system (Sensidyne, FL, USA). Pressurized air was also used, and it was cleaned with activated carbon and 5Å molecular sieves. It diluted the sample air to a ratio of 10:1. Thus, total volumetric flow was 4.4 liters per minute. The DMS device can handle an

air flow of 3 liters per minute, so approximately 1.4 liters per minute were lost. Each measurement lasted about 30 seconds, and each collection bag was measured three times while connected to the device. Therefore, the analysis of one bag lasted approximately 1.5 minutes. However, as the volume of the bags were 750 ml and the flow rate was 400 ml per minute, the analysis cycle would require a volume of 1.2 liters. The flow from the sample was not, however, a constant 400 ml per minute because of the potential resistance in the bags when the volume of air was diminishing.

Between measurements of the bags, we measured the room air aspirated through the DMS device. The measurement protocol is shown in figure 1. Of all the measurements, there were 60 nasal air sample measurements and 43 measurements of room air aspirated through the DMS device (termed: reference air). We also had 15 measurements of five bags of room air (termed: room air). However, one measurement was accidentally deleted from the device history, leaving 14 measurements. The day-wise numbers of measurements are presented in table 1.

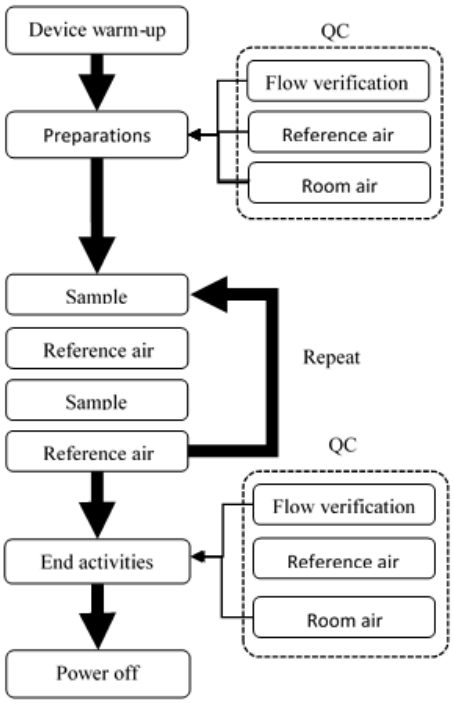

Figure 1. The measurement protocol of the air samples with the differential mobility spectrometry device

Table 1. Number of each measurement type per day.

	Measurement day					
Sample type	Day 1	Day 2	Day 3	Day 4	Day 5	total *n* per sample type
Nasal air	15	9	12	12	12	60
Room air	3	2	3	3	3	14
Reference air	9	7	8	8	11	43
Total *n* per day	27	18	23	23	26	

2.5 Data analysis

The data analysis was performed with a statistical software R [19] in RStudio environment [20]. Packages caret [21], sda [22] and lsa [23] were utilized.

2.6 Data pre-processing

The DMS data were pre-processed by row-wise normalization to emphasize the signals in the high-separation areas on the spectra (figure 2). Each row, corresponding to a fixed U_{SV} value, was scaled between 0 and 1 using the minimum and maximum value of the row. To avoid accidentally emphasizing background noise in the low intensity rows, all values below the pre-defined noise threshold were substituted with the global minimum of the spectra prior to the row-wise normalization. The noise threshold was defined by plotting the histogram of all the intensity data. When the histogram is visually inspected, a gaussian-shaped peak can be observed at the smallest end of the histogram. This is considered to be normally distributed background noise. The values below this visually chosen threshold were substituted with the threshold value.

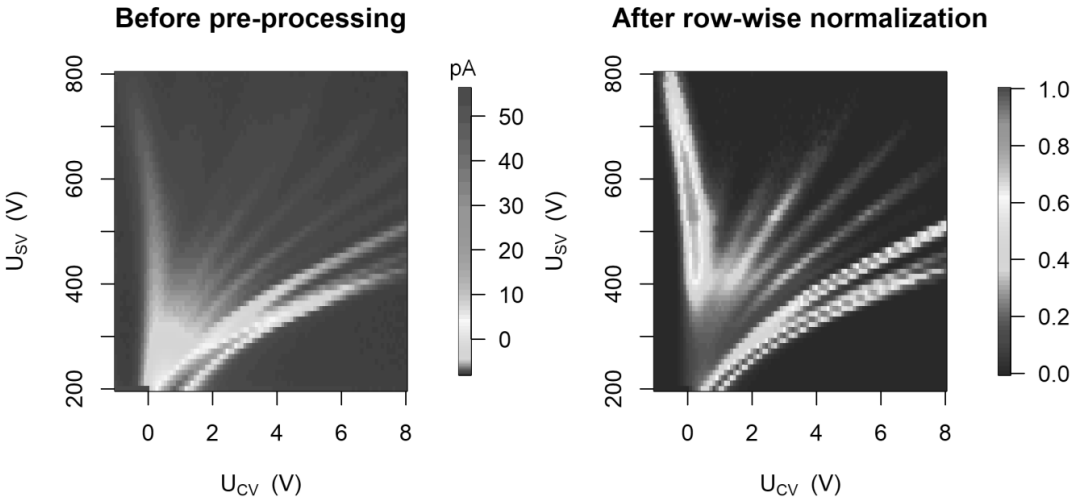

Figure 2. Averaged nasal measurements (negative side) before and after pre-processing and normalization. In the row-normalization, "fragment-like" peaks on the high U_{SV} values are the result of the normalization technique.
pA: picoamperes

2.7 Repeatability verification

To make reliable inference from the DMS measurements, the measurements must be repeatable. Thus, we need a method to compare the similarities and differences between the measurements. In the case of high-dimensional multivariate data (dimensionality d = 12 000), comparison of the measurements is not simple, and traditional univariate testing approaches cannot be used. Therefore, to estimate the repeatability and inter-class similarity of the DMS measurements, several difference and similarity measures were used.

The resemblance of the measurements can be measured with distance metrics, similarity metrics or dissimilarity measures. The distance between two identical measurements is 0, and this distance increases as the measurements are further away

from each other in their feature space. The distance metric used in this study was Euclidean distance, which is the distance between two p-dimensional data vectors x and y, is defined as follows:

$$d(x, y) = \sqrt{\sum_{i=1}^{p}(x_i - y_i)^2} \quad (1)$$

In theory, the upper limit for the distance does not exist. In contrast, similarity between two observations is 1 for identical observations and 0 for completely different observations. To have a comparable "similarity" metric for distance, a concept of dissimilarity (1-similarity) can be used. In this study, dissimilarity versions of cosine similarity as well as Pearson's and Spearman's rank correlation were used.

Our approach was to form an "archetype" for each sample type (nasal air, reference air, room air). In practice, an averaged dispersion plot of each data type was used for this (figure 3). In future studies, this kind of archetype could be used to calibrate the measurement device and the setup. The distance or similarity metric between the new measurements and the archetype can thus be calculated to see whether the new measurements are within the accepted distance/similarity interval.

The effectiveness of this approach was tested by comparing the within-group distances and dissimilarities to the between-group dissimilarities. The nasal air sample data were used to form the archetypes. To avoid bias, a separate archetype was calculated for the nasal air sample data of each measurement day, and the distance was then calculated between the archetypes and each individual measurement from the other days. The distributions of the within-group and the between-group distances and dissimilarities were then compared.

The statistical significance of the findings was tested with Kolmogorov-Smirnov test, which is a general non-parametric statistical test without any distribution assumptions.

2.8 Principal component analysis

Principal component analysis (PCA) is a dimensionality reduction method, where the data are linearly transformed into a feature space that maximizes the variance observed in the data [24]. The first two principal components of the dataset are visualized to illustrate how the data are naturally clustered.

