Novel views on endotyping asthma, its remission, and COPD

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Chapter 4

Assessing small airways dysfunction in asthma, asthma remission, and healthy controls using Particles in Exhaled Air (PExA)

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Introduction

Asthma is a chronic disease, characterized by variable airflow obstruction and airway inflammation [1]. Small airways are thought to be a major site of pathology in asthma [2,3]. There are different tools to assess small airways dysfunction (SAD), such as spirometry, body plethysmography, impulse oscillometry (IOS), multiple breath nitrogen wash-out (MBNW), alveolar fraction of exhaled nitric oxide (FeNO) and gas-trapping assessed by high-resolution CT (HRCT). However, there is no golden standard and some tests are difficult to perform [2,3]. PExA (Particles in Exhaled Air) is a recently developed technique with the potential to identify SAD phenotypes in asthma [4,5]. PExA measurements are non-invasive and easy to perform by subjects, even in severely obstructed patients. PExA captures the aerosol from exhaled breath, and specifically those endogenously generated particles in the size range 0.5-4 μm that are formed during airway closure and reopening. These particles contain water and non-volatile material originating from the respiratory tract lining fluid [6]. It is thought that SAD leads to impaired reopening of airways or altered composition of the respiratory tract lining fluid, causing less particles to be formed [7]. Therefore, severity of SAD is expected to be associated with a reduction of particles measured by PExA.

Some patients with asthma outgrow their disease and reach clinical asthma remission (ClinR); these individuals experience no asthma symptoms even without using asthma medication. Patients in ClinR, however, might still have (asymptomatic) bronchial hyperresponsiveness (BHR) or impaired lung function [8,9,10]. Broekema *et al.* demonstrated that subjects in ClinR still had ongoing airway inflammation [11]. In contrast, a smaller subset of asthma remission subjects may lack BHR and regain normal lung function, i.e. complete asthma remission (ComR) [10].

We hypothesized that more SAD leads to decreased exhalation of PExA particles and that this SAD is still present in ClinR-, but absent in ComR-subjects. Therefore, we compared exhaled PExA mass between ClinR- and ComR-subjects in relation to asthma patients and healthy controls. The second aim of this study was to investigate how PExA mass is associated with other measures of small and large airways function in these groups.

Methods

The study protocol was approved by the local ethical committee and all subjects gave informed consent (NL53173.042.15, Groningen). Included subjects were divided over four groups: subjects with childhood-onset asthma which persisted (PersA; subjects with wheezing and/or asthma attacks, asthma medication use, and a PC_{20} methacholine <8 mg/ml with 120s tidal breathing), or which had gone into clinical asthma remission (ClinR; subjects without wheezing/asthma attacks, no use of asthma medication in the last 3 years, with a documented history of asthma according to GINA guidelines, an FEV_1 % predicted <80% and/or PC_{20} methacholine <8 mg/ml), or into complete asthma remission (ComR; similar to ClinR, but with an FEV_1 % predicted ≥80%, PC_{20} methacholine ≥8 mg/ml and PC_{20} adeno-'5-monophosphate (AMP) ≥320 mg/ml), and healthy controls (Ctrl; similar to ComR, but without any history of asthma or use of asthma medication). All subjects were aged 40-65 years and were either never- or ex-smokers with a smoking history <10 pack years. Subjects were extensively characterized with the following tests: spirometry, body plethysmography, IOS, FeNO, MBNW, provocation tests, blood tests, sputum induction and CT-scans. PersA-subjects were withdrawn from inhaled corticosteroids six weeks prior to the clinical characterization.

PExA mass was collected using the PExA 2.0 device [5]. All subjects performed a similar breathing manoeuvre as described by Bake *et al.* [6]. To account for potential bias effects of circadian rhythm, all PExA measurements were performed in the morning.

Parametric Response Mapping (PRM) is a voxel-wise image analysis technique that was implemented on the CT-scans. PRM data was analysed according to the methods described in the literature [12,13].

