Research Plan

Pulmonary function test association with clinical, laboratory, histological and radiological characteristics – a Prospective study

Research legislation:	Ordinance on human research with the exception of Clinical trials (HRO) [1].
Type of Research Project:	Research project involving human subjects, in which health-related personal data is collected
Risk Categorisation:	Category A (according to ordinance HRO Art.7)
Project Leader:	Prof. Dr. med. Daiana Stolz Clinic of Pulmonary Medicine and Respiratory Cell Research University Hospital Basel CH-4031 Basel Phone: +41 61 265 51 84 Email: daiana.stolz@usb.ch

PROTOCOL SIGNATURE FORM

Project Title:	*Pulmonary function test association with clinical, laboratory, histological and radiological characteristics – a prospective study.*

Signature Page(s) of person(s) authorized to sign the protocol and the protocol amendment(s) for the sponsor or of the medical expert (if applicable), the investigator responsible for conducting the trial, the statistician (if applicable).

University Hospital Basel

Sponsor-Investigator: **Prof. Dr. med. Daiana Stolz**

Date: _____ Signature: _____

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GLOSSARY OF ABBREVATIONS

ABGA	Arterial blood gas analysis
BASEC	Business Administration System for Ethical Committees
CRF	Case report form
COPD	Chronic obstructive pulmonary disease
DLCO	Diffusing capacity of carbon monoxide
DTG-SBW	Double tracer gas single-breath washout
DTG	Double tracer gas
FOPH	Federal Office of Public Health
FOT	Forced oscillation technique
FeNO	Fraction of exhaled nitric oxide
FEV1	Forced expiratory volume in first second
FEV1/VC	Tiffeneau index
HRA	Human Research Act
HRO	Ordinance on Human Research with the Exception of clinical Trials
LCI	Lung clearance index
MCT	Methacholin challenge test, also known as bronchoprovocation test
N_2	Nitrogen
N_2*-MBW*	Multiple breath nitrogen washoutN_2*-SBW* Single-breath nitrogen washout
PD_{20}	Cumulative methacholin dose for fall of at least 20% of the FEV1
PD_{40}	Cumulative methacholin dose for increase of at least 40% of airway resistance
RV/TLC	Residual volume/Total lung capacity
Sacin	SIII derived from acinar compartments
SBW	*Single Breath Washout*
SIII	Phase III slope
$SIIIN_2$	SIII of Single-breath nitrogen washout
SIIIDTG	SIII of double tracer gas Single-breath washout
Scond	SIII derived from conductive compartments

1 BACKGROUND AND PROJECT RATIONALE

There is strong evidence indicating that the small airways are the main region where early physio-pathological changes occur before the development of symptoms in lung diseases such as cystic fibrosis, asthma, and chronic obstructive pulmonary disease (COPD)[1-3]. To date, we observe a reinvigorated interest in the assessment of small airways for the diagnosis and approach to different lung diseases.

The main function of lungs is gas exchange through gas transport. Due to the complexity of the lung structure, gas transport can only be optimized to a certain extent and is easily affected by changes in the small airways. There are currently five methods available to non-invasively assess peripheral/small airways: spirometry, plethysmography, oscillometry, nitrogen washout and other inert gas washout techniques such as double tracer gas (DTG) and heliox. The inert gas washout methods have shown the advantage of being sensitive in the early stages of obstructive diseases and to provide information about ventilation inhomogeneity[4]. Between the two nitrogen (N_2) washout techniques, the single-breath nitrogen washout (N_2-SBW) has been considered easier to be performed by the patient, whereas the multiple breath nitrogen washout (N_2-MBW) has the advantage of being less effort-dependent and providing more accurate information on the localization of the ventilation heterogeneity[4,5]. The N_2 exhalation occurs in four different phases at each breath, with phase III slope (SIII) alteration representing global ventilation inhomogeneity in N_2-SBW. Parameters such as the phase III slope (SIII) of single-breath washout tests are sensitive markers for small airway pathology, even when standard lung function is still unremarkable[6]. Whereas $SIIIN_2$ (SIII of nitrogen SBW) was suggested to reflect global ventilation inhomogeneity (central and peripheral ventilation inhomogeneity, convection-dependent and diffusion-dependent ventilation inhomogeneity) which derives from large and small airways, SIIIDTG (SIII of double tracer gas SBW) was proposed as a measure of acinar ventilation inhomogeneity (peripheral ventilation inhomogeneity, diffusion-dependent ventilation inhomogeneity)[7-9].