2.9 Classification

Different classification approaches were utilized to find out whether the different measurement clusters were distinguishable from each other. A commonly used way to estimate a classifier's generalization ability to unseen data is cross-validation (CV). In CV, the dataset is divided into k mutually exclusive subsets, and each subset is left out as an independent test set. The rest of the subsets are used to form the model. The overall performance can then be estimated from the combined test results of the subsets. If the measurements are independent, the subsets (folds) can be formed by random split (k-fold CV), or each instance can even form a subset of its own (in which case it is called leave-one-out CV, LOOCV). In our case, however, the measurements are not independent: the measurement order, the measurement day and the participant all compromise the independence. Thus, CV was performed by dividing the data into day-wise or participant-wise folds.

Linear discriminant analysis (LDA) is a classification method, where the classes are separated by hyperplanes maximizing the class separation. Due to the high dimensionality of the data (d = 12 000), regularization is required. The regularized version of LDA is shrinkage LDA (sLDA), which has previously been applied successfully to classify DMS data [25, 26], and was used in this study, too.

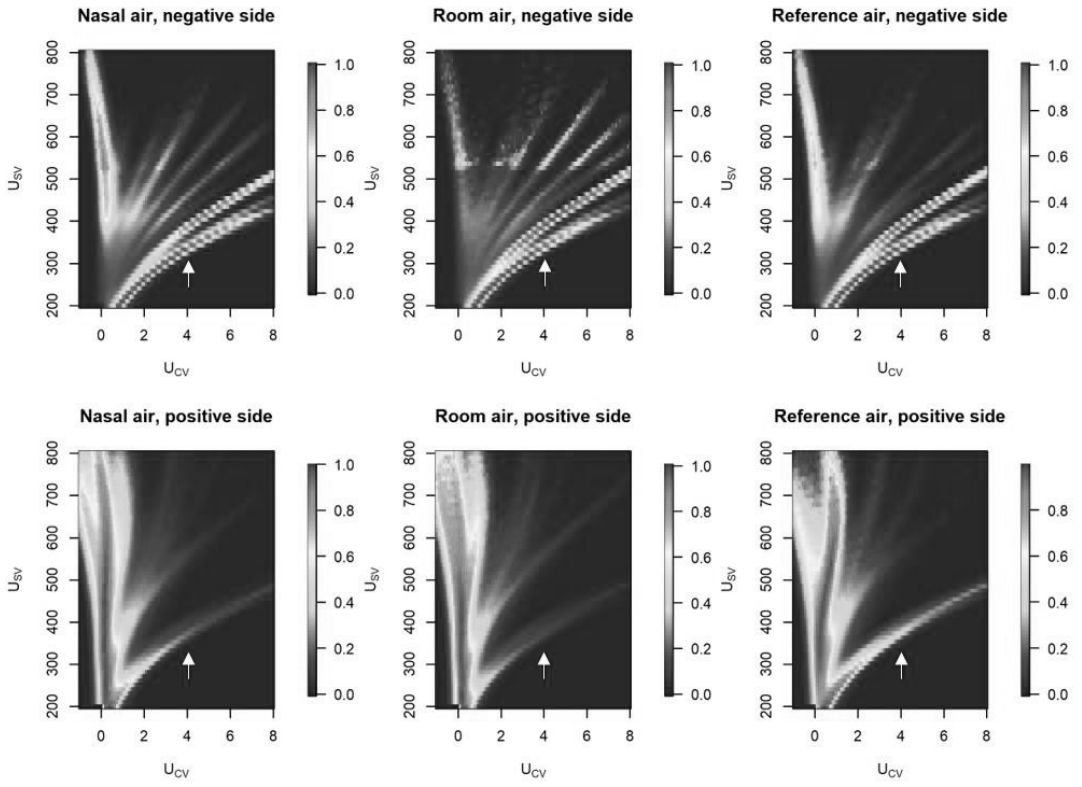

Figure 3. The averaged dispersion plots of each sample type after pre-processing with row-normalization technique. Reactant ion peak (a water peak) that resembles water in the spectrum is shown with a white arrow.

3. Results

PCA decomposition creates insight into the inherent clustering of the data (figure 4). The plots show that the nasal air measurements are distinguishable from the reference and room air measurements, while the measurement day also affects the measurements (figure 4a and 4b). In the nasal air data (figure 4c), it seems that even though the three nasal air measurements from the same bag are usually observed close together, there is no participant-wise clustering if the measurements of both bags of the same participant are studied.

The leave-one-day-out cross-validation results of the sLDA classifier for the sample types are shown in table 2. Each model was able to correctly classify 100% of the out-of-sample data. This means that the data were perfectly linearly separable.

Discrimination between study participants with sLDA was cross-validated by a 2-fold setup, where the first fold consisted of the first sample bags of each participant, and the second fold contained the latter measurement bags. The discrimination rate was 13.3% and, as such, does not significantly differ from the guess level of 10%. Visual assessment on the PCA plot (figure 4c) does not reveal significant clustering by a participant.

The boxplots of the distance and the dissimilarity metrics between the data groups and the nasal air sample archetype are shown in figure 5. The within-group distances and dissimilarities were notably lower than the corresponding between-group metrics in all cases. The differences between the distributions of the archetype class and the other types were statistically significant on a 95% significance level in all cases. Kolmogorov-Smirnov test showed statistically significance ($p < 0.05$) between all sample types.

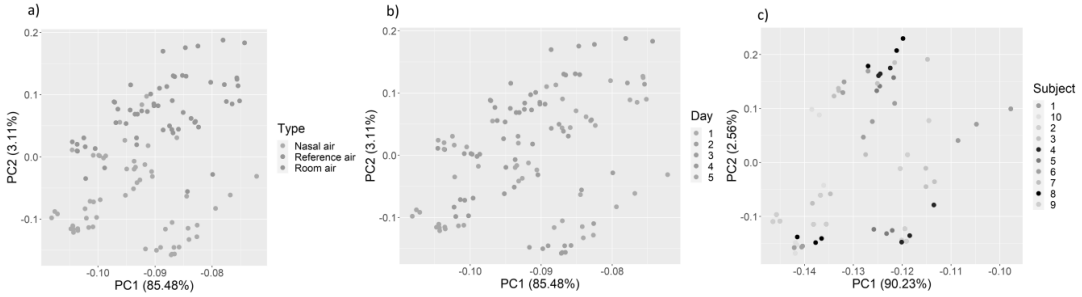

Figure 4. Two first principal components of a) the entire dataset, grouped by the measurement type, b) the entire dataset, grouped by the measurement day and c) the nasal air samples grouped by participants.

Table 2. Day-wise cross-validation results of the sample type classification with a shrinkage linear discriminant analysis model.

		True class		
		Nasal air	Room air	Reference air
Predicted class	Nasal air	60	0	0
	Room air	0	14	0
	Reference air	0	0	43

