Detection of inflammation by measuring volatile organic compounds in exhaled breath of patients with cystic fibrosis

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Layman summary

Cystic fibrosis (CF) is a progressive disease that is characterized by chronic infections and inflammation. Inflammation leads to worsening of symptoms and cause lung function decline and permanent tissue damage. To preserve quality of life in CF patients, adequate treatment is needed.

Volatile Organic Compounds (VOCs) are small particles that can be found in exhaled breath. Research has suggested the use of VOCs as a new tool for detecting the presence of bacteria in the lungs and pulmonary inflammation. Using VOCs in exhaled breath instead of current methods would allow for less invasive detection of inflammation and limit unnecessary treatment.

In this review, the potential of VOCs for predicting pulmonary inflammation is assessed based on comparing recent studies in this field. These studies were found to show variety in study objectives, study population, methodology and results.

Individually, the studies did show promising findings by being able to predict pulmonary inflammation in patients with CF by using VOCs. However, there was not one result shared among studies. This was probably because of variation between studies. Variation in results was thought to explain the fact that the use of VOCs is not yet ready for clinical use. To accelerate the implementation of VOCs in clinics, future research should make use of universal protocols, larger study groups and focus on discrimination between different types of VOCs to be able to tell what VOCs are specific for inflammation.

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Abstract

Cystic fibrosis (CF) is a progressive disease characterized by chronic infections and pulmonary exacerbations, causing lung function decline and permanent tissue damage. Adequate intervention, targeting exacerbation through antibiotic treatment is needed to preserve quality of life. Volatile Organic Compounds (VOCs) in exhaled breath have been suggested as a new biomarker for pathogen colonization and respiratory inflammation. Detection of pulmonary exacerbation through exhaled breath analysis, using gas chromatography with mass spectrometry (GC-MS) and electric nose (eNose), will allow for less invasive detection of inflammation and avoid inadequate or unnecessary treatment.

This review assessed the potential of VOCs for predicting pulmonary exacerbation in patients with CF based on recent studies in this field.

Studies included showed variety in study objectives, study population, methodology and results on VOC findings.

Results show promising findings in exacerbation identification and prediction. However, no ambiguous conclusion could be drawn after comparing the collected data, therefore not providing selected VOCs or VOC-patterns specific for exacerbation detection. Variation in results substantiates the observation that exhaled breath analysis is far from clinical implementation. Future research should make use of universal protocols, include larger cohorts and longitudinal data, and focus on discrimination between pathogen- and exacerbation-specific VOCs by setting up fitting cohort studies and comparing findings to in-vitro studies.

Introduction

Cystic fibrosis

Cystic fibrosis (CF) is an inherited and progressive disorder affecting mucociliary clearance, resulting in recurrent and chronic infection of the respiratory tract. Inflammation with pathogens in CF patients can result in pulmonary exacerbations, worsening clinical outcome and quality of life.[1] A common pathogen found in CF patients is Pseudomonas aeruginosa, responsible for chronic lung infection.[1] Chronic infection with Pseudomonas aeruginosa is associated with increased disease severity in both adults and pediatric CF patients.[2, 3]

Exacerbations in CF

Pulmonary exacerbations are characterized by episodes of acute worsening of symptoms and decline in lung function. Clinical presentation of exacerbation can be severe but also subtle with small changes in symptoms or little function decline.[4]

There is no consensus on defining exacerbations. In general, an exacerbation consists of a worsening of symptoms and a change in clinical parameters leading to additional treatment. Aiming for a universal interpretation of exacerbation, different organizations and research groups have suggested criteria for exacerbation definition based on clinical parameters, including symptom indication and measurements. These include the EPIC (Early Pseudomonas Infection Control) criteria by the EPIC program as well as the EuroCareCF Working Group definition for exacerbation, following changes in clinical parameters. [5-7] As to introducing a symptom defined exacerbation score like these ones, variability is likely to be introduced by the physician's opinion.[5] Both sets of criteria for the definition of an exacerbation are rather extensive and open to interpretation. The need to avoid variability in clinical assessment drives the need to more objective identification of exacerbations and more adequate treatment.[8]

