Assessing small airways dysfunction in asthma, asthma remission and healthy controls using particles in exhaled air

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ORIGINAL RESEARCH LETTER

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To the Editor:

Asthma is a chronic disease, characterised 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 washout (MBNW), alveolar fraction of exhaled nitric oxide (F_{ENO}) and gas trapping assessed by high-resolution computed tomography (CT). However, there is no golden standard and some tests are difficult to perform [2, 3]. Particles in exhaled air (PExA) is a recently developed technique with the potential to identify SAD phenotypes in asthma [4, 5]. PExA measurements are noninvasive and easy for subjects to perform, 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 nonvolatile 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 fewer 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 [8–10]. Broekema *et al.* [11] demonstrated that subjects in ClinR still had ongoing airway inflammation. 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 hypothesised 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.

The study protocol was approved by the local ethical committee and all subjects gave informed consent (NL53173.042.15; Groningen, the Netherlands). The included subjects were divided over four groups: the first three groups were subjects with childhood-onset asthma that 1) persisted (PersA; subjects with wheezing and/or asthma attacks, asthma medication use, and a provocative concentration causing a 20% fall in forced expiratory volume in 1 s (PC_{20}) for methacholine of <8 mg·mL^{-1} with 120 s tidal breathing), or that 2) 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 Global Initiative for Asthma guidelines, a forced expiratory volume in 1 s (FEV_1) <80% predicted and/or PC_{20} methacholine <8 mg·mL^{-1}), or 3) into complete asthma remission (ComR; similar to ClinR, but with an FEV_1 ⩾80% pred, PC_{20} methacholine ⩾8 mg·mL^{-1} and PC_{20} AMP ⩾320 mg·mL^{-1}); the fourth group was healthy controls (Ctrl; similar to ComR, but without any history of asthma or use of asthma medication).

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PExA mass can distinguish asthmatics from healthy individuals. Subjects with complete, but not clinical, asthma remission exhale more PExA mass compared to asthma. Higher PExA mass was associated with better function of both the small and large airways. http://bit.ly/2znHABg

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All subjects were aged 40–65 years and were either never- or ex-smokers with a smoking history <10 pack-years. Subjects were extensively characterised with the following tests: spirometry, body plethysmography, IOS, F_{ENO}, MBNW, provocation tests, blood tests, sputum induction and CT scans. PersA subjects were withdrawn from inhaled corticosteroids 6 weeks prior to the clinical characterisation.

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 were analysed according to the methods described in the literature [12, 13].

TABLE 1 Clinical characteristics for the subject groups and bivariate correlations between particles in exhaled air (PExA) mass and small and large airways parameters

Characteristics	Ctrl	ComR	ClinR	PersA	Kruskal–Wallis p-value
Subjects n	18	12	16	18	
Age years	56 (53–61)	46 (43–55)	54 (47–60)	60 (49–63)	0.044
Female	6 (33.3)	4 (33.3)	7 (43.8)	7 (38.9)	0.918¶
Smoking pack-years median (min–max)	0 (0–5)	0 (0–6)	0 (0–1)	0 (0–2)	0.104
FEV_1 % pred	113.6±12.0	108.1±9.5	84.5±23.1	81.3±17.2	<0.001
PC_{20} methacholine	>8	>8	0.8 (0.1–2.8)	0.6 (0.3–2.2)	
PExA mass ng·L^{-1}	5.68 (3.01–9.57)	4.87 (2.90–6.45)	3.15 (0.71–5.63)	2.67 (0.49–4.02)	0.017+
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 cells·L^{-1} #	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^{-1} #	Pearson	−0.483	<0.001		
PC_{20} AMP slope mg·mL^{-1}	Spearman	−0.441	0.001		
FEV_1/FVC ratio pre-salbutamol %	Spearman	0.355	0.004		
FEV_1 % pred pre-salbutamol	Pearson	0.417	0.001		
IOS R_{20} Hz	Pearson	−0.386	0.002		
Small					
IOS R_5–R_{20} Hz	Spearman	−0.308	0.014		
IOS AX Hz·kPa·L^{-1}	Spearman	−0.342	0.006		
RV % pred	Spearman	−0.431	<0.001		
RV/TLC % pred	Spearman	−0.340	0.006		
MBNW S_{cond}×V_T	Spearman	−0.380	0.003		
MBNW S_{acin}×V_T	Spearman	−0.250	0.056		
$FEF_{25–75\%}$ % pred	Pearson	0.340	0.006		
Alveolar F_{ENO} ppb	Spearman	−0.254	0.100		
CT PRM-fSAD %	Spearman	−0.051	0.717		
CT PRM inferior to superior gradient ΔHU	Pearson	−0.197	0.152		