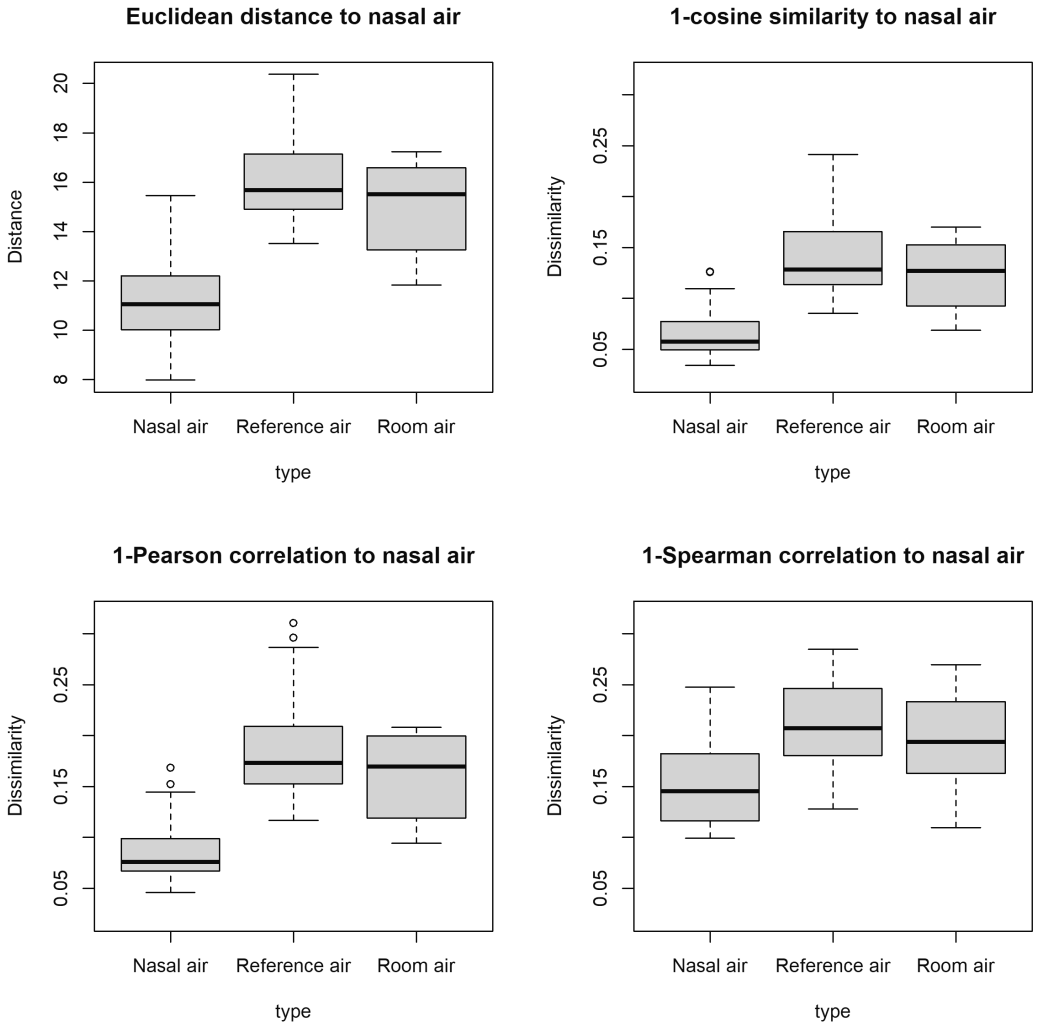

Figure 5. The boxplots of the cross-validated distance and dissimilarity metrics between the data groups and the nasal air sample archetype. The dissimilarity metrics all have the same scale on the vertical axis, whereas the Euclidean distance has its own, non-comparable scale.

The absolute humidity of the diluted sample types is shown in figure 6. Nasal air is more humid than reference and room air but no larger than variance of the humidity between measurement days. This is also illustrated by the visual intensity of a reaction ion peak in figure 3.

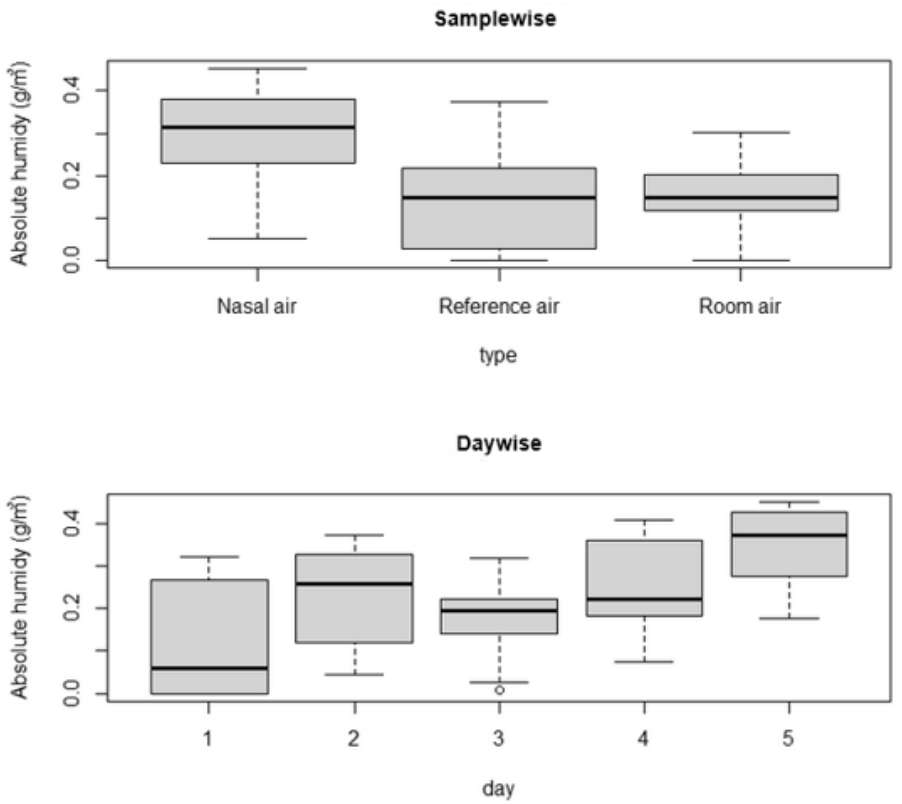

Figure 6. Absolute humidity in diluted air showing variance between samples and measurement days.

4. Discussion

In this study, we evaluated a method to collect and analyze nasal air that resembles the ATS/ERS guidelines for the measurement of nasal NO. Data analysis showed that nasal air, room air and reference air were clearly separable and established reference intervals for measurements from healthy participants. As the participants were not distinguishable from each other, there was no clustering of the data based on individual characteristics but only by sample type.

As we examined only healthy individuals, we do not know whether the analysis of nasal air can distinguish patients with conditions from healthy ones. To our knowledge, only a few studies have examined nasal air analysis in the diagnostics of diseases with eNoses [11-14]. Mohamed *et al.* [11] collected nasal outbreath into sterile plastic sacks from five chronic rhinosinusitis patients and five controls. Patients inhaled through the mouth and exhaled through the nose to fill the sack. In addition, the plastic sack contained a tampon that was first held in the middle meatus of the patient's nose to stimulate mucosal secretions. The contents of the sack were then analyzed using an eNose developed for research purposes (LibraNose, University of Rome Tor Vergata and Technobiochip) that utilizes quartz crystal microbalances covered with metalloporphyrins. The eNose had a sensitivity and specificity of 60% after leave-one-out cross-validation. Thus, the results did not differ much from the guess level of 50%. In the other study, breath samples from patients with acute rhinosinusitis symptoms and controls were obtained using a modified nasal CPAP mask that was connected to an eNose based on conducting polymer sensors. After leave-one-out cross-validation, the eNose could diagnose bacterial rhinosinusitis with an accuracy of 72% [12]. Both the aforementioned studies examined exhaled nasal air coming directly from the lungs, the pharynx and the oral cavity, which may have caused confusing results. However, Steppert *et al.* had more promising results in two recent pilot studies investigating nasal air with an IMS

coupled with a multicapillary column [13, 14]. In their first study, nasal air was aspirated during normal respiration. Samples were collected from individuals with confirmed influenza-A infection and then compared to persons with negative test results and to healthy volunteers. Influenza-A-infected patients were distinguished with perfect sensitivity and specificity [13]. Furthermore, the second study showed that an analysis of exhaled nasal air from patients with SARS-CoV-2 infection could be distinguished from patients with influenza-A infection and healthy controls with accuracy of 97% after cross-validation [14].

