MOLECULAR BREATH PRINT OF COPD PATIENTS WITH EXACERBATIONS DESPITE TRIPLE INHALATIONAL THERAPY (TRIPLEEX)

Research legislation:	Ordinance on human research with the exception of Clinical trials [HRO]
Study Type:	Observational study
Study Categorisation:	Risk category A according to HRO Art. 7
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Study Identifier:	NCT04638920
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Protocol Version and Date:	V 1.1, 02.09.2020

SIGNATURE PAGE

Study number	not yet registered (*ClinicalTrials.gov*)
Study Title	MOLECULAR BREATH PRINT OF COPD PATIENTS WITH EXACERBATIONS DESPITE TRIPLE INHALATIONAL THERAPY (TripleEX)

The Sponsor-Investigator (Principal Investigator) has approved the protocol version 1.1 dated 02.09.2020, and confirms hereby to conduct the study according to the protocol, current version of the World Medical Association Declaration of Helsinki and the local legally applicable requirements.

Sponsor-Investigator (Principal investigator):

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Sponsor-Investigator	Prof. Dr. med. Malcolm Kohler

Zürich, 7.9.2020

Place/Date

Prof. Dr. med. Malcolm Kohler

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GLOSSARY OF ABBREVIATIONS

BASEC	Business Administration System for Ethics Committees
BP	Blood Pressure
CA	Competent Authority (e.g. Swissmedic)
CEC	Competent Ethics Committee
COPD	Chronic obstructive pulmonary disease
CRF DBI FeNO	Case Report Form Deep Breath Initiative Fractional exhaled nitric oxide concentration
FEV1	Forced Expiratory Volume at 1 Second
FVC	Forced Vital Capacity
GCP GOLD	Good Clinical Practice Global Initiative for Chronic Obstructive Lung Disease
H	Hours
HFG	Humanforschungsgesetz (HFG = HRA)
HFV	Humanforschungsverordnung (HFV = HRO)
HRA	Federal Act on Research involving Human Beings
HRO ICS LAMA LABA	Ordinance on Human research with the exception of clinical trials Inhaled corticosteroids (e.g Budesonid, Fluticasonfuroat, Fluticasonpropionat) Long acting muscarinic antagonist (e.g. Aclidium, Umeclidium, Glycopyrronium, Tiotropium) Long acting β-2 agonist (e.g. Formoterol, Indacaterol, Salmeterol, Olodaterol, Vilanterol)
MSMS	Tandem mass spectrometry
PI	Principal Investigator
PTR-MS	Proton-transfer reaction - mass spectrometry
SABA	Short acting β-2 agonist (e.g. Terbutalin, Salbutamol)
SAE SAMA SESI-HRMS	Serious Adverse Events Short acting muscarinic antagonist (e.g Ipatropium) Secondary electro-spray ionization high resolution mass spectrometry
SIFT-MS	Selected ion flow tube - mass spectrometry
SNCTP	Swiss National Clinical Trial Portal
SOP	Standard Operating Procedure
TMF USZ	Trial Master File UniversitätsSpital Zurich
V	Version
VOC	Volatile Organic Compounds
WHO	World Health Organisation

STUDY SYNOPSIS

Sponsor	Prof. Dr. med. Malcolm Kohler
Study Title	MOLECULAR BREATH PRINT OF COPD PATIENTS WITH EXACERBATIONS DESPITE TRIPLE INHALATIONAL THERAPY (TripleEX)
Study ID	*Not yet available*
Protocol Version and Date	Version 1.0 of 27.05.2020
Trial registration:	*Not yet registered (SNCTP, clinicaltrials.gov)*
Study category and Rationale	Risk category A according to HFV
Background and Rationale	COPD is an inflammatory and progressive disease of the lungs and airways primarily characterized by irreversible airflow limitation. COPD is the third leading cause of death worldwide.(1) On-line breath analysis in COPD would allow continuous monitoring of metabolic health, disease progression, and medication in short time intervals. Results are obtained without delay which is of high importance in situations like COPD exacerbations.(2) The findings of a recent matched case control study in patients with frequent vs patients without frequent COPD exacerbations (3) provided data on metabolic breath-prints using untargeted real-time mass spectrometry in COPD. The study showed that real-time breath analysis by mass spectrometry allows distinguishing patients with frequent exacerbations from patients without frequent exacerbations on the basis of molecular analysis. The study provided biochemical insights in COPD patients at risk for exacerbations. However, because the study was performed in between COPD exacerbations (in a stable phase) it provided no information on biochemical processes during an actual COPD exacerbation. The identification of molecular changes during a COPD exacerbation could help to optimize treatment, identify new therapeutic targets and, is perhaps of diagnostic value. GOLD guidelines recommend treating symptomatic COPD patients with frequent exacerbations with a triple inhalational therapy i.e beta-2-sympathicomimetics, anticholinergics and steroids (e.g. Trelegy®).(4) However, despite triple inhalational therapy a substantial number of patients continues to have exacerbations and thus are at high risk for disease progression, morbidity and mortality.(5) Some of these patients have elevated levels of eosinophils and may therefore, in theory, be eligible candidates for antibody treatment (e.g. with IL-5 blocking compounds such as Mepolizumab).(6) However, apart from eosinophil counts there is very little information on biochemical processes in COPD patients with exacerbations despite triple inhalational therapy. Thus, the identification of molecular changes could help to better understand relevant pathways and furthermoreto identify new therapeutic targets. Particulary those patients who are most likely to respond to an antibody therapy would benefit.
Objective	The objective of this study is to determine specific molecular breath patterns by secondary electrospray ionisation mass spectrometry (SESI-HRMS) during and 8 weeks after a COPD exacerbation. Furthermore, breath patterns will be assessed for correlation and association to clinical outcomes and treatment.