Treatment of exacerbations

Going through pulmonary exacerbations is not without consequence for CF patients, as exacerbations in CF accelerate lung function decline and cause tissue damage. The inflammation in CF is associated with neutrophils releasing oxidants and proteases like elastase. Presence of elastase in the airway precedes the appearance of bronchiectasis, exacerbations, and lung function decline. This increases the need for adequate detection methods and anti-inflammatory and antibiotic treatment of exacerbations in CF.[9]

CF patients that present with pulmonary exacerbations generally receive a two-week antibiotics treatment. In severe cases, or if oral treatment proves insufficient, intravenous antibiotics are prescribed. When colonized with Pseudomonas aeruginosa, standard of care includes long term suppressive therapy with antibiotics.[10] Research data is lacking on optimal duration of treatment as well as the benefits of corticosteroid treatment. Consequently, the treatment of exacerbations in CF patients varies among medical centers. Furthermore, treatment of pulmonary exacerbation fails to achieve complete recovery of pulmonary function parameters in 25% of cases.[11] Also, experiencing multiple exacerbations over time, the rate of lung function (FEV1) decline increases.[12]

Absent consensus on defining and treating pulmonary exacerbations as well as their destructive nature highlight the need for early detection of exacerbations in CF patients. Additionally, current methods of detecting underlying pathogens in CF patients like cough swabs and sputum samples analyses are invasive and show low sensitivity and specificity.[13, 14] Therefore, research aims for identification of new biomarkers indicating pulmonary inflammation by analyzing blood serum/plasma-, sputum- and exhaled breath samples.[15]

VOCs

Volatile Organic Compounds (VOCs) are carbon-based molecules with high volatility at room temperature. They are present in exhaled breath and reflect products of bacterial and human metabolism.[16] By reflecting in vivo environment of for example the respiratory tract, VOCs (or VOC patterns) could be used as a biomarker or diagnostic tool in specific disease. In this manner, detecting VOCs might be used as a method for detecting known pathogens in the respiratory tract of CF patients to monitor infection status.[17]

The golden standard for exhaled breath analysis and VOC identification is gas chromatography with mass spectrometry (GC-MS), a technology enabling detection and

identification of individual compounds. Unfortunately, it is a rather expensive, complex and time-consuming technique.[16] Challenges in GC-MS led to the development of the Electronic nose (eNose) technology. Exhaled breath analysis by eNose is fast, cheap and allows for point-of-care testing. The technique is based on cross-gas-sensors, identifying VOC patterns rather than specific VOC concentrations.[18] In recent years, studies have been performed to test the ability of eNoses to serve as a noninvasive diagnostic tool, mostly in case control settings regarding obstructive airway diseases. These studies generally show good accuracy and support testing eNose technology in clinical settings.[19]

Implications of VOCs

Studies have shown promising results in differentiating CF patients from healthy controls as well as detecting Pseudomonas Aeruginosa using VOCs in exhaled breath samples of CF patients using both GC-MS and eNose.[20-22] More clinically relevant would be identification of inflammation markers in the lungs, as colonialization with pathogens can be harmless, and the inflammatory process during exacerbation is responsible for tissue damage and condition decline in CF patients.[8] Accordingly, studies have suggested VOC analysis as a tool for predicting chronic and acute lung diseases, including CF.[23] Exhaled pentane and ethane have been reported to show elevated concentrations in inflammatory diseases, and in CF patients. These VOCs have been described to be useful as non-invasive markers for oxidative stress.[24, 25] Furthermore, Isoprene has been described in CF exacerbation. [26]

What is important to consider is to carefully differentiate between pathogen-specific VOCs and inflammation-specific VOCs. The presence of inflammation-specific VOCs will most likely indicate the presence of pathogen-specific VOCs since inflammation is generally accompanied by pathogen presence. Contrarily, colonization can exist without inflammation, producing pathogen-specific VOCs without indication of inflammatory compounds.