We consider it crucial to investigate aspirated nasal air in studies concerning rhinologic diseases to exclude confounding factors as much as possible. Guidelines suggest the application of aspiration in the measurement of nasal NO [17], which is also applicable for nasal air analysis. This method excludes sample contamination with air originating from the lungs, the pharynx or the oral cavity, as it could affect the VOC profile. For instance, Smith *et al.* [27] examined ammonia levels in breath air and found that levels are significantly less in nose-exhaled breath than mouth-exhaled breath. The same applies for ethanol and hydrogen cyanide [28]. Because ion mobility spectrometry is sensitive for these compounds, they could cause significant bias if left unchecked [29-31]. Therefore, an analysis of nasal air could reduce contamination by endogenous VOCs that originate in the oral cavity. In exhaled breath sampling, VOCs depend on which portion of breath is analyzed. Alveolar samples, for example, show different VOCs than mixed expiratory samples [15]. Also, expiratory flow rate and breath hold influence the eNose pattern [16]. Aspiration of the nasal air does not suffer from these problems. However, one should note the potential confounding factors of aspiration. First, we did not measure nasal CO_2, which, when remaining low, would verify the closure of the soft palate. Nevertheless, blowing against resistance of a minimum of 10 cm H_2O is approved to be adequate [17]. Second, the flow of the air was not measured, and it could be affected by nasal aerodynamics. As the batteries of the pump were running out, a

reduction in the flow of the air was observed. Measurements of nasal NO suggest a targeted airflow, otherwise the values of NO are affected [17]. Similarly, the signal patterns of the eNose could be altered.

Many patient-related factors can affect the breath analysis regardless of whether the sample is collected through the mouth or the nose. For example, the consumption of certain foods can affect VOCs [32]. Some studies even advocate fasting before sample collection [7, 33, 34], but the role of fasting or diet on VOCs is unclear [35]. Moreover, we are unaware of any previous studies that have compared the effects of diet on the nasal and oral sampling of air. Other possible covariates that alter VOCs include age, gender, smoking status, and comorbid diseases. However, controversy exists as to which of these covariates should be adjusted for breath analysis [4, 34, 36-39]. Also, medication, such as nasal sprays, could affect the VOC profile. Indeed, nasal decongestants and corticosteroid sprays have been shown to decrease nasal NO [40-42]. In our study, we advised the volunteers to refrain from using their nasal sprays for a week prior to sample collection, but a shorter period would probably have been sufficient. However, corticosteroid sprays have a prolonged effect on inflammation, and the time the sprays take to wear-off is unknown.

Room air is a source of exogenous VOCs that might interfere with the results. A typical way to exclude the impact of room air is to use an inspiratory VOC filter and to rinse the patient's lungs with filtered air [43]. In our study, this would have demanded the use of a filter attached to the patient's open nostril. To our knowledge, it is not known how much time would be enough to rinse the nasal cavity with purified air. As we used an estimate of 32 ml for the volume of both nasal cavities [18], we expect that a few seconds of aspiration would replace the room air in the nasal cavity with purified air. Nevertheless, one should note that use of a clean air supply might be an additional confounding factor since it might reduce concentrations of likely endogenous VOCs and increase exogenous [44]. Furthermore, breath collection devices can release contaminant VOCs [45], which

also applies to our pump. Therefore, if one would want to eliminate its effect, an airtight container should be used. The container has the sampling bag inside and two airtight ports. One port connects to a pump outside the container and the other to a bag to supply sample air from the patient via a tube. When the pump is turned on, the air in the container is drawn out, which produces a differential pressure, and air is then drawn into the bag via the port from the patient's nose. This method would not, however, allow cleaning of the nasal cavity from air of the oral cavity while the patient is blowing against resistance at the beginning of nasal air aspiration. In addition to the pump, the tubes are also a potential source of contamination. In the present study, we used Teflon tubes which were disposed of after the test. Teflon is a suitable material due to it being inert and is suitable for use with the eNose [46]. Teflon is, however, quite rigid and requires more adjustment with the pump compared to silicone.

It should be borne in mind that in the present study air samples were stored in the collection bags. Previous studies have shown that VOCs adsorb from bags over time, which affects the storing time [47, 48]. Therefore, we analyzed all samples as quickly as possible and within at least 6 hours, as instructed by the manufacturer of the bags. Furthermore, we did not re-use the collection bags, although with cleaning protocols it would have been possible and would have reduced costs [48, 49]. However, cleaning may still fail to remove some compounds [50]. During the analysis of the bags with the DMS device, the flow from the sample was set to 400 ml per minute, but the resistance in the bags during emptying varied and affected the flow rate. Therefore, clean air was most likely present in different volumes in the three measurements of the one bag. This did not, however, seem to have a significant effect since the PCA composition shows that the measurements are usually close to each other.

Since some environmental factors, such as temperature, humidity and air quality, cannot always be controlled in a clinical setup, they can be expected to affect the

DMS measurements in some way. The DMS is sensitive to humidity and therefore measurements of the same compound in different humidity levels might produce different results. However, the air in the nasal cavity is saturated to between 90% and 100% [51, 52]. Therefore, changes in the humidity of room air supposedly does not significantly affect the measurements of nasal air. The measurement device itself can also produce dynamically changing baseline noise to the measurements due to system stabilization. The changes in the baseline can be compensated by using various normalization methods. The row-wise normalization used in this study highlights the higher parts of the DMS dispersion matrices, where the peak separation is the highest but the signal is the weakest. With this method, the sample types became perfectly linearly separable.

A potential reason for the separability of nasal air from the reference and room air is that nasal air contains endogenous VOCs and the concentration of some VOCs of the room air might change during the air flow through the nasal cavity. Most importantly, the air is humidified in the nasal cavity during aspiration to the collection bag. As seen from the figure 6, nasal air is more humid than other samples but the humidity also varies greatly between measurement days. Although the reaction ion peak that resembles water in the spectrum differs between different sample types, there is significant variation caused by other compounds in other areas of the spectrum as well (figure 3). Because the measurement device was located in a different location compared to the collection of the nasal air and room air samples (hospital environment), the VOCs in the reference air were different, which may explain the differences in the box plots. Although we recognize that the strength of our study is limited by the small sample size, the study still manages to achieve good, unbiased results despite this limitation.

The data analysis also had possible bias factors. The most obvious bias factor results from the nasal air sample bags, each of which were measured three times on the same

day. They are expected to be highly similar to each other, and this is also supported by the PCA transformation of the nasal air data, where the measurements from the same bag are usually observed close together. Furthermore, the measurement order of the different samples (figure 1) was always the same and could therefore cause bias and affect the distances and classification results. Another factor is the measurement day. The measurement conditions during a measurement session are similar between measurements, which is why measurements from the same session tend to cluster together. Thus, to avoid bag-wise and day-wise bias, the cross-validation was performed by leaving each day as a test set at a time. However, since the nasal air of each participant was measured twice on separate days, each test day contained data from the same participants that were also present in the training data. Even though the participant-wise measurements did not form participant-wise clusters in the visual inspection of the PCA, this is still a possible bias factor in the sLDA classification. However, since the measurement types did not differ significantly between days, this is unlikely to be a great disadvantage.

The distance comparisons show that the distance and dissimilarity metrics, especially Euclidean distance where the relative differences between the archetype group and the other groups are most prominent, could be used to study the repeatability of the DMS data and in device calibration. The absolute values of the Euclidean distances cannot be directly compared to the dissimilarities since the scales differ. However, as Euclidean distance is widely used in different fields and it is intuitively simple to understand, we recommend its use. Moreover, all the presented metrics are computationally cheap to evaluate.

Breath analysis for disease detection is an exciting and promising field of research. Although it is important to find disease-specific biomarkers using, e.g., mass spectrometry, the qualitative analysis of breath based on pattern recognition better suits fast and cheap point-of-care use. As there is a lack of standardization in breath

sampling, we evaluated a method that is similar to the validated method for the measurement of nasal NO to diminish confounding factors. We believe that this kind of approach is suitable for use in the diagnostics of rhinologic diseases, such as acute and chronic rhinosinusitis, allergic rhinitis and sinonasal cancers.

5. Conclusion

Although numerous studies on exhaled breath analysis exist, this is the first study to examine the eNose analysis of aspirated nasal air with soft velum closed. The study shows that the concept of collecting nasal air into a breath collecting bag by aspiration and the subsequent analysis of the nasal air using DMS works well. Indeed, DMS distinguishes sample types perfectly but the difference in the humidity of the samples might contribute to the results. We believe that the analysis of aspirated nasal air with DMS brings more potential for the use of the method in disease detection studies.