Outcomes	Primary Outcome Measure The primary outcome will be the change in exhaled breath metabolites during vs after COPD exacerbation. Secondary Outcome Measures Correlation of breath metabolites with symptoms (mMRC and CAT®), inflammation markers (CRP and eosinophils, exhaled NO), causative agents (sputum microbiology and viral swabs) and treatment (inhalational drugs, steroids, antibiotics).
Study design	40 patients with COPD (staged according to GOLD recommendations, aged ≥18 years) on adherent triple inhalational therapy are to be included in a longitudinal study. Patients will undergo two measurements of exhaled breath; at baseline during a hospitalization due to COPD exacerbation and 8 weeks after the COPD exacerbation. Real-time breath analysis will be performed using SESI-HRMS coupled to a Q-exactive Orbitrap mass spectrometer (Thermo Fisher).
Inclusion criteria (inclusive) for all participants	• Informed Consent • age ≥ 18 years • staged according to GOLD recommendations • GOLD stage 2-4, risk groups A-D • hospitalization due to COPD exacerbation • subjects adherent to triple inhalational therapy (β-2-sympathomimetics, anticholinergics, steroids) • suitable for follow-up assessment • <48 hours after initiation of antibiotic therapy or systemic steroid therapy
Exclusion criteria (exclusive) for all participants	• physical or intellectual impairment precluding informed consent or protocol adherence • known pregnancy • congenital defects with direct impact on central metabolism e.g. amino acid metabolism defect • uncontrolled diabetes (e.g. HbA1c >11% or Glc >20mmol/l). • acute or chronic pulmonary disease other than COPD • renal failure or renal replacement therapy (GFR < 15 mL/min)
Number of Participants	N = 40
Study Duration	01.08.2020 – 31.07.2023

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Study Centre	Pulmonary Division, University Hospital Zurich, Rämistrasse 100, 8091 Zurich, Switzerland
Statistical Considerations	To test the hypothesis if there is a significant difference in the values of exhaled metabolites during and after COPD exacerbation, we will perform a paired-sample t-test to evaluate differential concentrations of such metabolites. False discovery rate and q-values will be subsequently computed as defined by Storey.(7) Well-established data reduction techniques such as principal component analysis will be used for visualization purposes. Finally, high mass resolution/ high mass accuracy data obtained will allow us to query online databases (e.g., human metabolome database) to provide tentative identification of the subset of most informative exhaled compounds. The pre-processing of the raw mass spectra and the statistical analysis will be implemented using MATLAB (MathWorks, Massachusetts, US).
GCP Statement	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki(8), the principles of Good Clinical Practice and as well all national legal and regulatory requirements in Switzerland.

1. GENERAL INFORMATION & STUDY ADMINISTRATIVE STRUCTURE

1.1 Sponsor, Sponsor-Investigator (Principal-Investigator)

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2. BACKGROUND AND RATIONALE

2.1 The role of exhaled breath analysis

The ancient Greeks already knew that using exhaled breath as an indicator of disease can be revealing.(9) The famous physician Socrates mentioned characteristic smells for different types of diseases such as a sweet smell in diabetes, a fishy smell in patients with liver problems, a urine-like smell in patients with kidney problems, or a putrid smell in patients with a lung abscess.(10) The primary opportunity of using exhaled breath as source for biomarker discovery is the ease with which breath can be collected for analysis (Figure 1). Patients are only required to exhale into a device or container and recent technological advancements have enabled a real-time analysis of exhaled breath, where data are available on the spot. Breath analysis offers a unique opportunity to non-invasively retrieve potentially unlimited sampling of relevant information on the ongoing internal biochemical processes, as parts of the most volatile components of blood reach the gas phase and are subsequently exhaled (e.g. detection of ethanol in breath).(11) Simple methods of breath analysis have made use of this, e.g. for the assessment of bronchial inflammation by measuring fractional exhaled nitric oxide (FeNO).(12) Despite these advantages of exhaled breath testing, its use in clinical practice is still limited. This is mostly due to a lack of thoroughly validated techniques.