Clinical characteristics and PExA mass in the subject groups were compared using independent sample T-test for normally distributed data (including log2-transformed variables), Mann-Whitney U tests for non-normally distributed data and Fisher's exact tests for categorical variables. Likewise, PExA mass was correlated with small and large airway parameters using either Pearson or Spearman. Last, a stepwise multivariate regression analysis was performed to assess independent associations with PExA mass.

Results and discussion

Clinical characteristics of the subject groups are presented in figure 1A. ComR-subjects were significantly younger than PersA-subjects (P=0.027). The FEV_1 was significantly higher in Ctrl- and ComR- compared to PersA-subjects, and higher in ComR- compared to ClinR-subjects.

Table 1: baseline characteristics

	Ctrl (n = 18)	ComR (n = 12)	ClinR (n = 16)	PersA (n = 18)	Kruskall-Wallis
Age (years)	56 [53 - 61]	46 [43 - 55]	54 [47 - 60]	60 [49 - 63]	0.044
Female (n, %)	6 (33.3)	4 (33.3)	7 (43.8)	7 (38.9)	0.918
Smoking pack years (min-max)	0 (0 - 5)	0 (0 - 6)	0 (0 - 1)	0 (0 - 2)	0.104
FEV_1 % predicted (%)	113.6 (12.0)	108.1 (9.5)	84.5 (23.1)	81.3 (17.2)	<0.001
PC_{20} methacholine threshold (mg/ml)	>8	>8	0.8 [0.12 - 2.83]	0.56 [0.27 - 2.16]	-
PExA mass (ng/L)	5.7 [3.0 - 9.6]	4.9 [2.9 - 6.5]	3.2 [0.7 - 5.6]	2.7 [0.5 - 4.0]	0.017

Ctrl: *healthy controls;* **ComR:** *complete remission subjects;* **ClinR:** *clinical remission subjects;* **PersA:** *persistent asthma patients;* **FEV_1:** *forced expiratory volume in one second;* **PC_{20}:** *substance provocative concentration causing a 20% drop of FEV_1. Age, pack years and PC_{20} methacholine presented as median, interquartile range. PExA mass presented as median, interquartile range and P-value based on ANOVA. FEV_1 presented as mean, standard deviation. Female presented as number, percentage and P-value based on Chi-Square test.*

PExA mass was significantly lower in PersA- compared to ComR- and Ctrl-subjects (P=0.028 and P=0.003 respectively). In addition, PExA mass was significantly lower in ClinR- compared to Ctrl-subjects (P=0.018). Comparison of particle size distribution per group did not yield additional information. This is the first study investigating exhaled particles in asthma remission subjects, showing a similar PExA mass in ComR compared to healthy controls and a decrease in PExA mass in ClinR compared to healthy controls, even though these individuals experience no wheeze or asthma attacks. Our findings are in concordance with the previously stated hypothesis that more SAD leads to decreased exhalation of particles. The fact that ClinR-subjects exhale less particles suggests that these subjects still have ongoing SAD similar to persistent asthmatics. In contrast, ComR-subjects exhale similar amounts of particles compared to healthy controls, possibly due to outgrown SAD.

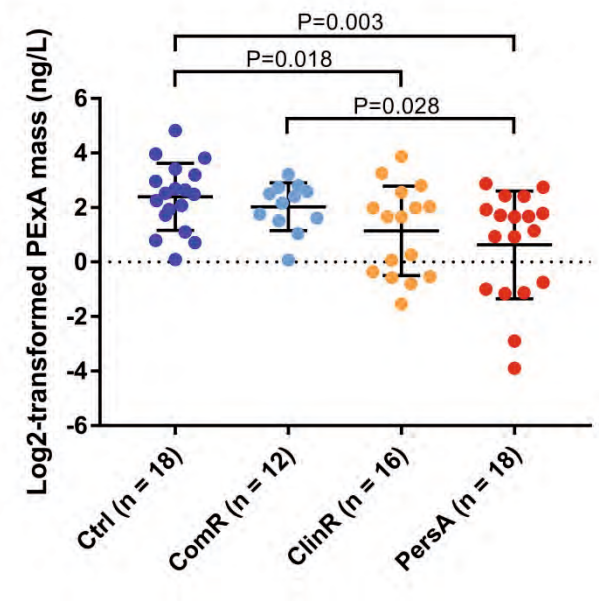


Figure 1: log2-transformed PExA mass in ng/L per subject group, with independent T-test P-values.