Acknowledgements

Conflicts of interest

Markus Karjalainen, Anton Kontunen, Niku Oksala and Antti Roine are shareholders in Olfactomics Ltd, which is about to commercialize proprietary technology for the detection of diseases using ion mobility spectrometry. Anna Anttalainen is an employee in Olfactomics Ltd. The remaining authors have no conflict of interests to declare.

Funding

Jussi Virtanen declares funding from the Doctoral School of Tampere University, the Finnish Medical Foundation, the Finnish ORL-HNS Foundation and Tampere Tuberculosis Foundation. This study was financially partly supported by the Competitive State Research Financing of the Expert Responsibility area of Tampere University Hospital. These organizations had no role in the design or execution of the study or in the writing process of the manuscript.

Ethical statement

All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was conducted according to medical device trial regulations and was approved by the National Supervisory Authority for Welfare and Health. All volunteers provided written informed consent.

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PUBLICATION IV

Identifying chronic rhinosinusitis without nasal polyps by analyzing aspirated nasal air with an electronic nose based on differential mobility spectrometry

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Acta Otolaryngologica. 2022;142(6):524-531
doi: 10.1080/00016489.2022.2093397

ACTA OTO-LARYNGOLOGICA
https://doi.org/10.1080/00016489.2022.2093397

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RESEARCH ARTICLE

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Identifying chronic rhinosinusitis without nasal polyps by analyzing aspirated nasal air with an electronic nose based on differential mobility spectrometry

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ABSTRACT

Background: The diagnosis of chronic rhinosinusitis (CRS) is a complicated procedure. An electronic nose (eNose) is a novel method that detects disease from gas-phase mixtures, such as human breath.

Aims/Objectives: To determine whether an eNose based on differential mobility spectrometry (DMS) can detect chronic rhinosinusitis without nasal polyps (CRSsNP) by analyzing aspirated nasal air.

Materials and methods: Adult patients with CRSsNP were examined. The control group consisted of patients with septal deviation. Nasal air was aspirated into a collection bag and analyzed with DMS. The DMS data were classified using regularized linear discriminant analysis (LDA) models with 10-fold cross-validation.

Results: The accuracy of the DMS to distinguish CRSsNP from patients with septal deviation was 69%. Sensitivity and specificity were 67 and 70%, respectively. Bonferroni-corrected statistical differences were clearly noted. When a subgroup with more severe inflammatory disease was compared to controls, the classification accuracy increased to 82%.

Conclusions: The results of this feasibility study demonstrate that CRSsNP can potentially be differentiated distinguished from patients with similar nasal symptoms by analyzing the aspirated nasal air using DMS. Further research is warranted to evaluate the ability of this novel method in the differential diagnostics of CRS.

ARTICLE HISTORY
Received 9 May 2022
Revised 13 June 2022
Accepted 15 June 2022

KEYWORDS
Chronic rhinosinusitis; differential mobility spectrometry; electronic nose; nasal air

Introduction

The diagnosis of chronic rhinosinusitis (CRS) is a complicated procedure that is based on patient history and clinical findings. It is therefore difficult to estimate the true prevalence of CRS. In Europe, a prevalence of 11% was obtained in a symptom-based questionnaire study [1]. Symptoms alone, however, tend to overestimate the prevalence and are therefore not sufficient for the diagnosis of CRS [2]. The endoscopic visualization of purulence or mucosal inflammation in the middle meatus or the presence of polyps improves the specificity of the symptom criteria. Computed tomography (CT) is the gold standard in the diagnostics of CRS and is recommended for symptomatic, endoscopy-negative patients [3]. New innovative and non-invasive methods are, however, needed to help identify patients with CRS.

The analysis of human breath is an interesting field of research. The measurement of exhaled nitric oxide (NO), for example, can be used in the diagnostics of asthma. In addition to specific molecules, the non-targeted analysis of gas-phase compounds can also be used in disease diagnostics. The electronic nose (eNose) attempts to mimic mammalian olfaction. The device consists of an array of gas sensors combined with pattern recognition software and performs a qualitative analysis of gas-phase mixtures. The result is a measurement signature of the volatile organic compounds (VOCs) contained in the sample, which could represent the VOC pattern of a certain disease. Thus, different diseases could potentially be differentiated by comparing their VOC patterns and, as a result, eNose technology has gained interest in research. In many previous studies, exhaled breath has been used as a sample material [4]. Furthermore, studies have shown that a 'breathomics'-based approach can be used to diagnose and even determine the phenotype of asthma [5]. To date, only a few studies have examined the diagnostics of rhinosinusitis using an eNose. However, the accuracy reported in these studies has varied between 60 and 85% [6–8].

Although differential mobility spectrometry (DMS) is not an eNose in the strictest sense, it provides comparable information on the samples. In DMS, ionized molecules are separated from each other using an alternating electrical field. An *in vitro* study revealed that DMS can distinguish five

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Table 1. Demographics of the patients and comparison of the groups.

	CRSsNP ($n = 27$)	DS ($n = 27$)	p Values
Gender male/female	13/14	22/5	.021*
Age, median (range)	51 (24–71)	43 (23–69)	.140**
BMI, median (range)	28.4 (22.0–39.1)	27.1 (19–39.8)	.169***
SNOT-22 score, median (range)	38 (9–57)	29 (9–67)	.345***
Lund–Mackay score, median (range)	8 (4–19)	–	–

BMI: body mass index; CRSsNP: chronic rhinosinusitis without nasal polyps; DS: deviated septum; SNOT-22: Sino-Nasal Outcome Test.
*Fisher's exact test.
**Independent samples *t* Test.
***Mann–Whitney *U* test.

common rhinosinusitis bacteria from each other with an accuracy of 79% [9].

The aim of this feasibility study was to investigate whether patients with chronic rhinosinusitis without nasal polyps (CRSsNP) could be distinguished from patients with nasal symptoms due to deviated septum (DS) by analyzing the aspirated nasal air of these patients using DMS.

Materials and methods

Participants

In this prospective study, we consecutively recruited eligible patients into two groups: the CRSsNP group and the control group.

The following inclusion criteria were used for the CRSsNP group: age $\geq$ 18 years, symptoms meeting European Position Paper on Rhinosinusitis and Nasal Polyps criteria for CRSsNP [2], and paranasal mucosal changes visible in cone beam tomography or multislice CT. As incidental mucosal changes are possible in CT scans, Lund–Mackay (LM) scores were calculated and only those patients with LM scores of four or more were included. Nasal endoscopy was not performed.

Patients were recruited to the control group if they were aged $\geq$ 18 years, had experienced nasal congestion, and DS was diagnosed based on anterior rhinoscopy, but no evidence of purulence or polyps was observed. Again, nasal endoscopy was not performed.

The exclusion criteria for both groups were pregnancy or lactation, smoking during the past month, nasal polyps, prior paranasal surgery, acute upper respiratory tract infection during the past two weeks, severe immunodeficiency, or malignant disease treated in the previous five years.

All patients were examined and recruited by a single ENT specialist. After giving written informed consent to participate, patients filled out the 22-item Sino-Nasal Outcome Test (SNOT-22). The test is validated in the Finnish language and has revealed that healthy controls have a mean score of 8.9 [10]. Therefore, patients were included if they had a score of nine or more. No prior reparations, such as fasting and no exercise, were required. Information about the patient's current medication, allergies, height, weight, and any drinking or eating during the past two hours was obtained during an interview.

In total, 58 patients were asked to participate and two declined. Of the two patients who declined to participate, one had CRSsNP and the other had DS. Furthermore, two patients (one with CRSsNP and one with DS) were excluded because they had used nasal sprays within the past 24 h. Thus, 54 patients were enrolled in the study. The demographics of the patients are presented in Table 1. All the procedures performed in this study were in accordance with the Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of Tampere University Hospital (R16103). The study was conducted according to medical device trial regulations and was approved by National Supervisory Authority for Welfare and Health. All patients provided written informed consent.