In N_2-MBW, the individual breathes at tidal volume until the N_2 concentration falls to about 2%. The phases of subsequent breaths are jointly analyzed to obtain a normalized SIII, according to the lung turnovers. Measures of inhomogeneity can be obtained from the lung turnovers needed for the N_2 concentration to fall, through lung clearance index (LCI), or from SIII derived from conductive (S_{cond}) and acinar (S_{acin}) compartments.

Small airways are often the first anatomical compartment to be affected in diverse lung diseases. Therefore tests that access such compartments may be more sensitive. As an example, Cosio et al.[4] demonstrated significant differences between patients with minimal structural pulmonary disease and those with more advanced disease using N_2-SBW. Recently, Boeck et al.[3] were able to show that the SIII of the N_2-SBW correlated with FEV1, air trapping and DLCO in COPD patients. Also, comparing patients with mild-to-moderate versus severe asthma, Van Veen et al.[10] found that the slope of the SBNW test significantly differed between the two groups, while alveolar nitric oxide and plethysmography RV/TLC ratio did not.

Nyilas et al[11] currently demonstrated a sensitivity improvement of about 30% compared to the traditional lung function method with N_2-MBW in patients suffering from bronchiolitis obliterans after hematopoietic cell transplantation.

There is an increase in interest regarding screening for lung cancer to detect the disease in an earlier and probably curable stage. At the moment, low-dose CT-scans are used as a screening tool for lung cancer in high risk groups but this method has a high false-positive ratio. To increase the efficiency of lung cancer screening, we would like to evaluate the 'smell' of exhaled breath as a biomarker for the presence of lung cancer. Volatile organic compounds (VOC's) in exhaled breath are a result of metabolic changes due to tumor growth and the host's immune response. Electronic noses can recognize and classify these VOC's in terms of presence or absence of a disease. The early diagnosis of any lung disease is fundamental in order to correctly treat these patients.

The overall aim of this study is to prospectively associate various pulmonary function tests with clinical, laboratory, histological and radiological characteristics.

2 PROJECT OBJECTIVES AND DESIGN

2.1 Hypothesis and primary objective

To associate various diagnostic tools with clinical, histological, laboratory and radiological characteristics

Primary objective: To assess washout and Aeonose measurements in patients with clinical suspicion of lung disease and in patients with known lung disease with different stages of disease severity.

Secondary objective:

- To compare outcomes of participants with no respiratory disease.
- To compare the various diagnostic tools for specific lung diseases.

2.2 Primary and secondary endpoints

Primary Endpoint

- The primary endpoint for washout measurements: LCI as the main measurement in N_2-MGW and SIII for N_2-SBW and DTG-SBW.
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Secondary Endpoints

- S_{cond}, S_{acin}, area under the curve of DTG-SBW
- Bronchoprovocation test outcomes such as PD_{20} and PD_{40}.
- Comparing washout and Aeonose measurements with known clinical, laboratory, histological and radiological characteristics.
- Endpoints for Aeonose is the sensitivity, specificity, negative and positive predictive values and area under the curve.
- Determine techniques to detect respiratory disease and sleep apnea early.
- Detect acute exacerbations early

2.3 Project design

3 EXPLORATORY HYPOTHESIS GENERATING STUDYPROJECT POPULATION AND STUDY PROCEDURES

3.1 Project population, inclusion and exclusion criteria.

- Patients ≥18 years referred to the University hospital Basel,
- Adults with or without respiratory symptoms,
- Adults with or without a diagnosed respiratory disease.
- Able to answer the questionnaires
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3.2 Recruitment, screening and informed consent procedure

The participants will be recruited in the University Hospital Basel.

3.3 Study procedures

We would like to recruit a small percentage of 5 % (1000 patients/year) of our 20000 patient contacts per year for our exploratory prospective project. The project will run for approximately 5 years (February 2019 to February 2023).
The project-specific non-invasive measurements, occur on the same day as the diagnostic examinations not specific to the project

Non-invasive measurements specific to the project and performed in all participants, include:

