

# Non-invasive sampling from the deepest of our lungs

- a novel opportunity for Precision Respiratory Medicine



Jörgen Ostling • Jan 2024

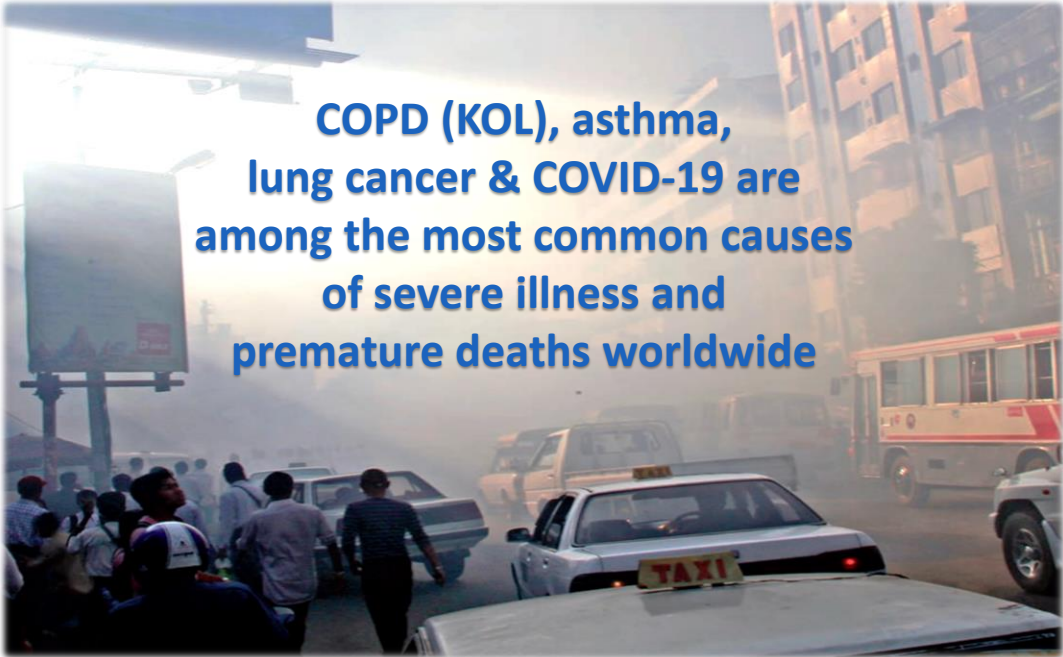
PEXA founded by among others Prof. Anna-Carin Olin, Gothenburg University, Sweden

**PEXA**  
Particles In Exhaled Air

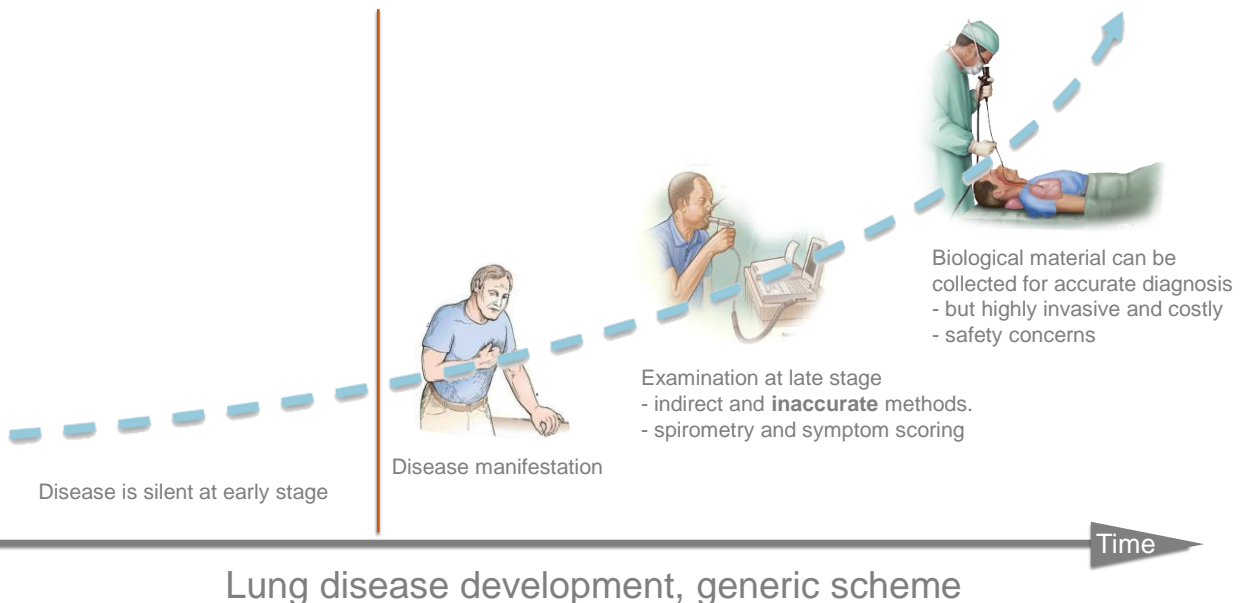
PEXA is a Swedish early-phase company that develop a unique technology that enables Non-invasive sampling from the deepest of our lungs.