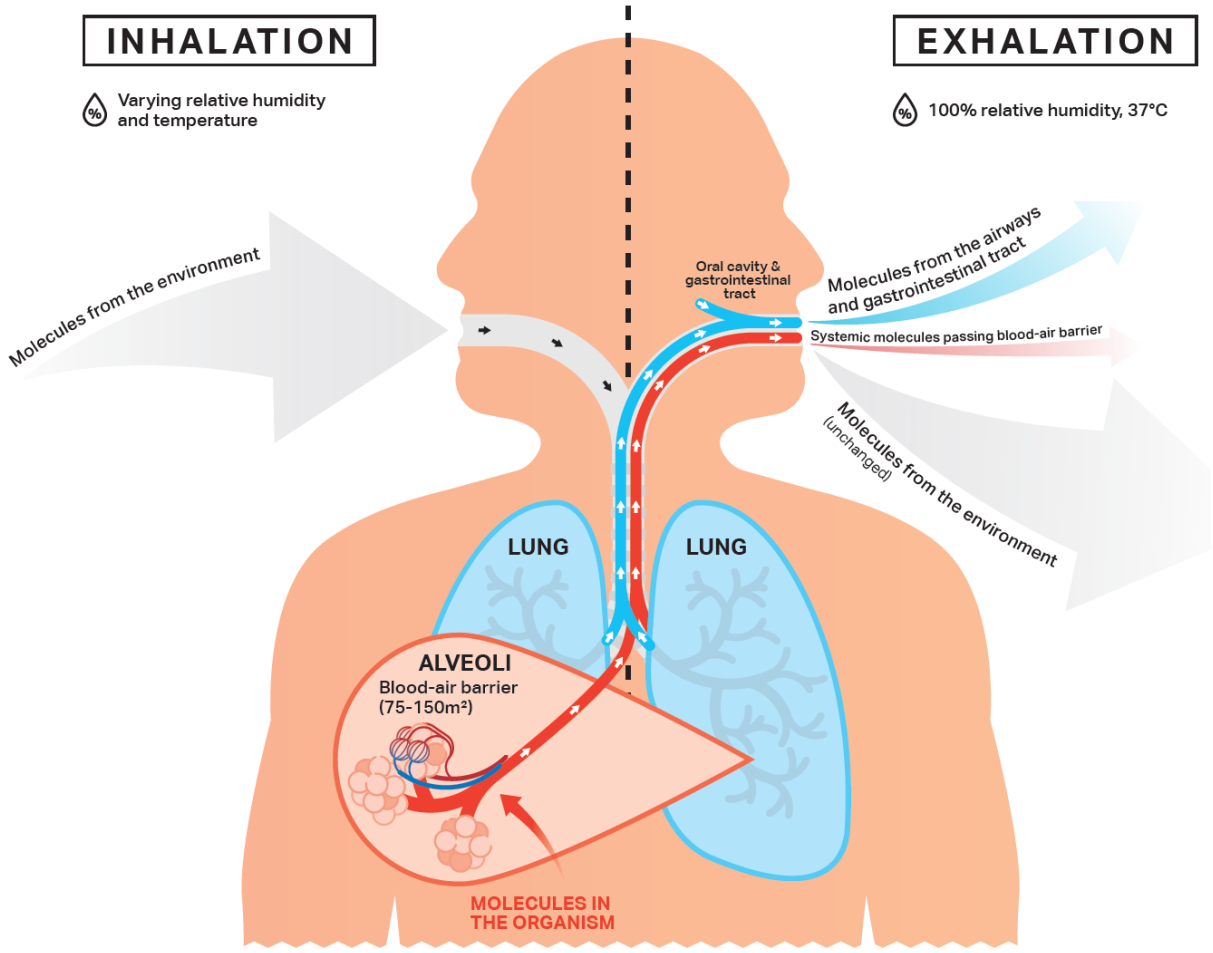

Figure 1: Composition and origin of exhaled breath

2.2 Exhaled breath analysis by mass spectrometry

Analysis of exhaled breath gives biochemical information about the metabolism and the pathophysiological state. Chemical analysis of exhaled breath with mass spectrometry identified numerous volatile organic compounds (VOCs) [low molecular weight-components: < 500 Dalton (Da)].

The origin of these VOCs is diverse. VOCs can either be endogenous (arise from the respiratory tract), systemic (passing the blood-air barrier) or can be of exogenous origin (inhaled/exhaled environment air).(2) The alveoli are intimately connected with the systemic circulation through the alveolar capillaries and its large blood-gas-interface. It was demonstrated that exhaled human breath contains a molecular fingerprint (also referred to as breathprint(9)). Breath composition changes during the course of the day (intra-individual variations). These temporal fluctuations may reflect circadian changes (13-15). Despite these changes, it was shown that each individual shows a distinct breathprint stable in time (individual core pattern of volatile and semi-volatile metabolites)(16). The findings of preliminary studies by others and our group suggest that diseases of the airways such as e.g. chronic obstructive pulmonary disease

(COPD)(17, 18) may be diagnosed by a specific exhaled breath pattern.

2.3 Chronic Obstructive Pulmonary Disease (COPD)

COPD is an inflammatory and progressive disease of the lungs and airways primarily characterized by irreversible airflow limitation. COPD is the third leading cause of death worldwide.(1) Its prevalence among heavy smokers can reach up to 50%. According to the GOLD guidelines (Global Initiative for Chronic Obstructive Lung Disease) the diagnosis of COPD can be established by a fixed ratio of post-bronchodilator forced expiratory volume (FEV1) and forced vital capacity (FVC) below 0.7 measured by spirometry (see http://www.goldcopd.org). The severity of airflow limitation is graded according to the percentage of FEV1 predicted (GOLD stage I-IV). Furthermore severity of symptoms and risk of exacerbation is categorized by the risk groups A - D. This functional definition has been used to characterize the disease for many years. However, the degree of airflow limitation is only one aspect of the disease and COPD is being recognized as a heterogeneous disease, which requires tailored therapeutic approaches.(19) While the disease course involves a slow progressive deterioration in airflow, it is punctuated by exacerbations (acute deterioration of the patient's respiratory symptoms, i.e. worsening of dyspnea, cough and/or increased sputum production), many of which are serious, requiring hospitalization, and are associated with high mortality and extensive costs. The risk for exacerbations increases with higher GOLD stage.(20-22) After the second episode of severe exacerbation, the course of COPD is often associated with a rapid decline in health status. Furthermore, the mortality risk increases in the weeks following every severe exacerbation (23). Because of the complexity of COPD the disease management is mainly characterized in supporting prevention and maintenance therapy, stable COPD management and exacerbation management.

2.4 Exacerbation

An exacerbation of COPD is defined as an acute worsening of respiratory symptoms that results

in additional therapy.(24, 25) COPD exacerbations are complex events usually associated with increase in airway inflammation, increased mucus production and marked gas trapping. These changes contribute to increased dyspnea which is the key symptom of an exacerbation.(4)

Exacerbations are classified as:

- Mild (treated with short acting bronchodilators only, SABDs)
- Moderate (treated with SABDs plus antibiotics and/or oral corticosteroids)
- Severe (patient requires hospitalization or visits the emergency room). Severe exacerbations

may also be associated with acute respiratory failure

During a COPD exacerbation, the worsening of the symptoms usually lasts between 7 to 10 days, but some events may last longer. It is well established that COPD exacerbations contribute to disease progression.(26) Disease progression is even more likely if recovery from exacerbations is slow.(27) Exacerbations can also cluster in time and once COPD patients experience an exacerbation, they will show increased susceptibility to further events.(20, 28) Depending on the frequency of exacerbations, COPD patients are divided into infrequent (0-1 exacerbations within the last 12 months) and frequent exacerbators (2 or more exacerbations within the last 12 months). Identifying COPD patients at risk for exacerbations, and especially frequent exacerbations, would be highly desirable to prevent further deterioration of early disease stages.

2.5 Exhaled breath analysis in COPD

Breath analysis in COPD is attractive because breath is available in nearly unlimited quantities. The analysis is non-invasive, and it presents no burden to the subject being measured. On-line breath analysis in COPD would allow continuous monitoring of metabolic health, disease progression, and medication in short time intervals. Data are obtained immediately which is of high importance in situations like COPD exacerbations.(2)

The diagnostic “gold standard” for COPD is based on spirometry and subjective symptom assessments, similar to asthma with respective COPD guidelines.(29) As it is primarily a disease of the lungs, a high number of breath-analysis studies are available for COPD; however, only a minority of the studies employed on-line methods.(30) Most studies used a case-control design with an untargeted approach in order to identify putative discriminative markers for the disease or disease severity. Three studies used ion mobility separation to identify COPD-specific biomarkers in a cohort of 119, 130, and 96 patients, respectively.(31-33) While two studies reported a 70−79% accuracy in the diagnosis of COPD (based on 6−10 VOCs), the other study reported 10 biomarkers, most of which were unidentified.(31) None of the identified substances overlapped between the two studies or was validated in an external cohort. Finally, two untargeted studies (17, 18) compared the breath print of COPD patients against healthy controls using SESI-MS with a real-time approach. Both studies yielded a Receiver Operating Characteristic (ROC) curve with an area under the curve of 0.88−0.91. However, in this case, with the aid of MS/ MS and complementary pre-separation methods some of the discriminate biomarkers could be identified and attributed to be metabolites of oxidative stress processes, such as fatty acids,

aldehydes, and amino acids.(17, 18) Most studies tried to control for the effects of smoking and there was evidence that lung capacity, body mass index, and certain inflammatory blood markers seem to play a role in the discrepancies observed between groups.(33, 34) In conclusion, studies on this topic are extremely heterogeneous and data processing and statistical methods are not standardized. As a result, none of the reported biomarkers overlaps between the studies and none of the biomarkers has been validated in an external cohort yet.