N2 washout (single and multiple breath):
Single breath - the measurements are carried out with the patient sitting upright and breathing normally. The test subjects breathe through a mouthpiece with a nose clamp that is attached to the measuring device. A bacteria filter is installed upstream of the measuring device. The filter, mouthpiece and nose clip are replaced after each patient. The measurement device is based on an ultrasonic flow head, the Exhalyzer® (Eco Medics AG, Dürnten, Switzerland), connected to a bypass element that provides constant air intake. The gas used as part of the study (100% O_2, DTG) is supplied to the patient via this bypass element. During the measurement procedure, the accurate recording of the flow volume curve and the gas signals (O_2, CO_2 and the derived N_2, molar mass signal) enable good online monitoring of the measurement quality. Any changes to the patient's breathing pattern, hyperventilation or leaks are identified immediately and the measurement procedure is terminated.
Multiple breath - for N_2 MBW, the supply of 100% O_2 flushes out the nitrogen in the lungs. The test ends when < 2% end-expiratory N_2 (1/40 of the starting concentration) is reached over three consecutive breaths. For the evaluation, all signals are aligned in terms of time, and the respiratory flows and derived breathing volumes are corrected for body temperature, ambient pressure and humidity. To calculate the functional residual capacity (FRC), the ratio (net volume of expirated tracer gas) / [(end-expiratory N_2 concentration at start of washout measurement) – (end-expiratory N_2 concentration at end of the washout measurement)] is calculated. Over the course of the three requisite measurements per patient, the FRC must not deviate by more than 20%; otherwise, the measurement must be rejected. The LCI is calculated via FRC as the ratio of the cumulative expirated volume.

Forced oscillation technique (FOT) - the sound waves, generated with the help of a loudspeaker are transmitted into the lungs of the subject. These sound waves, which are essentially pressure waves, cause changes in the pressure and this change in pressure drives changes in airflow. By measuring the magnitude of change in the pressure and flow, one can determine the mechanical properties of the lung. Waves of lower frequencies travel deep into the lungs till and into the alveoli and are reflected back while those of higher frequencies are reflected from the larger airways. Thus, the parameters calculated at different frequencies give measures of different regions in the lungs.

Aeonose – Measured data consists of an individual breath-print of volatile organic compounds. Patients breathe into the portable Aeonose for five minutesThe system learns from each data entered and is updating consistently. We would like to perform Aeonose for lung cancer and asthma/COPD as well. The system learns from each data and is updating consistently until the system has enough data and can't improve any more. Therefore it's difficult to indicate how long we will perform the Aenonose measuring. The device is CE-certified and therefore there is no risk in using it for our patients.

Sleepiz One – A contactless, non-invasive device that measures vital parameters while patient is at rest. It is a radar-based sensor that does not pose any risks. The electromagnetic emission is around 100 times lower than that of a typical mobile phone.

3.4 Criteria for withdrawal/discontinuation

1. Repeated failure to comply with instructions for successful measurements. Patients will have 3 attempts to perform successful measurements for N2-SBW, 2 attempts for N2-MBW and 7 attempts for Lung function tests.
2. If subject faints or experiences respiratory distress.
3. If subject withdraws their consent.

4 STATISTICS AND METHODOLOGY

4.1. Statistical analysis plan

Data will be analyzed using the Stastistical Package for Social Sciences (SPSS Inc., IL; version 22). A p value ≤ 0,05 will be considered as statistically significant. Differences in dichotomous variables will be evaluated using the Chi-square test or Fischer's exact test, as appropriate. Normally distributed parameters will be analyzed using the Student's t-test for equality of means. All other continuously non-normally distributed parameters will be evaluated using the non-parametric Mann-Whitney U test or Kruskal-Wallis test, as appropriate. Kaplan-Meier curve and cox-regression will be used to determine time-dependent predictors.

4.2. Coding

Once the participants have signed the informed consent, they will be given a code. The code will consist of 5-digits starting at 80000. Only the principal investigator, the study nurse and the person analysing the data will have access to the key.
The code may only be broken if it is necessary to avert an immediate risk to the health of the person concerned or to guarantee the rights of the person (e.g. in revoking the consent) or a legal basis exists for breaking the code.

4.3. Handling of missing data

Only factual data will be analyzed. No imputation is foreseen. Sensitivity analyses for the main outcome will be performed.

5 REGULATORY ASPECTS AND SAFETY

5.1 Local regulations / Declaration of Helsinki

This research project will be conducted in accordance with the protocol, the Declaration of Helsinki[12], the principles of Good Clinical Practice, the Human Research Act (HRA)[13] and the Human Research Ordinance (HRO)[14] as well as other locally relevant regulations. The Project Leader acknowledges his responsibilities as both the Project Leader and the Sponsor.

5.2 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.

5.3 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. 21[1].

5.4 Radiation

X-Ray, CT-scan or SPECT will be performed where needed for the routine diagnostic work-up in patients or in subjects with an indication for CT-screening for lung cancer and are **not** study-specific events.

5.5 Amendments

Substantial changes to the project set-up, the protocol and relevant project documents will be submitted to the Ethics Committee for approval according to HRO Art. 18 before implementation. Exceptions are measures that have to be taken immediately in order to protect the participants. Substantial amendments are changes that affect the safety, health, rights and obligations of project participants, changes in the protocol that affect project objective(s) or changes of project site(s) or of project leader and sponsor.