Collection of nasal air

We collected nasal air into a collection bag by aspiration in the same manner as described earlier [11]. This resembles the standardized method for the collection of nasal air for the measurement of NO [12]. In brief, nasal air was aspirated using a suction pump SP 625 EC-LC-DU (Spiggle & Theis Medizintechnik GmbH, Overath, Germany). A Teflon tube with a metal Politzer nasal olive was inserted into the patient's nostril while the other end was attached to the suction pump. The collection bag was a metalized polyester 750 ml GaSampler Single-Patient Collection Bag (Quintron Instrument Company Inc., Milwaukee, WI), which was connected *via* a Teflon tube to the outlet port of the pump. The patient blew against resistance of 15 cmH_2O to close the soft palate, allowing only the aspiration of nasal air. This was simply performed by measuring 15 cm of tap water into a plastic bottle and then asking the patient to blow bubbles in the water. Less than ten seconds is required to fill the bag. The contents of the bag were then analyzed with the DMS device within 6 h. The set up for the collection of nasal air (without the plastic bottle) and attachment of the full bag to the DMS device is shown in Figure 1.

DMS device

The DMS device used in this study is a differential mobility spectrometer (Ionvision, Olfactomics Ltd, Tampere,

Figure 1. Above: the pump used to aspirate nasal air. A collection bag at the end of Teflon tube and a nasal olive at the end of another Teflon tube. Below: the DMS device attached to the full collection bag and ready for a measurement.

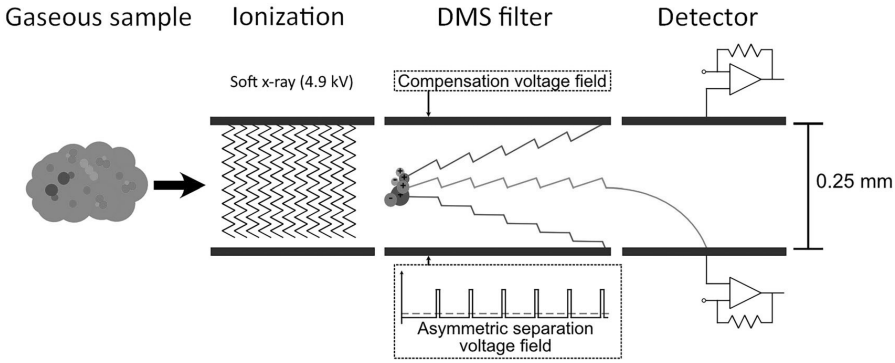

Figure 2. A schematic representation of the differential mobility spectrometer (DMS) used in this study. kV: kilovolts; mm: millimeters.

Finland). In DMS, gas phase molecules are ionized and driven into a drift chamber formed by two electrodes (Figure 2). These electrodes create an oscillating electric field that is perpendicular to the ions' motion in the carrier gas. The separation field of DMS alternates between high and low voltages asymmetrically. This causes ion swarms to travel in a saw-like motion that separates ion clusters based on their mobility characteristics. An additional compensation voltage field is applied to selectively prevent certain ions from colliding with the chamber electrodes. At the end of the chamber is a detector into which ions collide, creating an electric current signal. When different electric fields and compensation voltage values are scanned, the result is a measurement signature called a dispersion plot. The measurement is conducted simultaneously for both positive and negative ions.

Analysis of the samples

Samples were collected and analyzed within 28 separate days. Each sample bag was analyzed three times while connected to the DMS device. The dispersion plots were

measured using separation voltages (U_{SV}) of 200–800 V with 60 increments and compensation voltages (U_{CV}) of −1–8 V with 100 increments, resulting in a 6000-dimensional dispersion plot for positive and negative ions. The baseline of the device was evaluated by inspection of the dispersion plot using an analysis of the room air aspirated through the device between each patient sample at the beginning and the end of the measurement session. Further, bags of room air were collected with the pump and analyzed at least once during a measurement session. However, if significant carry-over was observed in the dispersion plots, the bags of room air were analyzed more often.

Data analysis

The baseline characteristics (age, gender, body mass index (BMI), SNOT-22 scores) were compared to evaluate whether there were any statistical differences between the two groups. Analyses were performed using IBM® SPSS® Statistics for Windows version 27 (IBM, Armonk, NY). First, we evaluated whether the data were normally distributed by visual inspection of the histograms and by Shapiro–Wilk test. For normally distributed variables (age), the comparison was made with independent samples *t* test and for non-normally distributed variables (BMI, SNOT-22) with Mann–Whitney *U* test. Gender was compared with Fisher's exact test.

The DMS data were classified using regularized linear discriminant analysis (LDA) models with 10-fold cross-validation. Regularized LDA has been used in the classification of DMS data in previous studies [11]. The classification models were created in MATLAB version 2019a (MathWorks, Natick, MA). The differentiation of the nasal air samples from the room air samples was first conducted using every available measurement to ensure that there was no methodological problem with the sampling. For the classification of the sample types, the average spectrum of the three DMS measurements from each patient was utilized. Each separation voltage row was normalized to values of 0–1 to further accentuate the differences in the spectra in the higher separation voltages, where the absolute signal intensities are lower.

Spectral archetypes were created by averaging each measurement into a single spectrum for CRSsNP and DS. The statistical differences between the features of the archetype spectra were studied with the Kolmogorov–Smirnov test. The statistical significance was determined by the Bonferroni-corrected *p* value for each feature ($p < .05/6000$). To study the similarity of the measurements within and between classes, the Euclidean distance from the CRSsNP and DS archetype was also calculated for each measurement.

In addition to distance analysis, a subset of patients that had an LM score of at least 10 ($n = 11$) was classified separately against 11 randomly selected patients with DS to assess the effect of the severity of CRSsNP on the classification results.

The performance of the classification models was evaluated using the accuracy, sensitivity, specificity, negative, and positive predictive values. The Wilson score interval method was used to calculate 95% confidence intervals (CIs).

Results

Both groups consisted of 27 patients. There were 13 males and 14 females in the CRSsNP group and 22 males and five females in the DS group. Comparison of the groups shows that only gender was statistically significantly different ($p = .021$) but others (age, BMI, and SNOT-22 score) were not ($p > .05$) (Table 1).

Nasal air vs. room air

Nasal air samples were differentiated from room air samples with a classification accuracy of 94% (CI 91–96%), and visual comparison of the Euclidean distances (Figure 3) revealed that they are clearly distinguishable from the sample archetypes.

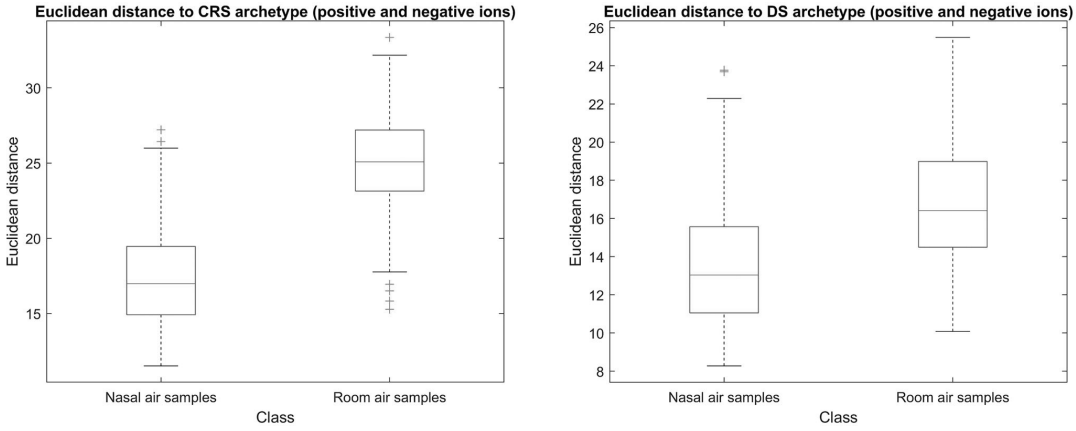

Figure 3. Euclidean distances of each measured sample type from sample archetypes. CRSsNP: chronic rhinosinusitis without nasal polyps; DS: deviated septum.