Welcome to this presentation in which we provide a short description of the technology. If you want we can provide a narrated version

**COPD (KOL), asthma,  
lung cancer & COVID-19 are  
among the most common causes  
of severe illness and  
premature deaths worldwide**



## Lung disease develop slowly and is often not noticed until in a late stage



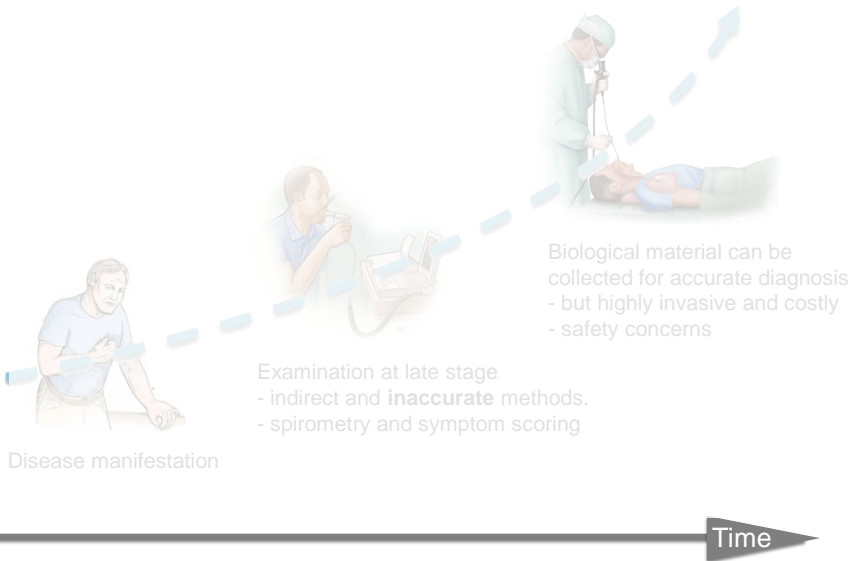
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Our lung have a considerable excess capacity, and it's function declines gradually, with noticeable reduction typically occurring only in old age. Even if a pathological process initiate and certain lung segments cease to function, a lack of awareness persists due to the compensatory function of other areas. While this redundancy may initially seem advantageous, it means that lung diseases often are detected at a too late and irreversible stage. Detecting the disease at an early stage, before manifestation when the pathological process is less complex, leads to more efficient treatment. Early detection may even allow lifestyle changes to reverse the disease or at least halt further progression.

Early detection → Treatment before disease become too complex → Improved outcome



PEX sampling have the potential to provide samples for early detection

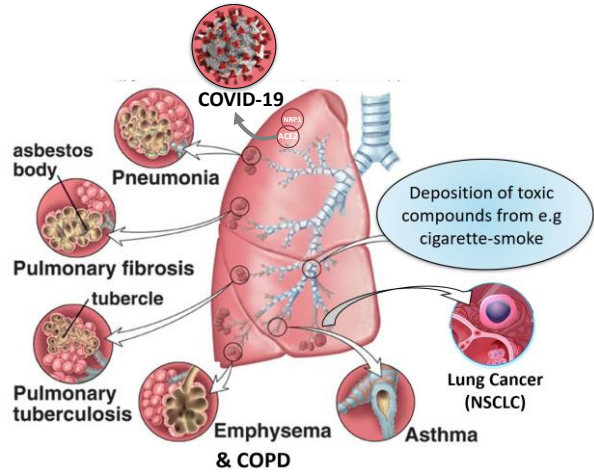
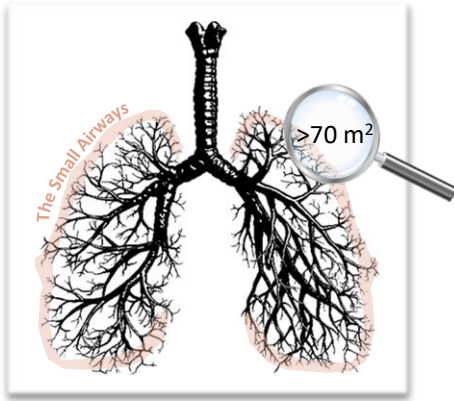


Lung disease development, generic scheme

PEXA is an innovative technology enabling non-invasive collection of a biological sample precisely from the region where many lung diseases originate. This non-invasiveness, coupled with the local sample origin, offers unique opportunities for precise and early detection of lung diseases.