Relevance

Clinical implication of VOC analysis on exhaled breath samples in patients with CF could help in monitoring patients and predicting exacerbations as well as identifying pathogen colonization in an objective manner. Consequently, this could limit unnecessary antimicrobial treatment and allow for timely pathogen eradication or adequate intervention in case of pulmonary exacerbation. This will prevent lung tissue damage and increase quality of life for CF patients.

Also, breath sample analysis allows for feasible, straightforward, and non-invasive sample collection with unlimited sample availability, making it a strong competitor for current sampling methods like cough swabs and sputum cultures.

This review assesses the potential of VOCs for predicting pulmonary exacerbation in patients with CF based on recent studies in this field. Different approaches of researching inflammation detection through breath analysis will be compared and evaluated. This includes study- set up, study population and duration (cross-sectional vs longitudinal) and research aim, targeting pathogen-specific VOCs or also including inflammation biomarkers. This assessment will lead to recommendations for future research on breath analysis.

Methods

Literature search process

A literature search was done on September 19th, 2023, on MEDLINE (PubMed) using the terms: *VOC /Volatile Organic Compound* AND *Exacerbation* OR *Inflammation* AND *cystic fibrosis / CF*. Selection of articles was based on title, abstract, study design and research method.

Reviews and meta-analyses were excluded for analysis. However, the reference lists of these articles were searched for relevant articles that were not found in the search but did meet the search criteria.

Clinical cohorts addressing exacerbation-specific VOCs were included. This was represented by analyses comparing non-exacerbation tot exacerbation patients. Studies solely researching specific pathogens were excluded as well as studies exclusively assessing the use of VOCs to discriminate between CF patients and healthy controls.

No preset definitions for CF diagnosis or exacerbations were used since it was expected that this would limit the number of studies found. As for research methods, only studies using GC-MS and eNose were included, as GC-MS is the golden standard for assessing VOCs and eNose is showing promising results in recent years.

Data synthesis

After selection, a table was made for comparing aims, study set-up, study population, analytical methods, and main result. Regarding all these variables, only data relevant for exacerbation detection was selected.

For defining study population, cohort size, number of patients with pulmonary exacerbations (PEX), age (pediatric, adult or both), CF diagnosis criteria and exacerbation definition were included, and inclusion criteria were investigated and compared.

Results

Searching MEDLINE (PubMed) generated four results. One review was excluded. The other three studies were included based on title, abstract, study design and research method. Additionally, three studies were included that were found browsing the library of the review that was excluded. Therefore, for investigating exacerbation prediction in patients with CF, a total of six studies was included.[27-32] Table 1 shows aims, study set-up, study population, analytical methods, and main results for these studies, discussing exacerbation prediction in CF.

Study objectives

Study objectives varied among the studies included. McGrath et al. and Barker et al. targeted specific VOCs, based on previous research findings, hereby strengthening known correlations, and looking for result validation. McGrath et al. investigated isoprene as a marker for PEX in CF and Barker et al. were looking to compare 12 different VOCs; ethane, propane, n-pentane, methanol, ethanol, 2-propanol, acetone, isoprene, benzene, toluene, dimethyl sulphide (DMS) and limonene. On the other hand, Paff et al., Joensen et al., van Horck et al., and Woollam et al. performed explorative studies, aiming for identification of either specific VOCs or VOC patterns.

