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RESEARCH ARTICLE

Exhaled nitric oxide and nasal tryptase are associated with wheeze, rhinitis and nasal allergy in primary school children

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Abstract

Rhinitis and asthma are the most common respiratory diseases in children. We assessed whether airway inflammation markers were associated with nasal allergies and self-reported symptoms of wheeze and rhinitis in 130 children 6–12 year old in an epidemiological context. Independent of sex and age, the fraction of exhaled nitric oxide (FeNO) and nasal mast cell (MC) activation (tryptase ≥ 5 ng/mL) were positively associated with wheeze, rhinitis and with nasal allergy. Nasal eosinophil cationic protein (ECP) and exhaled breath condensate (EBC) markers (pH, 8-isoprostane, interleukin-1 β) were not associated with symptoms or with nasal allergy. In conclusion, FeNO and nasal tryptase reflect allergic inflammation in the respiratory system.

Keywords

Eosinophil cationic protein, exhaled breath condensate, mast cells, Rhinostick

History

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Introduction

In the developed world, the prevalence of allergic rhinitis and asthma is estimated to be 20–25% and 8–12%, respectively (Lotvall et al., 2012). Rhinitis and asthma are the most common respiratory diseases in children. Asthma is characterized by obstruction, inflammation and hyperresponsiveness of the airways which can cause episodes of wheezing, breathlessness, chest tightness and cough (Bharadwaj & Agrawal, 2004). Rhinitis is defined by an acute or chronic presence of nasal symptoms such as runny nose, itching, sneezing, or a stuffy nose due to nasal congestion. Rhinitis can be both allergic and non-allergic in nature (Sin & Togias, 2011).

Because of the heterogeneous factors underlying airway diseases, it remains a challenge to correctly diagnose and treat these patients (Calamelli et al., 2012). Accordingly, many studies focus on biomarkers that might help in the diagnosis and disease management. Measuring the fraction of exhaled nitric oxide (FeNO), reflecting eosinophil inflammation in the lower airways, has proven to be of additional value in the diagnosis and management of childhood asthma (Piacentini et al., 2012). Additional markers are studied in exhaled breath condensate (EBC) (Bloemen et al., 2011).

EBC consists of water vapour and airway lining fluid (ALF) droplets. Non-volatile compounds in EBC are assumed to originate from the ALF (Effros et al., 2012). Aerosolisation of ALF occurs as a result of ALF film rupture in the respiratory bronchioles during inhalation (Johnson & Morawska, 2009). EBC can be easily collected during tidal breathing, even in children as young as three years old (Bloemen et al., 2010). EBC can be collected outside a clinical setting and it is a non-invasive way to study the airways. According to a recent review, an increased EBC acidity and higher oxidative stress markers in EBC are consistently associated with childhood asthma (Thomas et al., 2013). Acidity of EBC is associated with airway inflammation (Davis & Hunt, 2012) while 8-isoprostane, an oxidation product of arachidonic acid, is a marker of oxidative stress (Kuban & Foret, 2013). Other markers such as cytokines and chemokines are also analysed in EBC (Bloemen et al., 2007; Thomas et al., 2013). Interleukin (IL)-1 β can be detected in EBC (Robroeks et al., 2010) and is possibly involved in allergic inflammation (Krause et al., 2012).

Nasal secretions can be obtained in a non-invasive way and may contain mediators of cells involved in allergic processes such as mast cell-derived tryptase and eosinophil cationic protein (ECP), reflecting eosinophil activity (Diamant et al., 2010). Furthermore, allergen-specific immunoglobulin E (sIgE) in nasal secretions can be analysed to diagnose nasal allergies (Marcucci & Sensi, 1989).

The aim of the current study was to assess whether FeNO, EBC pH, 8-isoprostane in EBC, IL-1 β in EBC, nasal ECP

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and nasal tryptase were associated with nasal allergies and self-reported symptoms of asthma, wheeze and rhinitis in a population of 130 primary school children.