5.6 End of project

Upon project termination, the Ethics Committee is notified within 90 days. All biological material and health-related data are anonymized upon termination of data analysis.

5.7 Insurance

In the event of project-related damage or injuries, the liability of the Clinic of Pneumology, University Hospital Basel provides compensation, except for claims that arise from misconduct or gross negligence

6 FURTHER ASPECTS

6.1 Overall ethical considerations

With the recent questioning of appropriate lung function tests, the results of this study may help to answer which, if any, lung function test is the best diagnostic tool for lung disease. The study-specific tests are non-invasive with no known risks to the patients.

Controls will have an idea of their lung function and there is a possibility of incidental findings. In the informed consent, participants are required to mark what should happen with incidental findings. In this group of participants, any incidental finding would be to their benefit in that treatment could be started quickly, thus delaying the progression of the disease.

6.2 Risk-Benefit Assessment

As most of the interventions are not study-specific, and the three study-specific interventions, ie. The N2-washout (single-breath and multiple-breath), the forced oscillation and the Aeonose measurements are non-interventional measurements with no known side effects, the benefit of the knowledge obtained, outweighs the risks.

6.3 Rationale for the inclusion of vulnerable participants

[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, and which:
a. requires inpatient treatment not envisaged in the protocol or extends a current hospital stay;
b. results in permanent or significant incapacity or disability; or
c. is life-threatening or results in death.

n/a

7 QUALITY CONTROL AND DATA PROTECTION

7.1 Quality measures

The procedures performed are used in the Pneumology clinic on a daily basis, so all personnel are well-trained in performing their duties. 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.

7.2 Data recording and source data

Data will be recorded with paper case report forms. Data will be transferred to SPSS for later analysis.
Source data are all information in original records, certified copies of original records of clinical findings, questionnaires, observations, or other recorded activities. The routinely collected data will be transferred to the participant's CRF.

7.3 Confidentiality and coding

Project data will be handled with utmost discretion and is only accessible to authorized personnel who require the data to fulfil their duties within the scope of the research project. On the CRFs and other project specific documents, participants are only identified by a unique participant number.
The database is password-protected and can be accessed only by the lead investigator and the co-lead investigator. The documents stated above are stored in a lockable cupboard in the study room at University Hospital Basel. Raw data, questionnaires and the signed consent forms are archived in encrypted form for up to 10 years following successful publication.

7.4 Retention and destruction of study data and biological material

Health-related data are stored for at least 20 years after publication of the research project. Biological material will be retained for at least 10 years at the University Hospital Basel.

8 FUNDING / PUBLICATION / DECLARATION OF INTEREST

We strive to publish all data at conferences and/or in peer-reviewed journals.

The project will be funded by the University Hospital Basel and there are no conflicts of interest

9 AMENDMENT 1

9.1. BACKGROUND AND PROJECT RATIONALE

Patients with asthma are often plagued by nasal polyposis which may contribute to respiratory symptoms, decreased quality of life and poorer asthma control[15]. Current therapy for nasal polyposis includes topical corticosteroids, systemic glucocorticosteroids and repeated sinus surgery. Topical corticosteroids are unable to treat the disease, systemic glucocorticosteroids are not recommended for long-term use and repeated surgery is not feasible. Biotherapeutics with monoclonal antibodies, such as mepolizumab and dupilumab, have shown promising results with a decrease in nasal polyp score, a decrease in CT-scan opacification and a decrease in symptoms of disease after treatment[16-18]. Some patients, however, do not respond to the treatment and the polyposis persists[17]. It is unclear, why some patients are responders whereas other do not show any

clinical improvement on specific therapies with IL-5 or IL-4 blockers. Thus, it would be helpful to understand the pathophysiology of the nasal polyposis in patients undergoing treatment with biologics for severe asthma.

9.2. Project Objectives and design

9.2.1. Hypothesis and primary objective

Primary objective:

- To assess pathology and biochemical composition of nasal polyp biopsies in patients receiving biologics for severe asthma

Secondary objectives:

- To compare patients who have depicted clinical response to treatment with biologics with patients who have not responded to treatment
- To assess changes in remodeling using endobronchial biopsies in patients receiving biologics for the treatment of severe asthma

9.2.2. Primary endpoints

Primary endpoint – Assessment of clinical, histological, laboratory and radiological characteristics of patients with severe asthma receiving biologics

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2. Study Procedures

Severe asthma patients with and without nasal polyps on biologicals will be included in the study. Routinely performed polyps and/or endobronchial biopsies will be examined for histological and biochemical changes. Comparisons across substances and clinical responses will be performed.

10 REFERENCES

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