Data are presented as median (interquartile range), n (%) or mean±SD, unless otherwise stated. Ctrl: healthy controls; ComR: complete asthma remission subjects; ClinR: clinical asthma remission subjects; PersA: persistent asthma patients; FEV_1: forced expiratory volume in 1 s; PC_{20}: provocative concentration causing a 20% fall in FEV_1; FVC: forced vital capacity; IOS: impulse oscillometry; R_{20}: resistance at 20 Hz; R_5: resistance at 5 Hz; AX: area of reactance; RV: residual volume; TLC: total lung capacity; MBNW: multiple-breath nitrogen washout; S_{cond}: conductive ventilation heterogeneity; S_{acin}: acinar ventilation heterogeneity; V_T: tidal volume; $FEF_{25–75\%}$: forced expiratory flow at 25–75% of the pulmonary volume; F_{ENO}: fraction of exhaled nitric oxide; CT PRM: computed tomography parametric response mapping; fSAD: functional small airways disease. #: data were log2-transformed to obtain normal distribution; ¶: p-value based on Chi-squared test; +: p-value based on ANOVA.

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 tests. Last, a stepwise multivariate regression analysis was performed to assess independent associations with PExA mass.

Clinical characteristics of the subject groups are presented in table 1. 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.

PExA mass was significantly lower in PersA compared to ComR and Ctrl subjects (p=0.028 and p=0.003, respectively) (figure 1). 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 fewer 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. Next, we assessed the correlations between PExA mass and known small and large airway parameters. Results of these bivariate correlations are presented in table 1. Increased PExA mass was associated with less severe BHR and parameters of both large airway function (higher FEV_1 % pred and higher ratio of FEV_1 to forced vital capacity (FVC)) and small airway function (higher forced expiratory flow at 25–75% of the pulmonary volume % pred, less hyperinflation as reflected by lower residual volume % pred, lower IOS resistance at 5 Hz minus resistance at 20 Hz (R_5–R_{20}) and decreased MBNW conductive ventilation heterogeneity multiplied by tidal volume ($S_{cond}\times V_T$)). No correlation with PExA mass and PRM-defined (functional) small airways disease was observed. Finally, a stepwise multiple regression analysis was performed, including all variables significantly associated with PExA mass in the bivariate analysis (table 1). This analysis showed that MBNW $S_{cond}\times V_T$ was independently associated with PExA mass.

Soares *et al.* [4] 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 R_5–R_{20} (R=0.257, p<0.05). In accordance with these findings of Soares *et al.* [4], we show that increased PExA mass is associated with better function of both the large and the small airways.

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 subjects. 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 SAD 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 SAD as reflected by S_{cond}. This indicates that PExA mass could potentially be used as a tool to assess SAD. Future research