Methods

Study population

Children 6–12 years old were recruited from two primary schools in Antwerp, a city of approximately 500 000 inhabitants located in the northern part of Belgium (Western Europe). Invitations were sent to 744 children of whom 242 were willing to participate. Subjects were eligible for participation when they had attended the current school for at least one year, had no plans to change school or home residence within the next year, had lived for at least one year in the Antwerp agglomeration, were not exposed to tobacco smoke inside their house (although children with smoking parents were not excluded) and were willing to donate biological samples. After exclusion of 68 children not meeting these criteria, 130 subjects were selected so that the population was equally distributed over schools, grades, age and sex. The biomonitoring was performed in two seasons (May–June and November–December 2011), each campaign lasting six weeks. Biomonitoring was performed at the schools on ten different days in the first period and on ten different days in the second period. All children were sampled between 9:00 and 13:00 h. In the first period 130 children participated, while in the second period six children dropped out of the study because they changed schools or the permission to participate was withdrawn.

In the first period, information on asthma and rhinitis symptoms was obtained through a questionnaire and FeNO, EBC pH, 8-isoprostane in EBC, IL-1 β in EBC and nasal sIgE were analysed. In the second period, FeNO, EBC pH, 8-isoprostane in EBC, IL-1 β in EBC, nasal ECP and nasal tryptase were measured. Use of medication was reported. In total, five children used anti-asthma or anti-allergic drugs (inhaled corticosteroids, long-acting beta-agonists or antihistamines) during the sampling period.

The study was approved by the ethical committee of the University of Antwerp and subjects' parents gave written informed consent to participate in the study.

Questionnaire

A written questionnaire on asthma, wheeze and rhinitis symptoms was completed by the parents. Questions were based on the standardised questionnaire provided by the International Study of Asthma and Allergies in Childhood (ISAAC) (Asher et al., 1995) to obtain information on wheeze ever [‘‘Has your child ever had wheezing or whistling in the chest at any time in the past?’’], current wheeze [‘‘Has your child had wheezing or whistling in the chest in the past 12 months?’’], asthma ever [‘‘Has your child ever had asthma?’’], rhinitis ever [‘‘Has your child ever had a problem with sneezing, or a runny or blocked nose when he/she did not have a cold or the flu?’’] and recent rhinitis [‘‘In the past 12 months, has your child had a problem with sneezing, or a runny or blocked nose when he/she did not have a cold or the flu?’’].

Nasal sIgE

In the first sampling period, nasal sIgE was measured using the Rhinostick test as previously described (Marcucci & Sensi, 1989). Briefly, a plastic stick containing five allergen-coated paper disks (grass pollen, mix of tree pollen, cat epithelium, mould or house dust mite) plus a negative control disk with chicken albumin (Sigma Aldrich, Milan, Italy) was inserted into the nostril for 15 minutes. After sampling, sticks were stored in a buffer (NaCl 0.9%, Tween 0.1% and NaN₃ 0.05%) at 4 °C. IgE levels were analysed using a previously described ELISA method (Marcucci & Sensi, 1989). After washing, the allergen-coated disks were incubated with a biotinated goat IgG anti-human IgE (VECTOR Laboratories, Peterborough, England) then washed and incubated with streptavidine-betagalactosidase (Boehringer Ingelheim, Milan, Italia), washed again and incubated with a fluorimetric development kit (CAP System FEIA Phadia). IgE levels were determined using a standard reference curve (CAP System FEIA IgE Phadia) performed with goat IgG anti-human IgE-coated paper disks (VECTOR Laboratories).

Nasal ECP and tryptase

In the second period, a Rhinostick was used to measure nasal tryptase and ECP according to Sensi et al. (1997). A plastic stick containing sponges coated with anti-tryptase and anti-ECP antibodies was inserted into the nostril for 15 minutes. Sticks were stored in buffer (NaCl 0.9%, Tween 0.1% and NaN₃ 0.05%) at –20 °C. Tryptase and ECP levels were determined by ELISA (UniCAP Tryptase System FEIA and UniCAP ECP System FEIA, Pharmacia, Uppsala, Sweden), as previously described (Sensi et al., 1997). The detection limit for both markers was 2.0 ng/mL. For ECP, all samples were above the detection limit while for tryptase 37% was below the detection limit. These samples were given a value of 1.0 ng/mL. A threshold of 5.0 ng/mL for tryptase was considered for nasal mast cell activity. Based on this, a new binary variable ‘mast cell (MC) activation’ was defined.

FeNO

FeNO was analysed using a portable NIOX MINO[®] (Aerocrine, Solna, Sweden) according to the manufacturer's protocol. Subjects inhaled through the mouthpiece and exhaled for 5 s at a flow of 50 mL/min. Two measurements were performed. When these differed more than 10% a third measurement was carried out. The two or three measurements were averaged to obtain a final FeNO value.