Tabel 1: Comparison of studies on identification of pulmonary exacerbation

Authors	Year	Aim / main study objective	Study population			Set up	VOCs	Analytical method	Result on exacerbation detection
			pwCF, n	PEX, n	Pediatric/ adult				
McGrath et al.	2000	To investigate isoprene as a marker for PEX in CF	12	12	Adult	Longitudinal case-control	Isoprene	TD-GC-MS	Decreased isoprene levels during PEX
Barker et al.	2006	To compare 12 specific VOCs in pwCF and healthy controls with a methodologically defined procedure	20	5	Both	Cross sectional	ethane, propane, n-pentane, methanol, ethanol, 2-propanol, acetone, isoprene, benzene, toluene, dimethyl sulphide (DMS) and limonene	GC-MS	Higher alveolar gradient of n-pentane in breath samples from individuals with PEX.
Paff et al.	2013	To differentiate breath profiles in pwCF, PCD patients and HCs, assessing PEX status according to breath profile	25	9	Pediatric	Cross-sectional case- control	VOC pattern	eNose	Discrimination of pwCF with and without PEX with 89% sensitivity and 56% specificity
Joensen et al.	2014	Explorative eNose-based analysis of pwCF, PCD patients and HCs	64	10	Both	Cross-sectional case-control	VOC pattern	eNose	Discrimination of pwCF with and without PEX (90% sensitivity, 50% specificity)
van Horck et al.	2021	To determine whether exacerbations can be predicted by VOC	49	31	Pediatric	Longitudinal observational	Discriminating VOCs defined by Random Forrest modelling	GC-MS	A model including nine discriminatory VOCs was found to correctly predict 79% of children with upcoming PEx (sensitivity 79%, specificity 78%)
Woollam et al.	2022	To identify VOCs indicative for PEX in CF	18	7	Pediatric	Cross-sectional study	3,7-dimethyldecane, durene, 2,4,4-trimethyl-1,3-pentanediol 1-isobutyrate and 5-methyltridecane	GC-MS	Identification of specific VOCs correlating to PEX classification

pwCF; people with cystic fibrosis
PEX: pulmonary exacerbation
PCD: primary ciliary dyskinesia
HCs; healthy controls

Study population

All studies included people diagnosed with CF. The criteria for CF diagnosis differed among studies. McGrath et al. based CF diagnosis on genotype and or sweat testing (chloride >70 mmol/L). Joensen et al., Paff et al. and Horck et al. included clinical symptoms in CF diagnosis in combination with abnormal sweat test (sodium >60 mmol/L) and/or two CF mutations (in both CFTR-gene alleles). In contrast, Barker et al. and Woollam et al. did not define the criteria for CF in their study population.

In the study by McGrath et al., CF patients were only included when showing chronic colonization by more than one organism associated with CF, based on sputum cultures. Similarly, Joensen et al. prioritized inclusion of chronically infected CF patients with the intention to achieve an even distribution of chronically infected and non-infected patients. This reflects their multiple study objectives besides solely assessing exacerbation. In most studies, patients with pulmonary exacerbations were included by chance since they were recruited in stable state during outpatient clinic visits.

When further comparing study cohorts, the amount of pwCF included ranged from 12 in McGrath et al. to 64 in Joensen et al.. In McGrath et al., Paff et al. and Joensen et al. a group of healthy controls was included allowing for a case-control set up.

As for age, McGrath et al. only included adult patients, Paff et al, van Horck et al. and Woollam et al. included solely pediatric patients and Barker et al., and Joensen et al. included both adult and pediatric patients.

Defining exacerbation

As described in table 3 Barker et al. does not disclose to the reader what their criteria were when defining exacerbation. The criteria used for defining exacerbation in the other studies included similar components, among which the need to start with antibiotics and decrease in lung function (FEV1) of 10% or more. Partly covered by the need for antibiotic treatment is introduction or worsening of respiratory symptoms, also included in the criteria for exacerbation definition. Furthermore, additional clinical parameters like weight loss, temperature, physical examination findings, fatigue or lethargy, radiographic changes were included. In Joensen et al., Paff et al., en van Horck et al. Paff et al. refers to the Dutch national CBO guidelines from 2009.[6] Van Horck et al. uses the EPIC trial criteria.[7] Van Horck et al. included patients when receiving antibiotics for increase in clinical symptoms, regardless of meeting the exact EPIC criteria, in order to prevent biased results. Also, van Horck et al. were the only ones considering symptom duration while defining exacerbation.