Table 2. A confusion matrix presenting the classification results of differential mobility spectrometry between chronic rhinosinusitis without nasal polyps (CRSsNP) and deviated septum (DS).

		Predicted class		
		CRSsNP	DS	
Actual class	CRSsNP	18 True positives (TP)	9 False negatives (FN)	Sensitivity = TP/(TP + FN) = 67%
	DS	8 False positives (FP)	19 True negatives (TN)	Specificity = TN/(TN + FP) = 70%
		PPV = TP/(TP + FP) = 69%	NPV = TN/(TN + FN) = 68%	

NPV: negative predictive value; PPV: positive predictive value.

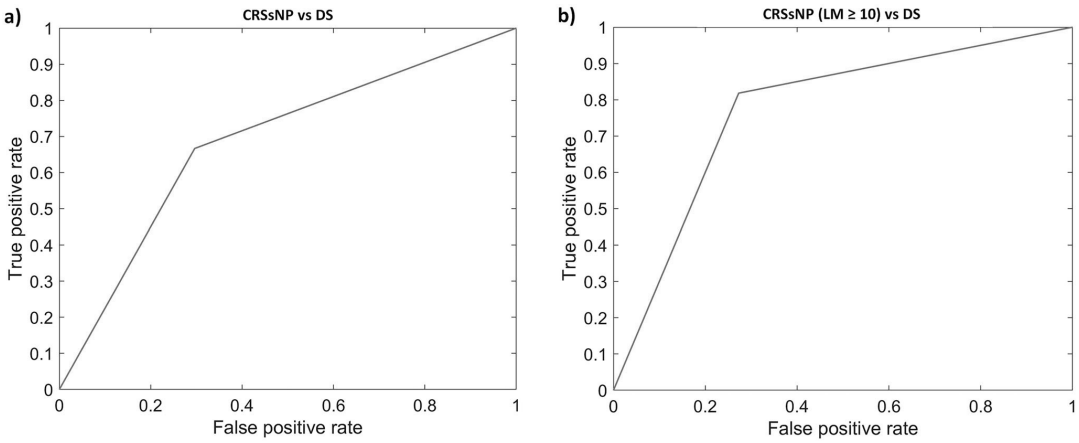

Figure 4. Receiver operating characteristic curves for the classification of CRSsNP and DS; a) for all patients and b) for patients with LM-score of at least 10. CRSsNP: chronic rhinosinusitis without nasal polyps; DS: deviated septum; LM: Lund–Mackay.

CRSsNP vs. DS

After row-normalization and 10-fold cross-validation, the accuracy of the DMS to distinguish CRSsNP from DS patients was 69% (CI 55–79%). Sensitivity and specificity were 67% (48–81%) and 70% (52–84%), respectively. The confusion matrix is presented in Table 2 and the receiver operating characteristics (ROC) curves in Figure 4. The archetype spectra for CRSsNP and DS are shown in Figure 5 and visual comparison of these reveal differences, especially with negative ions. Furthermore, the differences are visible with Bonferroni-corrected statistical differences in the spectra (Figure 6).

In subgroup analysis between CRSsNP patients with LM score $\geq$ 10 ($n = 11$) and randomly chosen DS patients ($n = 11$), the accuracy, sensitivity, and specificity were 82% (52–95%), 82% (52–95%), and 82% (52–95%), respectively.

Male vs. female

As there were statistical differences between males and females in the groups ($p < .05$), we performed a classification between genders. When comparing the samples from men ($n = 35$, labeled as negatives) and females ($n = 19$, labeled as positives), the DMS had an accuracy of 46%, a sensitivity of 26% (5/19), and a specificity of 57% (20/35), showing that classification is not biased due to gender.

Discussion

Our results show that by analyzing aspirated nasal air, DMS can distinguish CRSsNP with a sensitivity of 67% and a specificity of 70%. Moreover, the classification accuracy increases to 82% when a subgroup with more severe inflammatory disease is compared to controls. These findings are suggestive of a distinct VOC profile being associated with CRSsNP.

The previous literature on the eNose in a CRS context is heterogenous. Mohamed et al. examined five CRS patients and five controls. In their study, the eNose developed for research purposes had an accuracy of 60% after leave-one-out cross validation [6]. Thaler and Hanson performed a case-control study and examined rhinosinusitis patients and sampled breath air exhaled through the nose with a modified nasal continuous positive airway pressure (CPAP) mask. After leave-one-out cross validation, the eNose had an accuracy of 72%. However, in their study, most of the patients had acute exacerbation of CRS [7], and the acute infection could have affected the VOC profile. A more recent study by Broza et al. employed an array of

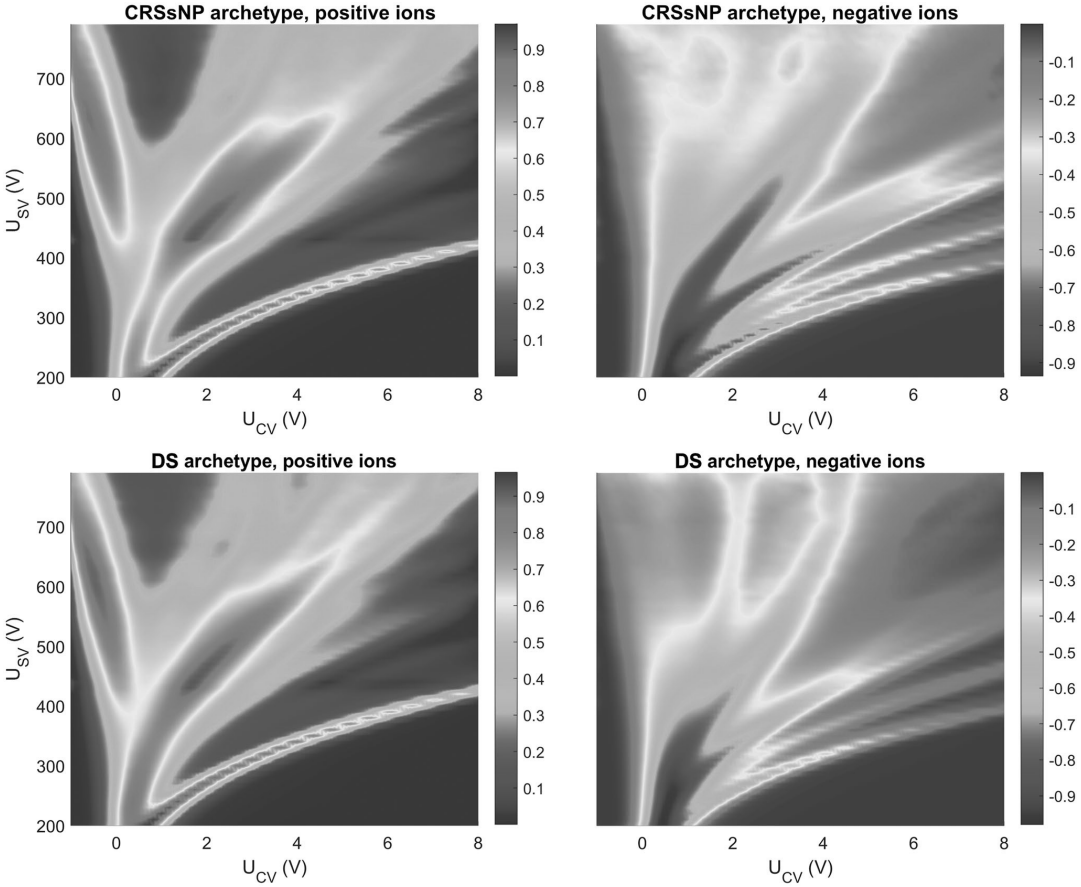

Figure 5. The row-wise normalized archetype spectra for chronic rhinosinusitis without nasal polyps (CRSsNP) and deviated septum (DS). U_{SV}: separation voltage; U_{CV}: compensation voltage; V: volt.