Next, we assessed the correlations between PExA mass and known small and large airway parameters. Results of these bivariate correlations are presented in figure 1B. Increased PExA mass was associated with less severe BHR and both parameters of large (higher FEV_1 % predicted and FEV_1/FVC ratio) and small (higher FEF25-75% % predicted, less hyperinflation as reflected by lower RV % predicted, lower IOS R5-R20 resistance and decreased MBNW Scond*VT) airway function. No correlation with PExA mass and PRM defined small airways disease (fSAD) was observed. Finally, a stepwise multiple regression analysis was performed including all variables significantly associated with PExA mass in the bivariate analysis (see figure 1B). This analysis showed that MBNW Scond*VT was independently associated with PExA mass.

Soares *et al.* found a correlation between mean number of particles per exhalation and FEV_1/FVC ratio (R=0.246, P=0.021), and between surfactant A PExA concentration and R5-R20 resistance (R=0.257, P<0.05)[4]. In accordance with the findings of Soares *et al.*, we show that increased PExA mass is associated with better function of both the large and the small airways.

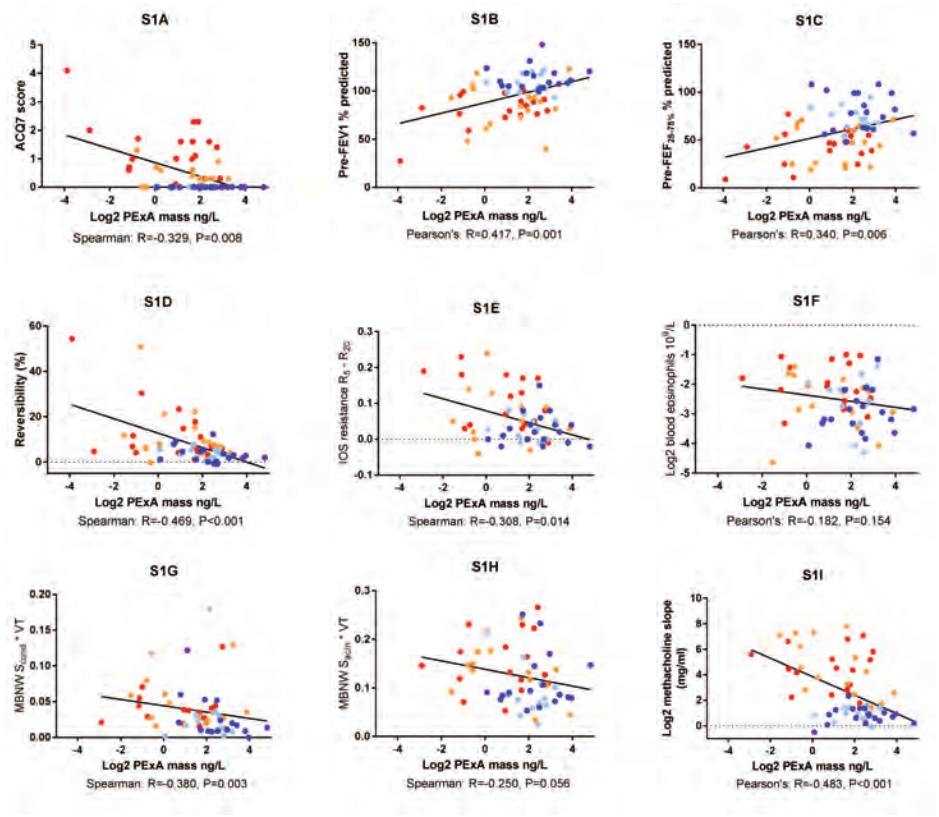
Table 2: PExA mass correlates with large and small airways parameters