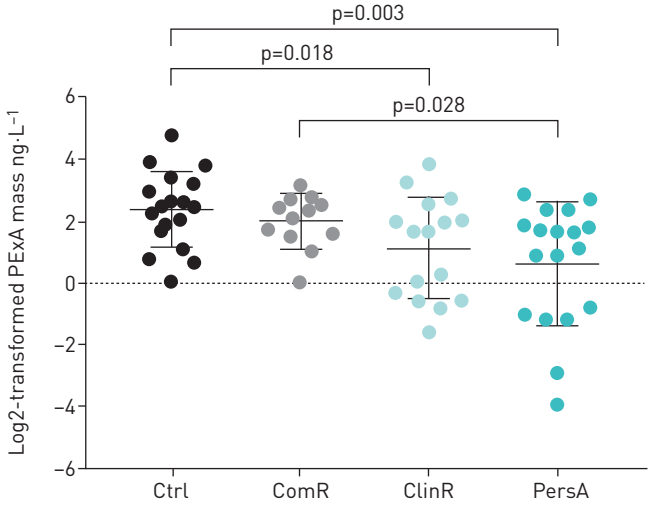

FIGURE 1 Particles in exhaled air (PExA) mass per subject group. Independent sample t-test p-values are shown. Ctrl: healthy controls (n=18); ComR: complete asthma remission subjects (n=12); ClinR: clinical asthma remission subjects (n=16); PersA: persistent asthma patients (n=18).

should focus on exploring the composition of exhaled particles to gain more insight into the pathophysiology of SAD in asthma persistence and remission.

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References

1 Lipworth B, Manoharan A, Anderson W. Unlocking the quiet zone: the small airway asthma phenotype. *Lancet Respir Med* 2014; 2: 497–506.
2 McNulty W, Usmani OS. Techniques of assessing small airways dysfunction. *Eur Clin Respir J* 2014; 1: 25898.
3 Postma DS, Brightling C, Baldi S, *et al.* Exploring the relevance and extent of small airways dysfunction in asthma (ATLANTIS): baseline data from a prospective cohort study. *Lancet Respir Med* 2019; 7: 402–416.
4 Soares M, Mirgorodskaya E, Koca H, *et al.* Particles in exhaled air (PExA): non-invasive phenotyping of small airways disease in adult asthma. *J Breath Res* 2018; 12: 046012.
5 Almstrand AC, Ljungström E, Lausmaa J, *et al.* Airway monitoring by collection and mass spectrometric analysis of exhaled particles. *Anal Chem* 2009; 81: 662–668.
6 Bake B, Larsson P, Ljungkvist G, *et al.* Exhaled particles and small airways. *Respir Res* 2019; 20: 8.
7 Larsson P, Lärstad M, Bake B, *et al.* Exhaled particles as markers of small airway inflammation in subjects with asthma. *Clin Physiol Funct Imaging* 2017; 37: 489–497.
8 Carpaij OA, Nieuwenhuis MAE, Koppelman GH, *et al.* Childhood factors associated with complete and clinical asthma remission at 25 and 49 years. *Eur Respir J* 2017; 49: 1601974.
9 Panhuysen CI, Vonk JM, Koëter GH, *et al.* Adult patients may outgrow their asthma: a 25-year follow-up study. *Am J Respir Crit Care Med* 1997; 155: 1267–1272.
10 Carpaij OA, Burgess JK, Kerstjens HAM, *et al.* A review on the pathophysiology of asthma remission. *Pharmacol Ther* 2019; 201: 8–24.

11 Broekema M, Timens W, Vonk JM, *et al.* Persisting remodeling and less airway wall eosinophil activation in complete remission of asthma. *Am J Respir Crit Care Med* 2011; 183: 310–316.
12 Galbán CJ, Han MK, Boes JL, *et al.* Computed tomography-based biomarker provides unique signature for diagnosis of COPD phenotypes and disease progression. *Nat Med* 2012; 18: 1711–1715.
13 Bell AJ, Foy BH, Richardson M, *et al.* Functional CT imaging for identification of the spatial determinants of small-airways disease in adults with asthma. *J Allergy Clin Immunol* 2019; 144: 83–93.
14 Lärstad M, Almstrand AC, Larsson P, *et al.* Surfactant protein A in exhaled endogenous particles is decreased in chronic obstructive pulmonary disease (COPD) patients: a pilot study. *PLoS One* 2015; 10: e0144463.