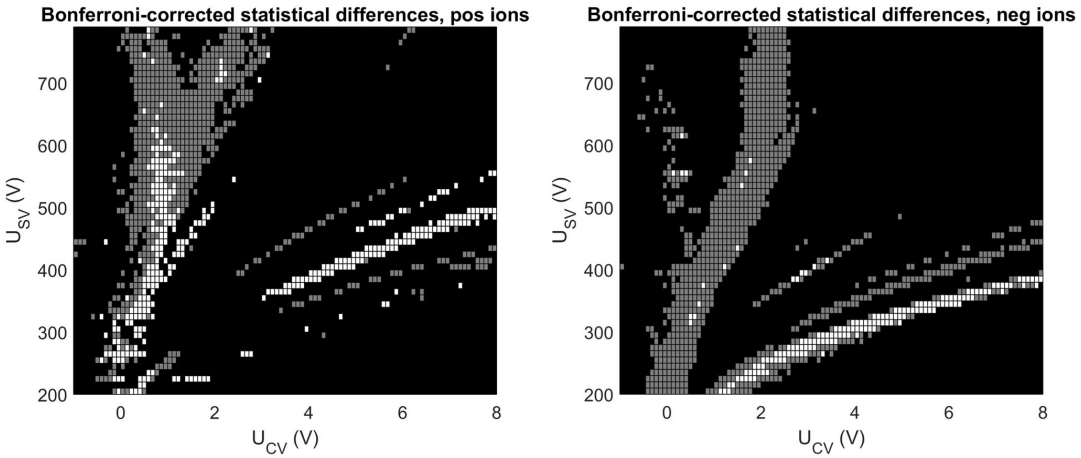

Figure 6. Features with Bonferroni-corrected statistical differences between the classes highlighted with grey for the complete dataset (54 patients) and with white for the subset of patients with Lund–Mackay score of more than 10.

nanomaterials-based sensors to examine exhaled breath through the mouth from CRS patients with polyps ($n = 24$) and CRSsNP ($n = 17$), and healthy controls ($n = 30$). After external validation, patients with CRSsNP were distinguished from healthy controls with a sensitivity of 80% and a specificity of 89% [8].

In all the above studies [6–8], patients with CRS were compared to healthy volunteers, which might have overestimated the diagnostic accuracy. Therefore, in this study, we aimed to distinguish two groups of patients with similar symptoms but with different etiology. In our control group, patients were symptomatic due to anatomical factors rather than inflammatory ones, although DS may predispose to chronic mucosal inflammation [13]. Though unproven, this may alter the measurement signature of the DMS and influence the accuracy. However, as the patients with DS did not undergo CT examination, some of the patients could have had mucosal changes in the paranasal sinuses, which may have also affected the DMS analysis. In addition, the exclusion of nasal polyps in the control group was conducted by anterior rhinoscopy which can, in some cases, result in false negative findings. Other paranasal diseases could also be confounding factors.

Our subgroup analysis revealed that patients with LM scores of 10 or more were more effectively distinguished from DS patients, which may have been due to a more severe inflammatory disease leading to a more prominent VOC profile. The sample size, however, was small and limits the drawing of any further conclusions. Furthermore, the results are limited to only CRSsNP. CRS has different endotypes and phenotypes and the signal patterns of the eNose could differ between them.

Although most eNose studies sample exhaled breath [4], few concentrate on nasal air. In this study, we collected nasal air using a similar method to the one validated for the measurement of nasal NO [12]. The method minimizes the confusing effects in the collected sample for evaluating rhinologic diseases with DMS. Previous studies have shown that exhaled nasal air contains less ammonia, ethanol, and hydrogen cyanide than breath exhaled through the mouth [14]. These compounds could therefore serve as confounding factors, since ion mobility spectrometry is sensitive to them [15,16]. Although collecting exhaled breath is simpler than collecting aspirated air through the nose, VOCs differ in terms of expiratory flow rate, breath hold, and the portion of exhaled breath analyzed [17]. These factors do not, however, affect aspirated nasal air, although targeted airflow during aspiration, such as in the measurements of nasal NO, would be preferable [12].

The comparison of room air samples to nasal air samples was distinguishable (accuracy of 94%) and shows that the measurement protocol works and day-to-day variation or baseline drift of the DMS do not exist to a significant degree. However, nasal air samples were not as well discriminated between patient groups, as the accuracy was 69%. Still, Bonferroni corrected statistical differences reveal that there were differences between patients with CRSsNP and patients with DS.

Patient-related factors can have an impact on breath sampling. For example, age, gender, and smoking, can interfere with the measurement signature of the samples, but it is unclear which of these factors should be adjusted in breath analysis [8,18]. In this study, we excluded some of the potential confounding factors, such as smoking. The groups were similar regarding age and BMI. Furthermore, our analysis showed that the classification was not based on gender. The differences in SNOT-22 scores were not statistically significant, but patients with CRSsNP had higher scores (the median values 38 *vs.* 29). Therefore, the severity of symptoms was not identical even though falling into the category of moderate disease in both groups [19]. Although higher LM scores improved classification accuracy in this study, they do not correlate with SNOT-22 scores [20]. Therefore, the impact of symptom severity on VOC profiles between groups is unclear. Furthermore, it remains unknown whether there are specific symptoms that affect the VOC profile in the spectra of the dispersion plots. Further studies are required to evaluate which covariates and symptoms can affect the VOC patterns.

In addition, the limitations of the data analysis need to be addressed. Even though cross-validation was used in this study to alleviate overfitting, the generalizability of the results could have been improved by using a completely independent set of patients for validation of the model. Furthermore, the sample size is limited, as demonstrated by the wide CI, and reduces the power of the study. Therefore, the results should only be interpreted as preliminary.

The potential use of a breathomics-based approach in addition to the instruments currently used to identify CRS is intriguing. However, it cannot replace clinical examination, such as observing intranasal findings. As phenotyping asthma from exhaled breath is already possible [5], the same could perhaps be done for CRS phenotyping by analyzing nasal air. At present, however, eNose technology is not ready for use in the diagnostics of CRS. Therefore, to evaluate the ability of DMS or other eNoses to diagnose CRS further, a larger study containing a control group of patients who have symptoms positive for CRS, according to established criteria, but negative CT findings should be undertaken. This would lead to a diverse etiology of symptoms, such as allergic and non-allergic rhinitis, DS, and hypertrophy of the inferior turbinates. These etiologies could have an impact on the nasal air profile and would require a larger number of samples to build a trustworthy classifier. Furthermore, patients with different endotypes and phenotypes of CRS should be evaluated as their VOC profile could differ from each other. Preliminary findings show that CRSsNP was distinguished from CRS patients with polyps with an accuracy of 67% [8]. In addition, the impact of disease severity based on symptoms and objective findings to the VOC profile should be further evaluated.

Conclusion

The results of this feasibility study demonstrate that CRSsNP can potentially be differentiated from patients with

similar nasal symptoms by analyzing aspirated nasal air using an eNose based on DMS. Further research is warranted to evaluate the ability of this novel method in the differential diagnostics of CRS.

Acknowledgments

The authors thank study nurse Merja Rumpunen for her help recruiting the patients and collecting the samples.

Disclosure statement

Anton Kontunen, Niku Oksala, and Antti Roine are shareholders in Olfactomics Ltd, which is about to commercialize proprietary technology for the detection of diseases using ion mobility spectrometry. The remaining authors have no conflict of interest to declare.

Funding

Jussi Virtanen declares funding from the Doctoral School of Tampere University, the Finnish Medical Foundation, the Finnish ORL-HNS Foundation, and Tampere Tuberculosis Foundation (Korvatautien Tutkimussäätiö; Suomen Lääketieteen Säätiö; Tampereen Tuberkuloosisäätiö). This study was also financially supported by the Competitive State Research Financing of the Expert Responsibility area of Tampere University Hospital.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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