Early detection → Early treatment → Improved outcome

**Pathological process often take place unnoticed in the small airways**



**Slow progression + difficult to sample -> Difficult to study and detect**

so., the main reason for why PExA have been developed is based on the notion that many lung disease initiate and develop in the most distal part of our airways and yet there have been no good way to get non-invasive access to biological material from this region.

## Lack of non-invasive methods for assessment of Small Airways pathology is often considered being a bottle neck for improved healthcare



Mayo Clinic Proceedings

REVIEW

### Why We Should Target Small Airways Disease in Our Management of Chronic Obstructive Pulmonary Disease

Omar S. Usmani, FRCP; Rajiv Dhand, MD; Federico Lavorini, PhD; and David Price, FRCGP

#### Abstract

Published: June 25, 2021

For more than 50 years, small airways disease has been considered a key feature of chronic obstructive pulmonary disease (COPD) and a major cause of airway obstruction. Both preventable and treatable, small airways disease has important clinical consequences if left unchecked. Small airways disease is associated with poor spirometry results, increased lung hyperinflation, and poor health status, making the small airways an important treatment target in COPD. The early detection of small airways disease remains the key barrier; if detected early, treatments designed to target small airways may help reduce symptoms and allow patients to maintain their activities. Studies are needed to evaluate the possible role of new drugs and novel drug formulations, inhalers, and inhalation devices for treating small airways disease. These developments will help to improve our management of small airways disease in patients with COPD.

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Mayo Clinic Proceedings DOI: (10.1016/j.mayocp.2021.03.016)

[https://www.mayoclinicproceedings.org/article/S0025-6196\(21\)00240-8/fulltext](https://www.mayoclinicproceedings.org/article/S0025-6196(21)00240-8/fulltext)

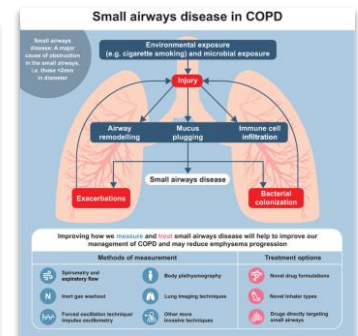
#### Assessment

Small airways disease can be difficult to assess because of the small size and inaccessibility of the airways.<sup>15</sup> Many methods are complex or invasive, and overall, there is no unanimously accepted approach. Different methods of assessment are described below and summarized in the Supplemental Table (available online at <http://www.mayoclinicproceedings.org>), with Figure 3 providing an infographic overview of these techniques and their outputs.

Other techniques to assess small airways are more invasive and typically only used for research purposes, for example, sputum induction after inhalation of hypertonic saline, bronchoalveolar lavage, and endobronchial and transbronchial biopsies.<sup>12</sup>