2.6 Rationale for the current study

The findings of a recent matched case control study in patients with frequent vs infrequent COPD exacerbations (3) provided data on metabolic breath-prints using untargeted real-time mass spectrometry in COPD. The study showed that real-time breath analysis by mass spectrometry allows distinguishing patients with frequent exacerbations from patients with infrequent exacerbations on the base of molecular analysis. Metabolite levels from the ω -oxidation pathway, namely ω -hydroxy, ω -oxo, and dicarboxylic acids, were consistently decreased in frequent exacerbators. Additionally, several new nitro-aromatic metabolites, which were significantly increased in frequent exacerbators, were identified. Thus, the study provided insights about ongoing biochemical processes in patients with COPD at risk for exacerbations. However, because the study was performed in between COPD exacerbations (in a stable phase) it provided no information on biochemical processes during an actual COPD exacerbation. The identification of molecular changes during a COPD exacerbation could help to optimize treatment, identify new therapeutic targets and, is perhaps of diagnostic value.

GOLD guidelines recommend treating symptomatic COPD patients with frequent exacerbations with a triple inhalational therapy i.e beta-2-sympathicomimetics, anticholinergics and steroids (e.g. Trelegy®).(4) However, despite triple inhalational therapy a substantial number of patients continues to have exacerbations and thus are at high risk for disease progression, morbidity and mortality. Some of these patients have elevated levels of eosinophils and may therefore, in theory, be eligible candidates for antibody treatment (e.g. with IL-5 blocking compounds such as mepolizumab).(6) However, apart from eosinophil counts there is very little information on biochemical processes in COPD patients with exacerbations despite triple inhalational therapy. Thus, the identification of molecular changes could help to better understand relevant pathways and furthermore help to identify new therapeutic targets. Particularly those patients who are most likely to respond to e.g. an antibody therapy would benefit from a deeper understanding of molecular mechanisms.

This will be the first study to investigate the acute effects of COPD exacerbations on molecular exhaled breath patterns assessed by SESI-MS. By identifying a distinctive breath pattern it may be possible to define biochemical processes specific of a COPD exacerbation and correlate it with causative agents, treatment and outcome.

2.7 Triple Inhalational Therapy in COPD

Triple therapy is most likely indicated in patients with COPD who are severely symptomatic, have moderate to very severe airflow obstruction and a history of frequent and/or severe exacerbations.(4) The step up in inhaled treatment to LABA plus LAMA plus ICS (triple therapy) can occur by various approaches.(35) This may improve lung function, patient reported outcomes and prevent exacerbations. Adding a LAMA to existing LABA/ICS improves lung function and patient reported outcomes, in particular exacerbation risk.(36-40) Double blind RCTs have reported benefits of single-inhaler triple therapy compared with LABA/LAMA combination therapy.(41, 42)

2.8 Role of eosinophils and targeting IL-5 in COPD

Novel approaches to COPD treatment focus on understanding and targeting molecular mechanisms of airway inflammation, airway obstruction, remodelling and lung destruction. The most promising biologic treatments at an advanced stage of development are agents blocking interleukin (IL)-5 or its receptor.(43) The inflammatory phenotype of COPD can be described as: 1) neutrophilic, 2) eosinophilic, 3) mixed eosinophilic and neutrophilic and 4) paucigranulocytic nature.(44) Neutrophilic airway inflammation is the most predominant type seen in COPD, but eosinophilic inflammation is present in up to 40% of COPD patients.(45) In particular eosinophils increase the risk of exacerbations and IL-5 expression.(45, 46) IL-5 expression is a key player in inflammatory processes of COPD. Therefore options such as targeting IL-5 with Mepolizumab/ Reslizumab or IL-5Rα with Benralizumab are encouraging. However, results of phase III clinical trials e.g. METREX(47), METRO(47), GALATHEA(48) or TERRANOVA(49) in eosinophilic COPD patients have not shown a breakthrough in exacerbation prevention. Breath analysis could lead to a deeper understanding of pathophysiological mechanisms of COPD exacerbations in context with IL-5 and thus help to find more suitable biomarkers and select those COPD patients who are most likely to benefit from IL-5 blocking therapy.

3. STUDY OBJECTIVES AND DESIGN

3.1 Hypothesis and primary objective

3.1.1 Hypothesis

Molecular breath patterns acquired by secondary electrospray ionization mass spectrometry (SESI-MS) are distinctively altered during COPD exacerbation.

3.1.2 Objective

The objective is to determine specific molecular breath patterns by secondary electro ionisation-mass spectrometry (SESI-MS) during and 8 weeks after a severe exacerbation requiring hospitalization. Furthermore, molecular breath patterns will be assessed for correlation and association to clinical outcomes and treatment.

3.2 Study Outcome

3.2.1 Primary Outcome

The primary outcome will be the change in exhaled breath metabolites during vs after COPD exacerbation.

3.2.2 Secondary Outcome Measures

Correlation of breath metabolites with symptoms (mMRC and CAT®), inflammation markers (CRP and eosinophils, exhaled NO), causative agents (sputum microbiology and viral swabs) and treatment (inhalational drugs, steroids, antibiotics).