Markers in EBC

EBC was collected using an RTube[™] sampling device (Respiratory Research, Inc., Austin, TX). The RTube[™] was mounted with an aluminium sleeve which was cooled on dry ice for at least 10 min before collection. Subjects were asked to breathe tidally through a mouthpiece connected to the tube for 15 min, yielding approximately 1 mL of EBC. No food was taken 1 h prior to collection. After collection the EBC was immediately divided in aliquots of 250 μ L, using 1.5 mL protein LoBind tubes (Eppendorf, Hamburg, Germany). Samples were kept on dry ice and stored at –80 °C until

further analysis. The pH was analysed exactly 5 min after EBC collection in the 1.5 mL tube using a microelectrode (SI Analytics GmbH, Mainz, Germany). IL-1 β was analysed using a Meso Scale Discovery (MSD) Ultra-Sensitive Kit. The 96-well plates were read using a SECTOR[®] Imager 6000 instrument (Meso Scale Discovery, Rockville, MD). The detection limit was 50 fg/mL. Samples below the detection limit (21% of all measured samples) were given a value of 25 fg/mL. 8-Isoprostane was measured using a Cayman Chemical 8-isoprostane enzyme immunoassay kit (Cayman Chemical Company, Ann Arbor, MI). All samples were above the detection limit of the assay, which was 2.7 pg/mL.

Statistics

The influence of age and sex on the biomarkers was tested using simple linear regression analysis. For this, all biomarkers except EBC pH were log10 transformed. For each sampling period, multiple logistic regression analysis adjusting for sex and age was performed to test the associations between biomarker levels and asthma ever, wheeze ever, current wheeze, rhinitis ever, current rhinitis and nasal sIgE. Except for nasal tryptase, results were expressed as the odds ratios with the 95% confidence interval (CI) per interquartile range (IQR) increase in biomarker level. For nasal tryptase, the ORs were calculated using the binary variable MC activation (≥ 5 ng/mL tryptase) since 37% of all samples were below the detection limit.

As a sensitivity analysis, all models were repeated excluding children taking asthma or allergy medication at the moment of sampling. To test whether biomarkers were associated with rhinitis symptoms in subjects without nasal allergy, logistic regression analyses were performed with exclusion of the children in whom nasal sIgE was detected.

Statistical analysis was performed using SPSS Statistics 20 (IBM, Armonk, NY). Effects were considered significant when the *p* value was lower than 0.05.

Results

The characteristics of the study population are displayed in Table 1. The study population was evenly distributed over all ages and an equal number of boys and girls participated. The Rhinostick test for detection of nasal sIgE was successfully performed in 117 children. In 13.7% of the subjects nasal sIgE could be detected; 87.0% of these children showed detectable sIgE levels for only one type of aeroallergen. The majority of children with nasal sIgE reported rhinitis symptoms. Only a quarter of the children with rhinitis symptoms had detectable levels of nasal sIgE, indicating that a large portion of the children suffered from non-allergic rhinitis. Based on nasal tryptase levels, 31.4% of the children were defined as having nasal MC activation.

Half of the currently wheezing children were diagnosed with asthma. The majority of the children that ever wheezed did not report recent wheezing symptoms. In contrast, children who reported ever having symptoms of rhinitis still displayed these symptoms during the last 12 months.

Table 2 displays the biomarker levels measured in the second period. The Rhinostick test for analysis of ECP and tryptase was not performed in three children since they were

Table 1. Population characteristics.

		<i>N</i> (%) or mean (SD)
Age (years)		9.5 (1.4)
Sex (boys)		61 (49.2)
BMI ^a	Underweight	19 (15.3)
	Normal	90 (72.6)
	Overweight	14 (11.3)
	Obese	1 (0.8)
Use of medication ^b		5 (4.0)
Nasal allergy ^c		16 (13.7)
MC activation ^d		38 (31.4)
Asthma ever		14 (11.3)
Wheeze	Current	8 (6.5)
	Past	22 (17.7)
	No	94 (75.8)
Rhinitis	Current	38 (30.6)
	Past	6 (4.8)
	No	80 (64.5)

^aCorrected for sex and age based on “Flanders 2004 children’s growth curves”.

^bInhaled corticosteroids, long-acting beta-agonists or antihistamines.

^cBased on detection of nasal sIgE in 117 subjects.