Tabel 2: Exacerbation definition per study

Author	Exacerbation definition
McGrath et al.	Reduction in forced expiratory volume in one second (FEV1) of >10% compared to the best in the previous year, an increase in respiratory symptoms and a decision to treat with intravenous antibiotica
Barker et al.	Unknown
Paff et al.	The need to start additional antibiotic treatment as a consequence of a recent change in at least two of the following clinical parameters: change in sputum volume or color, increased cough, increased dyspnea, increased malaise, fatigue or lethargy, temperature over 38°, anorexia or weight loss, change in sinus discharge, change in physical findings on examination, decrease in pulmonary function by 10% or more and radiographic changes. This was done according to ***national CBO guidelines***, based on internationally accepted criteria
Joensen et al.	The need to start additional antibiotic therapy and the presence of at least two of the following six criteria: change in sputum volume and/or color; increased coughing; increased lethargy, feeling unwell, or increased need for sleep; decreased appetite or weight loss; decrease in lung function ≥10%; increased shortness of breath or new acquired radiologic changes
Van Horck et al.	Defined in two ways. First, according to the ***EPIC trial***: established by one of the major criteria alone, or two of the minor signs, and fulfilment of symptom duration (duration of sign/symptoms ⩾5 d or significant symptom severity) And second, when the responsible paediatric pulmonologist started a course of therapeutic antibiotics (oral or intravenous) evaluating the clinical symptoms as an expression of a PEx, not meeting the exact EPIC criteria. This in order to prevent biased results.
Woollam et al.	The treating clinicians' choice to treat with antibiotics for new respiratory symptoms and/or a decline of FEV1pp >10% predicted from each individual subject's baseline

Set up and methodology

When investigating study set-up, Barker et al., Paff et al., Joensen et al. and Woollam et al. all performed cross-sectional studies while McGrath et al. and van Horck et al. included longitudinal data (>1 measurement/patient).

Gas chromatography mass spectrometry was the analytical method of choice in the studies of McGrath et al., Barker et al., van Horck et al., and Woollam et al.. The others, Paff et al. and Joensen et al., looked into VOC patterns making use of electric nose techniques. Both studies used the Cyronose 320, holding 32 sensors.

Results

Study results regarding detection of exacerbations through breath analysis can be found in table 1. McGrath et al. described a decreased isoprene level during exacerbation that increased significantly after treatment. The observed results were said to raise the possibility of isoprene not tracking oxidative stress or being indirectly related to oxidative stress.[27] Barker et al. measured a higher level of n-pentane in individuals experiencing exacerbations, but also in the presence of chronic Pseudomonas infection. According to the authors, these findings support the hypothesis that exhaled pentane reflects in vivo oxidative stress.[28] Paff et al. were able to significantly discriminate between CF patients with- and without exacerbation with a sensitivity of 89% and specificity of 56% using eNose technique.[30] Joensen et al. were able to reach similar results with a sensitivity of 90% and 50% specificity. These results were also obtained through VOC profiling using eNose.[29] Van Horck et al. were able to correctly predict exacerbations in pediatric CF patients in 79% of cases using a model with nine discriminatory VOCs; C_8H_{18}branched, C_9H_{20} branched, 2,4-dimethyl-1-heptane, 1,3-dimethylbenzene and/or 1,4-dimethylbenzene, p-benzoquinone, camphene, pentadecane, tetradecanal and 3-methyl-2-butanone. Van Horck et al. performed classification modelling using Random Forest multivariate analysis. With this model they reached a sensitivity of 79% and a specificity of 78%.[31] Lastly, Woollam et al. identified specific VOCs correlating with exacerbation in CF; 3, 7-dimethyldecane, durene, 2,4,4-trimethyl-1,3-pentanediol, 1-isobutyrate and 5-methyltridecane.[32]

Discussion

When comparing studies on prediction of exacerbation in patients with CF, there is no homogonous result that is shared among the studies included, meaning there is not one specific VOC, group of VOCs, or VOC pattern that was identified by multiple authors as being predictive for exacerbation. However, individually, studies were successful in identifying pulmonary exacerbation. Even though all studies aim for identifying a VOC/VOC- pattern in detection of exacerbations in CF, their approaches and set-up greatly vary, possibly explaining the variation in results. Supposedly, this observation contributes to the challenge of clinical implication of breath analysis, for example in CF patients.