3.3 Study Design

We will conduct a prospective, longitudinal, observational study including 40 patients with proven COPD staged according to GOLD recommendations and under adherent triple inhalational therapy. The treating physician will check during the general assessment the compliance to triple therapy treatment. A study flow chart is presented in Figure 2

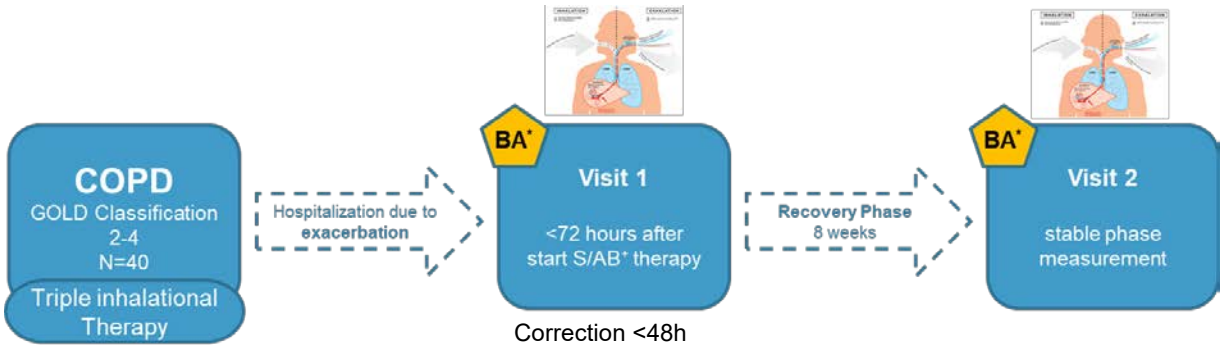

Figure 2: Study flow chart. COPD = Chronic Obstructive Pulmonary Disease; BA = Breath Analysis; GOLD = Global Initiative for Chronic Obstructive Lung Disease; * Breath analysis by SESI_MS; +steroids & antibiotics

4. STUDY POPULATION AND STUDY PROCEDURES

4.1 Project population and eligibility criteria

A population of subjects with COPD staged according to GOLD recommendations with exacerbation requiring hospitalization will be screened. Furthermore, only subjects adherent to triple inhalational therapy i.e beta-2-sympathicomimetics, anticholinergics and steroids are eligible. An exacerbation of COPD is defined as an acute worsening of respiratory symptoms that results

in additional therapy.(4)

4.2 Inclusion criteria

- Informed Consent
- Age ≥ 18 years
- GOLD stage 2-4, risk groups A-D
- hospitalization due to COPD exacerbation
- Defined exacerbation according to GOLD guidelines
- Subjects adherent to triple inhalational therapy (β-2-sympathomimetics, anticholinergics, steroids)
- Suitable for follow-up assessment
- <48 hours after initiation of antibiotic or steroid impulse therapy

4.3 Exclusion criteria

- Physical or intellectual impairment precluding informed consent or protocol adherence
- Known pregnancy
- congenital defects with direct impact on central metabolism e.g. amino acid metabolism defect

- uncontrolled diabetes (e.g. HbA1c >11% or Glc >20mmol/l).
- acute or chronic pulmonary disease other than COPD
- renal failure or renal replacement therapy (GFR < 15 mL/min)

4.4 Recruitment, screening and informed consent procedure

Recruitment

COPD patients will be consecutively recruited from the wards at the University Hospital Zurich. The treating physician will approach patients via personal communication. If interested, the treating physician will inform one of the investigators who then will contact the patient.

Participants will not be offered any material compensation for their voluntary agreement to take part in this study (Art. 14 HFG). Whilst the scientific benefit from participation will be explained to participants, no other incentives will be offered.

Patient Information and Informed Consent

The investigators will explain to each participant the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. The right of the participant to refuse to participate without giving reasons will be respected, and withdrawal of consent will not affect the participant's subsequent medical assistance and treatment. In the event of withdrawal of the participant's consent, data collected up to the date of the withdrawal will remain coded in the study database.

The participant must be informed that his/her medical records may be examined by authorised individuals other than their treating physician. The date and time of the consent will be recorded by the investigator (or his designee) and it will be retained as part of the study records.

The participant information sheet and the consent form will be submitted with the protocol for review and approval for the study by the CEC. The formal consent of a participant, using the approved consent form, must be obtained before that participant is submitted to any study procedure.

The participant should read and consider the statement before signing and dating the informed consent form, and should be given a copy of the signed document. The consent form must also be signed and dated by the investigator (or his designee) and it will be retained as part of the study records.

4.5 Study procedures

4.5.1 General study procedures

Upon participation in the study, participants will be requested to answer general questions on their

medical history. The participant will be asked to fill in questionnaires and vital parameters will be determined. Subsequent on-line breath analysis will be performed.

4.5.2 Medical history

General information: age, gender, weight, height, eating habits, activity habits, exacerbation history medication, co-morbidities, smoking history, disease specific images and clinical chemistry test results.

4.5.3 Vital parameters

Vital parameters are measured to reflect the basic function of the human body. We focus on blood pressure and heart rate, which are measured in sitting position after 3 min of rest. Furthermore, temperature and oxygen saturation will be measured.

4.5.4 Inflammatory markers

During the hospitalization of patients with COPD exacerbation blood sampling is routinely performed. CRP level and eosinophil level will be documented for this study during visit 1 and 2.

4.5.5 Spirometry

A spirometry will be performed if the physical sate of the patient allows at visit 1 and visit 2. Spirometry results (forced expiratory volume at 1 second [FEV1] and forced vital capacity [FVC]) will be expressed in percentage of predicted values according to the European reference equations.(50) Furthermore a FeNO Test will be used to investigate inflammatory characteristics more in detail. A FeNO Test is routinely performed in asthma patients. The patients should exhale into a to certified sensitive instrument where the NO concetration in the exhaled breath gives conclusion about inflammatyr celllines.

4.5.6 Causative agents

Sputum samples and viral smear is collected routinely during the clinical assessment during hospitalization (visit 1).

4.5.7 Online breath analysis

Real-time breath analysis will be performed with SESI-HRMS. SESI-HRMS is a new developed real time mass spectrometric method besides PTR-MS (51) and SIFT-MS. (52) The big advantage of the SESI method is that the ionisation takes place at atmosphere pressure. This characteristic brings the possibility to connect the SESI ionization source with high resolution mass analyser like the Orbitrap