Group	Parameter	Test	R-value	P-value
	Age (years)	Spearman	0.095	0.455
	Body Mass Index (kg/m^2)	Spearman	-0.203	0.107
Inflammatory	Blood eosinophils (10^9/L) #	Pearson	-0.182	0.154
	Sputum eosinophil differentiation (%)	Spearman	-0.449	0.013
Large	Reversibility pre-post (%)	Spearman	-0.469	<0.001
	PC_{20} methacholine slope (mg/ml) #	Pearson	-0.483	<0.001
	PC_{20} Adeno-'5-monophosphate slope (mg/ml)	Spearman	-0.441	0.001
	FEV_1/FVC ratio (pre-salbutamol) (%)	Spearman	0.355	0.004
	FEV_1 % predicted (pre-salbutamol) (%)	Pearson	0.417	0.001
	IOS R_{20} resistance (Hz)	Pearson	-0.386	0.002
Small	IOS R_5-R_{20} resistance (Hz)	Spearman	-0.308	0.014
	IOS AX (Hz kPa L^{-1})	Spearman	-0.342	0.006
	RV % predicted (%)	Spearman	-0.431	<0.001
	RV/TLC % predicted (%)	Spearman	-0.340	0.006
	MBNW S_{cond}*VT	Spearman	-0.380	0.003
	MBNW S_{acin}*VT	Spearman	-0.250	0.056
	$FEF_{25-75\%}$ % predicted (%)	Pearson	0.340	0.006
	Alveolar FeNO (parts per billion)	Spearman	-0.254	0.100
	CT PRM FSAD (%)	Spearman	-0.051	0.717
	CT PRM inferior-to-superior ventilation gradient (ΔHU)	Pearson	-0.197	0.152

#: log2-transformed, ***PC_{20}****: substance provocative concentration causing a 20% drop of* FEV_1*;* ***FEV_1****: forced expiratory volume in one second;* ***FVC****: forced vital capacity;* ***R_5****: resistance at 5 Hz;* ***R_{20}****: resistance at 20 Hz;* ***AX****: area of reactance;* ***RV****: residual volume;* ***TLC****: total lung capacity;* ***S_{cond}****: conductive ventilation heterogeneity;* ***S_{acin}****: acinar ventilation heterogeneity;* ***VT****: tidal volume;* ***$FEF_{25-75\%}$****: forced expiratory flow at 25-75% of the pulmonary volume;* ***FSAD****: functional small airways disease*

Conclusion

In conclusion, PExA mass can distinguish asthmatics from healthy individuals. In addition, we show that subjects with complete, but not clinical, asthma remission exhale more PExA mass compared to asthma. Our findings are in concordance with previous studies showing that decreased PExA mass is associated with more severe obstructive pulmonary disease [7,14]. These results reinforce the theory that clinical asthma remission subjects still have ongoing small airways disease and that subjects in complete asthma remission have completely outgrown their disease [10]. Our observations demonstrate that higher PExA mass is not only related to better large airway function, but also independently associated with small airways disease as reflected by Scond. This indicates that PExA mass could potentially be used as a tool to assess small airways dysfunction. Future research should focus on exploring the composition of exhaled particles to gain more insight on the pathophysiology of small airways dysfunction in asthma persistence and remission.



Supplemental figure 1: correlation between PExA mass and large or small airways parameters, red dots: asthmatics, yellow dots: clinical asthma remission subjects, light blue: complete asthma remission subjects, dark blue: healthy controls, **S1A:** log2-transformed PExA mass and Asthma Control Questionnaire Score, **S1B:** correlation between log2-transformed PExA mass and FEV_1 % predicted, **S1C:** correlation between log2-transformed PExA mass and $FEF_{72-75\%}$ % predicted, **S1D:** correlation between log2-transformed PExA mass and reversibility after 400mcg salbutamol, **S1E:** correlation between log2-transformed PExA mass and impulse oscillometry $R_5 - R_{20}$ in Hz, **S1F:** correlation between log2-transformed PExA mass and log2-transformed blood eosinophils in 10^9/L, **S1G:** correlation between log2-transformed PExA mass and Multiple Breath Nitrogen Wash-out S_{cond} * VT, **S1H:** correlation between log2-transformed PExA mass and Multiple Breath Nitrogen Wash-out S_{acin} * VT, **S1I:** correlation between log2-transformed PExA mass and log2-transformed methacholine slope in mg/ml.

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