Study population

The studies included in this review, assessing exacerbation detection in CF, differ in study population and inclusion criteria. In all studies, CF patients were included based on previous

diagnosis. However, the criteria for CF diagnosis differed among studies. Sometimes, the diagnosis reflected clinical symptoms. Other studies have based CF diagnosis solely on genotyping (mutations in both CFTR-genes) and sweat test results. Although this could have led to minor variations in study population between studies, it is not expected that this has greatly influenced research outcomes in terms of VOCs that are indicative for exacerbation. Barker et al. and Joensen et al. prioritized patients with chronic infections during patient inclusion, influencing the cohort formation and the representation of the average CF population. However, when analyzing exacerbation detection in this population, differences in cohort formation are not expected to have great implication on VOC outcomes.

As mentioned before, until present day there is no consensus on defining exacerbation in CF. This was reflected in the observation that the studies included differ in how they defined exacerbation. However, similar components were used in this definition. Besides similarities in how an exacerbation was defined, the use of a symptom defined exacerbation score for identification of pulmonary exacerbations is likely to introduce variability caused by the physician's subjective evaluation.[5] This, and the heterogenous use of exacerbation definition poses incongruency when comparing studies in this review and highlights the need for more reliable and objective diagnostic tool for exacerbation identification. Moreover, since the presence or absence of exacerbation defines group distribution when analyzing exacerbation identification, differences in defining exacerbation could have greatly influenced results and comparability between studies. Result implications could include variation in positive and negative predictive value when thinking of exacerbation detection as a diagnostic tool in CF.

Differences in methodology

Next to heterogenicity of study populations, factors that have been described to influence VOC measurements include diversity in techniques and hardware, and variety of modelling and reporting strategies. Also, VOC analysis results can be modified by temperature, humidity, phase of breath, expiratory flow rate and collecting materials. This variability inevitably generates VOC outcomes that are inconsistent between studies, while within-study variability can be closely monitored by for example aiming for identical measurement environments or pre-processing of data, as is done by van Horck et al. for example.[33]

The inability to reproduce research findings could be related to differences in methodology. For example, Barker et al. could not reproduce Paredi et al. and contributed this to differences in methodology and ambient VOC, ethane in this case. These differences were not specified by the author, but presumably concern differences in the collection of exhaled breath. The inability to reproduce research findings was also the case for isoprene, when looking at previous findings by Kharitonov et al.[25, 26, 28] Similarly, these differences pose challenges when comparing results, as is done in this review. Furthermore, interpreting GC-MS results in comparison to eNose outcomes can be challenging since the eNose is limited to certain sensors that can differ between devices. This relates to the observed differences in study objectives, reflecting limitations in possible study outcomes. Variation between eNose devices is not relevant for this review since studies making use of the eNose technique, Paff et al. and Joensen et al. both used the Cyronose.

It is hard to draw an unambiguous conclusion since results from the studies included are not directly comparable due to differences in methodology. However, studies do show

promising results with high specificity and sensitivity, suggesting relevant correlations can be found within certain research environments.

Influence of covariates

Next to differences in methodology, it is relevant to consider the influence of certain covariates that have been described to influence VOCs in exhaled breath, namely: stress, age, time of day, gender, activity, body mass index, disease status, diet, malnutrition and medication use.[33] The effect of body mass index, for example, can be explained because lipophilic volatile compounds accumulate in fat tissue, influencing the tissue distribution of VOCs and therefore what is measured in breath samples.[34] The influence of these variables contribute to the heterogenous distribution of results in this review because they introduce interpersonal differences. Since every study is subject to these differences, study comparability is not expected to be greatly affected. Besides, as is done in most studies, influences of these variables are corrected for during analysis. For example, Woollam et al. performed correction for age and BMI by removal of VOCs that correlated with these variables. Also, Van Horck et al. included a selection of covariates in their analysis. Especially interesting is the covariate colonization with Pseudomonas aeruginosa, because correction for this covariate strengthens the claim that the VOCs found for exacerbation prediction are specific for inflammation.