spectrometer by Thermo Fisher. This results in sensitive and selective, yet real-time, analysis of trace vapour species.(53) Our SESI-HRMS is situated in a separate, lockable room at the University Hospital Zurich. Access to the designated room is only permitted to members of the staff. Participants will be asked to refrain from eating, drinking, chewing gum, alcohol, tobacco, caffeine use or brushing their teeth at least 1 hour before the measurements will be performed. Room temperature and lighting will be set at the same level for all measurements. Participants will exhale through a disposable mouthpiece, into a heated tube (stainless steel, Silconert coated) (50 cm long, 4 mm in diameter) connected to the curtain AUX gas port of a Orbitrap spectrometer (Thermo Fisher, Waltham, Massachussetts, USA) that is provided by DBI. The sampling tube is surrounded with an isolated heating tape at 130 °C to prevent water condensation and to minimize losses of exhaled compounds onto the walls of the tube. While performing full exhalations, the subjects will keep the volume through the sampling line at 8L/min monitored by a digital flow meter. A nano electrospray plume (FIT, Madrid, Spain), provided by DBI, will be used, where compounds of exhaled breath get ionized and subsequently are mass analysed. The technique, by which breath is analysed in real time by injecting exhaled breath into electrospray plumes of pure solvent and thus causing ionization of relevant exhaled compounds(54-56), has been referred to as secondary electrospray ionization (SESI)(57). Breathprints will be collected in real-time recording six replicates through the mouthpiece. The raw mass spectra will be exported as mzXML files for further processing using MATLAB (Mathworks, Inc). The spectra of exhaled breath will be averaged. In this process, only the last few seconds (typically around 6 sec.) of each exhalation will be considered, thus the first part of the exhalation, which reflects mostly the dead space in the upper respiratory tract, will be excluded from the analysis. SESI-MS allows for real-time breath-printing by detection of both volatile and non-volatile trace components in breath without any sample pre-treatment. The de-identified mass spectrometric breath signatures of patients are subsequently analysed by DBI with AI algorithms for spectroscopic profiling.

4.5.8 Questionnaires

4.5.8.1 COPD Assessment Test (CAT) & midified Medical Research Council (mMRC)

For assessing symptoms, GOLD guidelines primarily recommend the use of the COPD Assessment Test (CAT) or the modified Medical Research Council (mMRC) dyspnea score. The CAT score is a patient-completed instrument to assess and quantify health-related quality of life and symptom burden in COPD patients. It comprises 8 questions, each is presented as a semantic 6-point (0-5) differential

scale, providing a total score out of 40. Scores of 0-10, 11-20, 21-30, 31-40 represent mild, moderate, severe or very severe clinical impact, respectively. The mMRC dyspnea score is a 5-point (0-4) scale based on the severity of dyspnea.(58)

4.5.8.2 QUIT

QUIT is a Questionnaire to assess the probability of therapy adherence, especially in inhaled therapies. The Test was developed by the healthcare research unit of the Federal Association of Pneumologists in Germany. The Questionnaire was initially designed with focus on asthma patients. In COPD the adherence to inhalational therapy is much higher due to the consistency of symptoms during the disease progression. We use an adapted version with focus on COPD therapy.(59)

4.5.9 Study visits

Participation in the study requires two on-site study visits. Visit 1 will be performed while the participant is hospitalized. This visit will have a duration of approximately 45 minutes. After approximately 8 weeks patients are invited to the hospital to perform visit 2. This visit will take again approximately 45 minutes. Further information in Appendix.

4.5.10 Study duration

Individual participation time in this study will be approximately 8 weeks. Overall recruitment of patients will last until the targeted sample size is reached.

4.6 Withdrawal and discontinuation

Consent withdrawal of the patient will lead to an exclusion of the study. Data obtained up to the withdrawal will be included for the analysis.

5. STATISTICS AND METHODOLOGY

5.1 Statistical analysis plan

For data processing, we will follow the same procedure as described in our previous studies [Gaugg, 2016]: raw mass spectra will be transformed to *.mzXML format using the online tool msConvert. The *.mzXML files will be imported into Matlab. Each mass spectrum is then interpolated (shape-preserving piecewise cubic interpolation method), mass calibrated using an iterative grid search algorithm, and the resulting spectra are centroided. To test the hypothesis that there is a significant difference in the values of exhaled metabolites during and after the exacerbation, we will perform a paired-sample t-test to

evaluate differential concentrations of such metabolites. False discovery rate and q-values will be subsequently computed as defined by Storey.(60) To account for instrumental drift (e.g., detector sensitivity), the mass spectra are normalized using standard methods such as interquartile range, total ion current. Well-established data reduction techniques such as principal component analysis will be used for visualization purposes. Finally, high mass resolution/high mass accuracy data obtained will allow us to query online databases (e.g., human metabolome database) to provide tentative identification of the subset of most informative exhaled compounds. The preprocessing of the raw mass spectra and the statistical analysis will be implemented using MATLAB (MathWorks, Massachusetts, US).

For correlation analysis, the Pearson's linear correlation coefficient and 95% bootstrap confidence intervals will be applied. Continuous data will be summarized by means (SD) or medians (quartiles) as appropriate. A two-sided p value of <0.05 will be considered to be statistically significant for all tests.

5.2 Sample Size calculation

As this is an exploratory study it is not possible to perform a conventional sample size calculation. Based on the results of our published previous study including a control group(18), a group size of 40 patients has been chosen for this longitudinal study.Overall recruitment of patients will last until the targeted sample size is reached and full data sets of 40 subjects are acquired (two visits with breath analysis).

5.3 Handling of missing data

Subjects with missing data are excluded if the data is considered relevant to the specific analysis. Drop-outs will be replaced.

6. REGULATORY ASPECTS AND SAFETY

6.1 Registration and Categorisation

The study will be registered in the study registry ClinicalTrials.gov (61). The study is in line with risk category A according to HFV(62) and entails only minimal risks and burdens.

6.2 Local regulations/Declaration of Helsinki

This research project will be conducted in accordance with the protocol, the Declaration of Helsinki(8), the principles of Good Clinical Practice, the Human Research Act (HRA) and the Human Research

Ordinance (HRO) as well as other locally relevant regulations.(62) The Principle Investigator acknowledges his responsibilities as both the Principle Investigator and the Sponsor.

6.3 Participant privacy and confidentiality

The investigator affirms and upholds the principle of the participant's right to privacy and that they shall comply with applicable privacy laws. Especially, anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals.

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Subject confidentiality will be further ensured by utilising subject identification code numbers to correspond to treatment data in the computer files.

For data verification purposes, authorised representatives of the Sponsor (-Investigator), a competent authority (CA) (e.g. Swissmedic), or an ethics committee may require direct access to parts of the medical records relevant to the study, including participants' medical history.

6.4 Notification of safety and protective measures (HRO Art. 20)

The project leader is promptly notified (within 24 hours) if immediate safety and protective measures have to be taken during the conduct of the research project. The Ethics Committee will be notified via BASEC of these measures and of the circumstances necessitating them within 7 days.

6.5 Serious events (HRO Art. 21)

If a serious event occurs, the research project will be interrupted and the Ethics Committee notified on the circumstances via BASEC within 7 days according to HRO Art. 211.