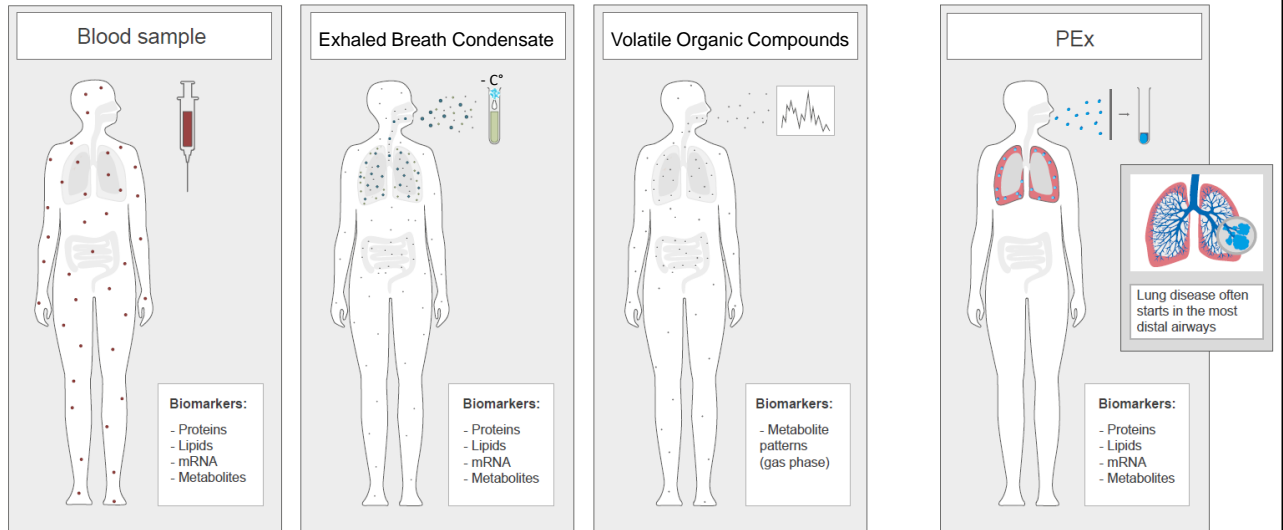
#### FUTURE DIRECTIONS

Despite growing insight into small airways disease, a number of questions have so far remained unanswered in our understanding of the area. Early detection remains the key barrier, although we have outlined several new approaches that have the potential to achieve this goal. Critically, if detected early, treatment with therapy options designed to target small airways should help slow disease progression, reduce symptoms, and help patients maintain their activities. It may therefore be beneficial to further investigate whether the ability to target treatments to the lung periphery and increase lung deposition could slow disease progression in patients with COPD and thereby become a viable treatment option for patients who have small airway involvement and early signs of COPD.



This review by Omar Usmani and colleagues from Imperial College describe the importance of increasing our understanding of pathobiological processes that takes place in the most distal airways. The authors describe why we need to target the Small airways, both in the context of novel drug targets and the urgent need for more precise and more early biomarkers.

## PEx originate specifically from the region where lung disease develops



**PEx is not the same as EBC or VOC**

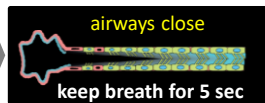
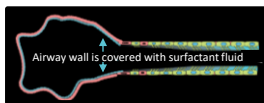
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When people hear about PExA, many think that PEx sample is just another Exhaled breath condensate or VOC sample. However this is incorrect. PEx sample originate directly from the respiratory lining fluid and surfactant that is present in the most distal part of our airways. Moreover, in contrast to EBC, the PEx sample is not contaminated with material from the upper airways or the oral cavity and it is undiluted. The fact that PEx originates exclusively from the lung as opposed to blood, EBC or VOC that originates from more or less the whole body and in undefined proportions, we believe that PEx sampling paves the way for more relevant, more accurate and less confounded biomarker data.

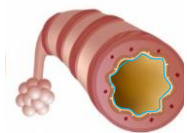
## The PExA breathing manoeuvre

### 1. Full exhalation

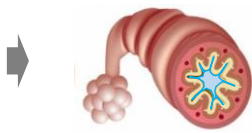
Side view



Sectional view

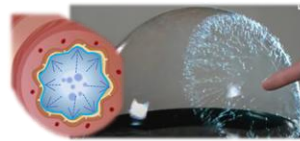
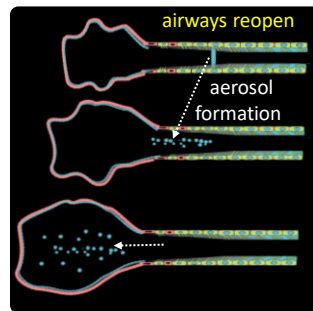


Open airway



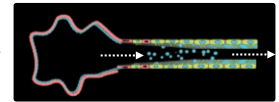
Closed airway

### 2. Full inhalation



Surfactant film stretch until it burst  
(like a soap bubble that burst)

### 3. Exhalation



Reproduced with permission from Per Larsson, Sahlgrenska Academy, University of Gothenburg, Sweden.

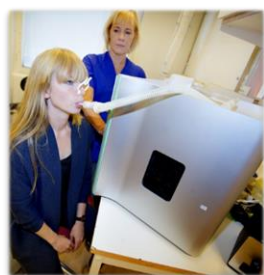
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One essential part of the PExA concept is the specially developed breathing maneuver.