Next to interpersonal differences, within-person variation occurs as well. Exhaled breath VOCs represent a fraction of the metabolome of a patient, also including indirect VOCs. These VOCs may have been subject to metabolization or have passed tissues with varying constitutions and affinities to these compounds. Also, they could originate not solely from the respiratory tracts and lungs, but from the mouth, nose, UGI and stomach as well. Therefore, VOC (patterns) in exhaled breath can present in variable ways, introducing challenges in interpretation and comparability to other breath samples.[33] As for exacerbation detection in exhaled breath, next to the change in metabolism that is expected due to inflammation, these within-person variations can introduce incongruency when comparing breath samples.

Future research

Similar to what is said about research on sputum biomarkers for pulmonary inflammation, it can be said that the use of VOCs in exacerbation detection in CF patients is not yet near introduction to clinical practice due to variability in study design and sampling methods.[35] To accelerate clinical implication of breath analysis for exacerbation prediction in CF patients, researchers should make use of universal protocols, include bigger cohorts, and use longitudinal measurements.

Future research should make use of similar inclusion- and definition criteria, allowing for better comparability in results, accelerating clinical implication of breath analysis. Furthermore, bigger cohorts will allow for minimization of inevitable methodological and personal variability. Also, taking into consideration the uncertainty of exacerbation occurrence at time of inclusion, expanding cohort size is beneficial.

In most of the studies included in this review, exacerbation detection was included as one of more research aims with pulmonary exacerbation being one or many outcome variables. Also, there was a limited number of patients experiencing exacerbations. Since these observations could have influenced research outcomes, future research might focus on

exacerbation detection as their main research objective. Like McGrath et al, van Horck et al., and Woollam et al., future research that aims for exacerbation prediction specifically, covariates and confounders can be assessed more carefully and elaborately.

Longitudinal research, including collection of multiple breath sample per patient will allow for better correlation of VOCs to exacerbation-specific clinical parameters while avoiding interpersonal heterogeneity. When monitoring patients in time, the chances of including exacerbation data will increase as well. Also, in this set-up, the effect of antibiotic treatment can be monitored in time. This is in line with what Barker et al. states in their discussion: 'the prognostic factor of pentane as inflammatory marker might be gathered through serial measurements, including antibiotic treatment.'[28]

As was mentioned before, it is important to carefully differentiate between pathogen-specific VOCs and inflammation-specific VOCs. It is relevant to consider the origin of specific VOCs and what they represent when steering towards clinical implementation of breath analysis. Suggested is a setup where colonized patients (with a known pathogen) are compared to non-colonized patients to be able to isolate VOCs specific for pathogen colonization. Hereafter, in similar research conditions, these results may be subtracted from VOC data obtained from patients experiencing exacerbations. In this way, like what is done by van Horck et al. by correcting for Pseudomonas aeruginosa colonization, VOCs found will be indicative of inflammation.

Moreover, analyzing exacerbation prediction in CF should focus on those VOCs specific for inflammation since exacerbations are causative of respiratory tissue damage. Reflecting tissue damage during inflammation, interesting is the presence of free radicals, since they have been described to cause structural damage. The presence of these free radicals are particularly relevant in CF, since CF patients have been described to show depleted antioxidant capacity.[36] Interesting could be comparing VOCs identified through in-vitro research to VOCs found in in-vivo studies like the ones in this review, hereby avoiding noise by pathogen-specific VOCs. In this approach, oxidative stress or could be used as a measure for inflammation as being responsible for tissue damage, and subsequently lung function loss, quality of life and patient survival.

Conclusion

Even though individual studies were successful in identifying pulmonary exacerbation in CF patients, this review does not provide with specific VOCs or VOC-patterns for prediction and identification of exacerbation as results varied among studies.

Although differences in procedures, analyses and lack of established reference values inhibit clinical implication of VOC measurements, present investigation of VOCs in CF exacerbation and other inflammatory diseases do support the concept of VOCs as a biomarker. With universal, unambiguous research designs, clinical implication of breath analysis can be accelerated and potentially allow for better exacerbation prediction and prognosis in CF and other pulmonary inflammatory diseases.

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