Due to the health state of our subjects and their life expectancy, we expect the subjects to have non-related serious events during the study time. Subject will be instructed to report serious events to the study team. Serious events else than "related" will not be reported to the Ethics Committee.

6.5.1 Definition of Seriouse Events

A serious event (SE) is any unfavourable event for which a causal relationship to sampling of biological material or the collection of health related personal data cannot be ruled out, and which:

- requires hospitalization or prolongation of an inpatients' hospitalization,

[1] A serious event is defined as any adverse event where it cannot be excluded, that the event is attributable to the sampling of biological material or the collection of health-related personal data.

- results in persistent or significant disability or incapacity, or
- is life-threatening or results in death,

If a serious event occurs the research project must to be set on hold.

6.5.2 Assessment of SEs

The assessment by the project leader regarding the causal relationship of an event to the project-specific measure is done according to the following definitions:

Unrelated	• The event started in no temporal relationship to the project-specific measures applied and • The event can be definitely explained by underlying diseases or other situations.
Related	• The event started in plausible time relationship to the project-specific measures applied and • The event cannot be definitely explained by underlying diseases or other situations.

All SEs are to be documented in the subjects´ file and on the SE report form.

6.6 Early termination of the study

The Sponsor-Investigator may discontinue the study prematurely according to certain circumstances:

- ethical concerns,
- insufficient participant recruitment,
- alterations in accepted clinical practice that make the continuation of a clinical study unwise

6.7 Protocol amendments

Substantial amendments are only implemented after approval of the CEC. Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human participants may proceed without prior approval of the sponsor and the CEC. Such deviations shall be documented and reported to the sponsor and the CEC as soon as possible.

6.8 End of project

Upon project termination, the Ethics Committee is notified within 90 days.

6.9 Insurance

Insurance is covered by “Versicherung für klinische und nicht-klinische Versuche des Kantons Zürich für das UniversitätsSpital Zürich (Policennummer 15.369.591)“. Any damage developed in relation to study participation is covered by this insurance. So as not to forfeit their insurance cover, the participants themselves must strictly follow the instructions of the study personnel. The investigator must also be informed instantly, in the event of health problems or other damages during or after the course of study treatment.

The investigator will allow delegates of the insurance company to have access to the source data/documents as necessary to clarify a case of damage related to study participation. All involved parties will keep the patient data strictly confidential. A copy of the insurance certificate will be placed in the Investigator’s Site File.

7. FURTHER ASPECTS

7.1 Overall ethical considerations

This will be the first study to investigate the acute effects of COPD exacerbations on molecular exhaled breath patterns assessed by SESI-HRMS. By identifying a distinctive breath pattern it may be possible to define biochemical processes specific of a COPD exacerbation and correlate it with causative agents, treatment and outcome. It may also be possible to identify those patients who need, and are most likely to respond to additional therapy such as e.g. antibody treatment.

The study procedure (contact to patients and relatives) will be conducted in accordance with internationally accepted quality criteria and entails only minimal risks and burdens.

7.2 Ethical Conduct of the project

The study will be carried out in accordance with principles enunciated in the current version of the Declaration of Helsinki (8), the principles of GCP, the Federal Act on Research involving Human Beings (HRA), the Ordinance on Human research with the exception of clinical trials (HRO)(62), and Swiss Academy of Medical Sciences’ ethics guidelines (63). Before this study will be conducted, the protocol, the proposed participant information and all other study-specific documents, which are recommended by swissethics(64), will be submitted to Competent Ethics Committee (CEC) of the Canton of Zurich in agreement with local legal requirements, for formal approval.

The decision of the competent authority concerning the conduct of the study will be made in writing to the Sponsor-Investigator before commencement of this study. The study can only begin once approval

from all required authorities has been received. Any additional requirements imposed by the authorities shall be implemented. No substantial amendments are made to the protocol without prior CEC approval, except where necessary to eliminate apparent immediate hazards to study participants.

The following are considered to be significant changes/substantial amendments:

- changes affecting the participants' safety and health, or their rights and obligations;
- a change of research site or conducting the research project at an additional site; or
- a change of the project leader or sponsor.

The regular end of the study or premature study end or interruption is reported to the CEC within 90 days.

7.3 Participant information and informed consent

Prior to the project start each participant has to give their written informed consent after he/she was comprehensively informed - verbally and in writing - on the nature, relevance and impact of the project. The content of this information should be documented on the informed consent. Each participant must be informed that the participation in the project is voluntary and that he/she may withdraw from the project at any time and that withdrawal of consent will not affect his/her subsequent medical treatment. The participants will be given enough time to make their decision whether they would like to participate or not.

The consent to participate into the research project should be signed and dated by both, the participant and the project leader. A copy of the signed and dated subject information and informed consent form has to be handed out to the participant.

No project procedures are to be conducted before a legally accepted written informed consent has been given.

7.4 Risk / Benefit Assessment

This study does not impose any potential threats or harms to the participants. Participants will undergo the regular diagnosis and treatment pathway for patients with COPD as it is standard in the University Hospital Zurich. The knowledge and insights emanating from this study will potentially advance research efforts focused on COPD and may ultimately lead to better diagnosis and treatment of this condition.

7.5 Compensation offered to participants

Participants will not be offered any material compensation for their voluntary agreement to take part in

this study. Whilst the scientific benefit from participation will be explained to participants, no other incentives will be offered.

8. QUALITY CONTROLE AND DATA PROTECTION

8.1 Quality measures

The Sponsor-Investigator is implementing and maintaining quality assurance and quality control systems with written SOP and working instructions to ensure that studies are conducted and data are generated, documented (record), and reported in accordance with the protocol, the Declaration of Helsinki(8), the Human Research Act (HRA) and the Human Research Ordinance (HRO)(62) as well as other locally relevant regulations. Trainings will be performed including all the study staff before start of the study. During the study, one independent internal quality control will take place. Designated study team members will be responsible for data collection, confidentiality, and data management.

For quality assurance the Ethics Committee may visit the research sites. Direct access to the source data and all project related files and documents must be granted on such occasions.