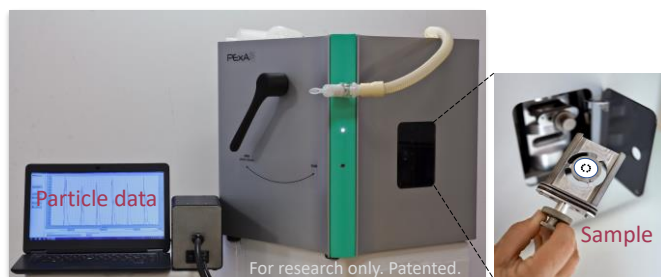
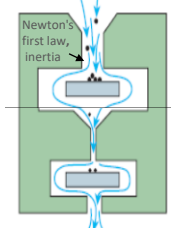
The breathing maneuver is easy to perform and usually not more cumbersome than spirometry, but it is different. It is a three-step maneuver, where the subject starts by exhaling as much air as possible and keeps breath for 5 seconds, such that all distal airways close. In this phase the respiratory lining-fluid and surfactant that is lining the airways form a film. Now we ask the subject to do a swift inhalation, meaning that the film will expand and eventually burst into small droplets. The process is similar to what happens when a soap bubble expands and bursts. Some of the droplets will be large and sediment by gravity whereas others will be small enough to float in the air, like an aerosol and therefore come out upon the next exhalation.



## PEx sample collection in short



Exhaled particles are collected by impaction



- Apply a series of PExA breathing manoeuvres
- Particles are collected on a removable substrate by impaction (no VOCs)
- Amount of collected droplet-particles is reported in real time
- Particle size distribution data is recorded for subsequent analysis (16 size bins, 0.4-7  $\mu\text{m}$ )
- A sampling session normally takes from 10 to 20 minutes.

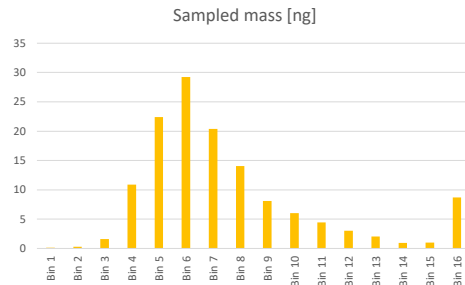
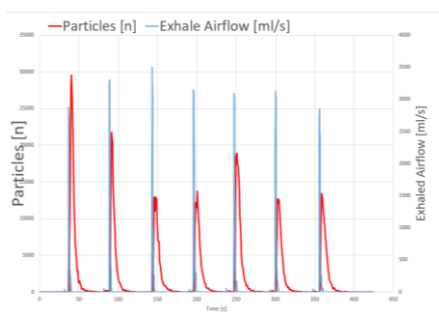
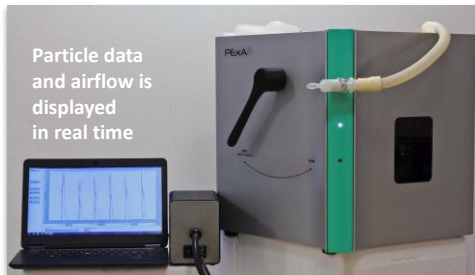
*PExA stands for Particles in Exhaled Air. PExA=name of instrument & company. PEx=name of the sample, matrix*

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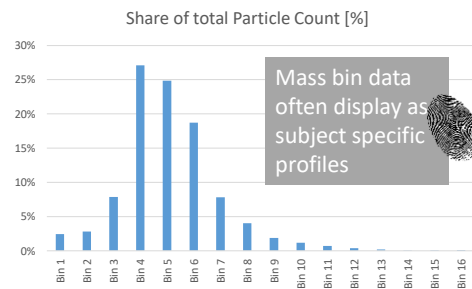
This slide illustrates what it looks like during a collection of a PEx-sample. A nurse guides the patient to perform and repeat the breathing maneuver. Usually, a sampling session takes from 10 to 20 minutes. The generated droplet particles are exhaled into the PExA instrument and collected on a removable substrate by impaction. The number and size of the particles are measured and reported in real time, such that the operator can see how much of the undiluted PEx material that is collected for each breathing maneuver. When enough material has been collected, the substrate is transferred to a sample tube that subsequently can be subjected to biochemical analysis to assess the molecular composition of the sample. Importantly, the reported amount of Pex material can be used to normalize the biomarker data.

# Particle data

## Particle data is automatically stored after each sampling session



Bin	$\mu\text{m}$
1	0.31
2	0.38
3	0.45
4	0.56
5	0.7
6	0.94
7	1.15
8	1.28
9	1.38
10	1.47
11	1.57
12	1.69
13	1.89
14	2.16
15	2.52
16	>3.42