8.2 Data management/ Data recording and Source data

Designated research team members will be responsible for data collection and data management. Data are registered in a case report form (CRF). Paper documents and informed consent forms will be stored in a lock-secured cupboard in a dedicated research office at the University Hospital Zurich. Only study team members have access to these documents. Authorized staff of the responsible ethics committee can request access to these data for monitoring and auditing purposes. Electronic data will be stored in a password-secured folder. The files will be stored within the University Hospital Zurich intranet server. It can only be accessed by authorized users from registered computers at the University Hospital Zurich. All personal and medical information obtained for this study is confidential and disclosure to third parties other than those noted below is prohibited. Confidentiality of the subjects will be maintained by assigning subjects a study number (ID), keeping identifiers separate from the data and storing data in a locked file and secure computer database in line with Swiss legal requirements. Patient identifier will be assigned with a code of letters and numbers (the first letter for the population, a consecutive number for the subject, the visit and the sample or data type resp. e.g. TX0001V1).

The USZ will handle administration of the study database. The data entry will be performed by the study

team, consisting of investigators and study nurses. Study researchers will only be given access to database exports and will not have direct system access. Only de-identified database exports will be made accessible to the project collaborators DBI.

8.3 Data handling and record keeping / archiving

The study will strictly follow the protocol. If any changes become necessary, they must be laid down in an amendment to the protocol. All amendments of the protocol must be signed by the Sponsor-Investigator and if essential submitted to CEC.

8.3.1 Case Report Forms

The investigators will use paper case report forms (CRF), one for each enrolled study participant, to be filled in with all relevant data pertaining to the participant during the study. All participants who either entered the study or were considered not-eligible or were eligible but not enrolled into the study additionally have to be documented on a screening log. The investigator will document the participation of each study participant on the Subject Master List.

CRFs must be kept current to reflect participant status at each phase during the course of study. Participants must not to be identified in the CRF by name. It must be assured that any authorized person, who may perform data entries and changes in the CRFs, can be identified. A list with signatures and initials of all authorized persons will be filed in the study site file and the trial master file, respectively. Documented medical history and narrative statements will be additionally maintained. For further analysis, data will be transferred into electronic data sheets (spreadsheets) including an audit trail and will be stored password-secured. The paper CRF as well as electronic data will serve as source for study data.

8.3.2 Record keeping / archiving

The study data (Trial Master File [TMF], CRF, etc.) will be archived in a dedicated research office at the Department of Pulmonology, University Hospital Zurich, Rämistrasse 100, CH-8091 Zürich. A designated research member will be responsible for data collection, confidentiality, and data management. The collected data will be stored in the Trial master folder, which will be stored in a designated research office (Room RAE C 25) in a lock-secured cupboard. Some data will be documented on paper sheets and may be stored as certified copies only. For further analysis, data will be transferred into electronic data sheets (spreadsheets) including an audit trail and will be stored

password-secured. The file will be stored within the Division of Pulmonology research-section of the University Hospital of Zurich intranet server. The file itself can be only accessed by researchers from registered computers at the University of Zurich. Access is electronically recorded and password-secured. The study personnel will preserve the confidentiality of participants taking part in the study according to HFV art. 5(62). All study data must be archived for a minimum of 10 years after study termination or premature termination of the clinical trial.

8.4 Details of the Secure Handling of Data connected to DBI

Data will be collected at University Hospital Zurich premises only by using Hardware and data acquisition tools (software), under the medical and administrative supervision and control of USZ. Data collection, data storage and data analytics will be described in more details in the following:

Medical records and de-identification of patients

All patients data will be de-identified by providing only a patient number with corresponding labels (gender, age, COPD stage/healthy,..) to DBI. This file is stored at DBI. DBI will not get any additional medical records other than these evaluated during the study visits.

Data collection and Data transfer

The unique patient identifier (number) will be used for the data collection as the only label received from USZ, added to the corresponding data acquired. The time series of data spectra will be locally encrypted on Hardware, transferred via internet to a Swiss-based, secure cloud storage, stored in encrypted state in the cloud, and transferred and finally decrypted at DBI. DBI will report the de-identified and clustered output data to USZ.

Data analytics at DBI

At DBI, data will be subdivided into temporal series of data and merged with the relevant labels (gender, age, GOLD status, …) Based on the subject-matter expert input, all data will be analyzed regarding specific VOCs in a targeted way (using a data subset with filtered VOCs levels) or broadband in a untargeted way (using the full spectrum). Various Machine Learning (“ML”) and Artificial Intelligence (“AI”) algorithms will be tested.

8.5 Audits and Inspections

A quality assurance audit/inspection of this study may be conducted by the CEC. The quality assurance auditor/inspector will have access to all medical records, the investigator's study related files and

correspondence, and the informed consent documentation that is relevant to this clinical study. The investigator will allow the persons being responsible for the audit or the inspection to have access to the source data/documents and to answer any questions arising. All involved parties will keep the patient data strictly confidential. As part of the informed consent, participants agree that authorized members of staff of the responsible ethics committee can obtain access to confidential data for monitoring and auditing purposes under strict maintenance of participant's confidentiality.

9. FUNDING /DECLARATION OF INTEREST

9.1 Funding & Support

This investigator initiated trial (IIT) will be conducted in collaboration with the Deep Breath Initiative (DBI). GlaxoSmithKline plc. will provide financial support. DBI will provide the mass spectrometer as well as the ionisation source, technical support and assist in the data analysis process by employing machine learning algorithms.

9.2 Declaration of interest

Professor Dr. med. Kohler declares that he owns shares of DBI, other than that none of the authors has any conflict of interest (independence, intellectual, financial, proprietary). Professor Dr. med. M. Kohler is cofounder of the Deep Breath Initiative (DBI), a company that provides services in the field of breath analysis (www.dbi.ch).

10. PUBLICATION AND DISSEMINATION POLICY

After the statistical analysis of this study every endeavour to publish the data via publication in peer-reviewed scientific media will be made. Dissemination of results will be independent of negative or positive findings and submission of results for publication will follow the consensus decision of all investigators. Authorship credit is based on 1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published. Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content.

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12. APPENDIX

Overview study procedures

Study Procedure	Parameter Analysis	V 1 (day 0)	V 2 (8 weeks)
ICF		x	
Medical history	age, gender, weight, height, eating habits, activity habits, medication, co-morbidities, smoking history, disease specific images and clinical chemistry test results, exacerbation history	x	x
Vital parameter	BP, HR, T, SpO2	x	x
Inflammatory markers	CRP, eosinophil level	x	x
Spirometry	FEV1, FVC	x2	x2
FeNO		x2	x2
Sputum & viral smear		x1	
Observational data collection	On-line breath analysis	x	x
Questionnaire (eg. COPD Assessment Tool)	CAT, mMRC, QUIT	x	x
SE review		x	x
1 collected routinely during the clinical assessment after hospitalization *2 if applicable*			