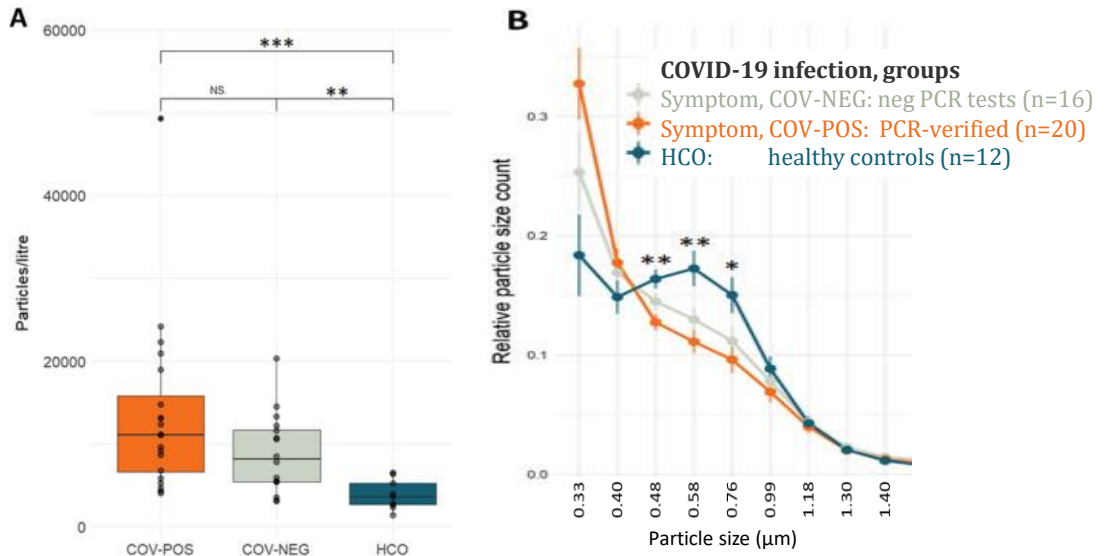


As previously mentioned, the PEXA instrument generate data that describe number and size distribution of the exhaled particles.

Some individuals generate more of smaller particles, others generate more of larger particles.. Also number of particles per exhaled air volume differ between subjects. The particle data is like a fingerprint..

Interestingly it has been shown that alterations in number of PEX particles and their size distribution is associated with development of lung disease and there are now several publications that describe how clinical signs correlate with change in PEX particle data. For instance, as seen in next slide one rather recent study found that size of particles generated by subjects with diagnosed COVID-19 were smaller than normal.

## Particle flow and size varies with lung pathophysiology



Hirdman, G. et al. Proteomic characteristics and diagnostic potential of exhaled breath particles in patients with COVID-19. *Clin Proteom* 20, 13, 2023

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This study by Hirdman et. al. show that number of PEx particles per exhaled volume of air was higher in patients with COVID-19. In addition, the generated PEx particles were smaller than those generated by healthy subjects

## PExA in ICU, mechanical ventilation



Particle Flow Profiles From the Airways Measured by PEXA Differ in Lung Transplant Recipients Who Develop Primary Graft Dysfunction

Ellen Broberg,<sup>1</sup> Snejana Hyllén,<sup>1</sup> Lars Algotsson,<sup>1</sup> Darcy E. Wagner,<sup>2</sup> Sandra Lindstedt<sup>3</sup>

Exp Clin Transplant, 2019 Dec;17(6):803-812

Patients with PGD showed a stepwise increase in particle count from day 0 until extubation.  
No adverse events related to the PEXA device was observed

<https://pubmed.ncbi.nlm.nih.gov/31615381/>

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There are also several studies that have been conducted in ICU units, where a mechanical ventilator have been connected to the PEXA, looping the air from the patient. These studies report that Pecks particle data change with development of disease or as in this case rejection after lung transplantation. These data is of great interest as there is an unmet need for surveillance systems that can alert if a patient start develop [Acute Respiratory Distress Syndrome](#) or other dangerous disease states while treated at an ICU unit.

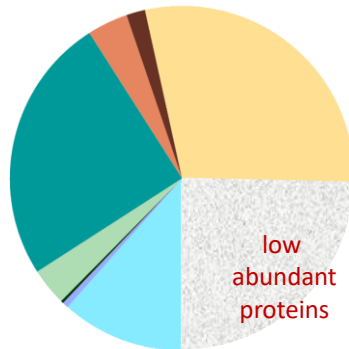
# Biochemical analysis ... some examples

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Here follows examples of data that illustrate what proteins can be detected and measured in PEx samples

## The molecular content of PEx

PEx sample contain large proportion of phospholipids but also proteins



Weight %

SpA (11.6%)	Surfactant protein A
SpB (0.6%)	
SpC (0.2%)	
CC16 (3.5%)	
Albumin (25%)	
Transferrin (3.8%)	
Alpha-1-antitrypsin (1.8%)	
Immunoglobulins (29%)	
Others (24.5%)	

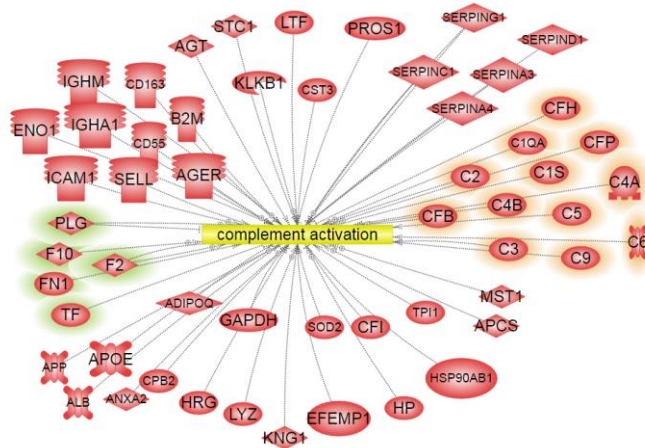
**Pilot experiment: SomaScan 1.3K, >200 protein could be detected**

*A novel non-invasive method allowing for discovery of pathologically relevant proteins from small airways*  
Östling et al. *Clinical Proteomics* (2022) 19:20 [pubmed.ncbi.nlm.nih.gov/35668386/](https://pubmed.ncbi.nlm.nih.gov/35668386/)

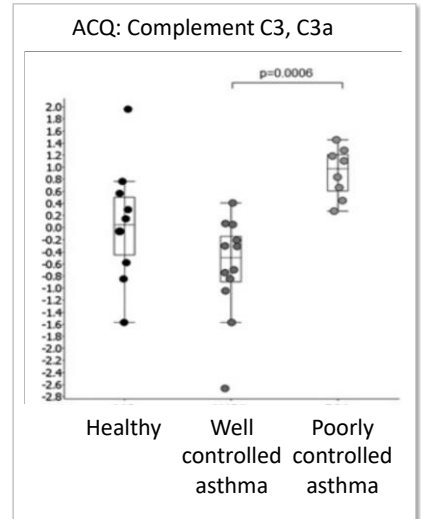
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One of the platforms that seem to be suitable for analysis of the protein composition of Pecks particles is SomaScan . With this platform over 200 different proteins could be detected and accurately measured in PEx samples

## Complement system AND its neighbors are well represented in PEx



### Matching the 207 proteins with Pathway Studio database



Östling et al, ERS 2017

Spela Kokelj et al, Respiration 2023;102:621–631

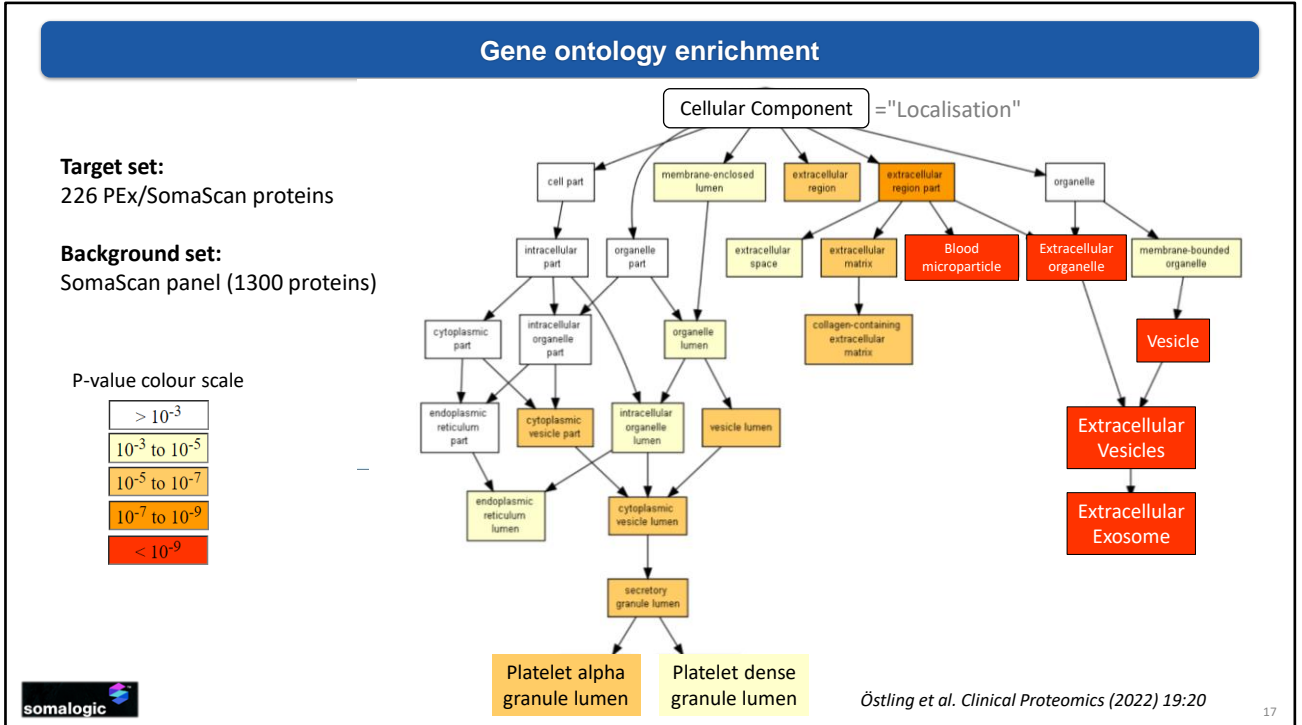


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Interestingly, when the 200 identified proteins were matched with protein databases it was revealed that Pecks samples constitute an overrepresentation of complement factors and not only the complement factors themselves but also the many proteins that are known to be associated with the complement-system and the innate immune response.

In spite of low n-number, in a recent study by Kokelj *et al.* it was shown that levels of Complement factor C3 and C3a were higher in PEx samples from a group of asthmatics that report that their asthma-medication does not help much. Note the clear differences between the groups.



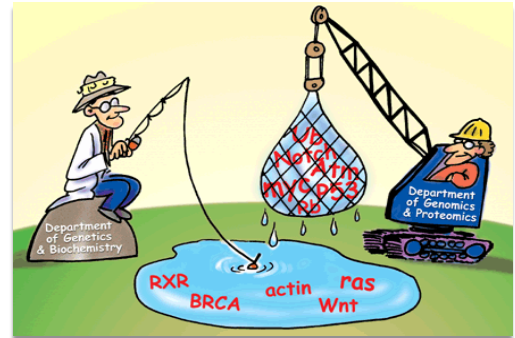


Furthermore, gene enrichment analysis based on GO-annotations revealed that the PEx proteome seem to constitute an overrepresentation of proteins known to be associated with exosomes. This observations is interesting in the light of the many studies that report the importance of exosomal content in search for novel biomarkers of disease. Another interesting observation was that Pecks samples also contain proteins that are involved in immune response that involve platelets, which is an emerging research field within respiratory-medicine.

<https://pubmed.ncbi.nlm.nih.gov/35668386/>

## Take home messages

- PExA is a technology that have been developed to enhance identification and development of actionable biomarkers in the field of precision respiratory medicine.
- PEx is not the same as EBC or VOC.
- PExA is a non-invasive method for collection of biological material from the distal airways, the region where many lung disease initiate and develop
- PEx contains if any, very little material from the upper airways
- The non-invasiveness open up for longitudinal study design where the Individual is it's own control.
- Biochemical analysis of the molecular content of PEx suggest that the sample resembles BAL but with the difference that it is non-invasive and undiluted
- PEx contain very small amount of biological material, but enough for proteomics and lipidomics
- SomaScan and Olink are examples of suitable proteomics platforms  
-other, even more sensitive proteomics platforms are under evaluation
- Preliminary data suggest that PEx contain high proportions of proteins  
- involved in innate immunity  
- associated with exosomes



Proteomics provide unique opportunities for Precision Medicine, but Proteomics is not only about measuring many analytes, selecting the "right lake for fishing" is also of high importance.

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So, to summarise,

- PExA is a technology that have been developed to enhance identification and development of actionable biomarkers in the field of precision respiratory medicine
- PExA is not the same as Exhaled breath condensate or VOC
- PExA is a non-invasive method for collection of biological material from the distal airways., the region where many lung disease initiate and develop
- PEx contains if any, very little material from the upper airways
- The non-invasiveness open up for longitudinal study design where the individual is it's own control.
- Biochemical analysis of the molecular content of PEx suggest that the sample resembles bronco-alveolar-lavage samples but with the difference that it is non-invasive and undiluted.
- Pecks contain very small amount of biological material, but enough for proteomics and lipidomics
- Soma-scan and Olink are examples of suitable proteomics platforms. Other, even more sensitive proteomics platforms are under evaluation
- Preliminary data suggest that PEx contain high proportions of proteins involved in innate immunity and are associated with exosomes

And as a final note, proteomics provide unique opportunities for Precision Medicine, but Proteomics is not only about measuring many analytes, selecting the right "lake for fishing" is also of high importance and this is where PExA provide unique opportunities.



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