^dDefined as nasal tryptase ≥ 5 ng/mL.

not comfortable with the test. FeNO data were missing for five subjects in period 1 and for two subjects in period 2 due to the inability of the children to perform the breathing manoeuvre. IL-1 β in EBC was not analysed in four and two participants in period 1 and 2, respectively because not enough sample was available. EBC 8-isoprostane was missing for one person in period 1 due to low sample volume.

In period 1, ten children had high FeNO values, above 35 ppb. High FeNO is likely to indicate significant airway eosinophilia (Dweik et al., 2011). In period 2, seven of these children had values which were also above this limit. Except for two subjects, all these children were diagnosed with airway allergies, explaining the consistent higher values. Single regression analysis showed that EBC pH and 8-isoprostane were not influenced by sex or age. Both IL-1 β in EBC and nasal ECP were significantly associated with age, but not with sex. An increase in age of one year resulted in a decrease in IL-1 β and ECP of 20.9% (95% CI: 8.2; 31.9%) and 13.5% (95% CI: 1.8; 24.0%), respectively. Age was also associated with a 14.3% (95% CI: 5.4; 23.9%) increase in FeNO. Tryptase was the only marker influenced by sex, and levels in boys were approximately 116.8% (95% CI: 32.4; 254.8%) higher compared to girls.

Table 3 displays the estimated ORs (95% CI) for asthma, wheeze and rhinitis symptoms and nasal allergies in relation to biomarker levels in the second period. All associations are adjusted for sex and age. Results for markers that were also measured in the first sampling period (FeNO, EBC pH, EBC 8-isoprostane, EBC IL-1 β) were similar to these of the second period (data not displayed). None of the measured EBC markers were associated with asthma, wheezing symptoms, rhinitis symptoms or nasal allergies. In contrast, FeNO and nasal MC activation showed a clear association with both symptoms and nasal allergy.

The respective estimated ORs (95% CI) for current wheeze and for wheeze ever were 1.43 (1.13; 1.82) and 1.25 (1.05; 1.50) per IQR increase in FeNO. For nasal MC activation,

Table 2. Stratified biomarker levels, displayed as median (P₂₅; P₇₅).

		FeNO (ppb)	EBC pH	EBC 8-isoprostane (pg/mL)	EBC IL-1β (fg/mL)	Nasal ECP (ng/mL)	Nasal tryptase (ng/mL)
Asthma ever	Yes	11.7 (7.7; 27.4)	6.21 (5.94; 6.29)	12.2 (7.5; 15.4)	337 (110; 830)	121.0 (29.3; 179.8)	3.9 (1.8; 11.3)
	No	10.5 (8.3; 15.3)	6.06 (5.96; 6.19)	12.0 (9.6; 14.8)	277 (106; 535)	74.1 (29.7; 133.5)	1.7 (1.0; 8.0)
Wheeze ever	Yes	15.0 (9.5; 27.8)	6.11 (5.93; 6.26)	12.3 (9.2; 15.3)	341 (100; 900)	89.2 (34.0; 163.0)	78.7 (31.1; 133.5)
	No	10.2 (8.0; 13.2)	6.05 (5.95; 6.17)	12.0 (9.4; 14.7)	263 (109; 494)	78.7 (31.1; 133.5)	2.3 (1.0; 11.8)
Current wheeze	Yes	23.0 (11.3; 50.8)	6.01 (5.95; 6.31)	11.9 (7.6; 14.6)	524 (109; 1051)	125.0 (80.9; 183.3)	11.7 (1.8; 33.2)
	No	10.3 (8.3; 14.6)	6.07 (5.95; 6.20)	12.0 (9.5; 14.9)	292 (106; 525)	72.9 (27.4; 135.0)	1.8 (1.0; 7.6)
Rhinitis ever	Yes	11.7 (8.3; 21.0)	6.03 (5.96; 6.24)	12.1 (9.8; 14.3)	277 (108; 708)	89.2 (42.1; 162.5)	2.9 (1.0; 14.1)
	No	10.3 (8.2; 14.1)	6.07 (5.93; 6.19)	12.2 (9.4; 15.2)	278 (105; 478)	74.9 (24.8; 134.0)	1.4 (1.0; 5.1)
Current rhinitis	Yes	10.0 (8.3; 25.7)	6.07 (5.97; 6.24)	12.1 (9.6; 14.7)	277 (110; 781)	89.2 (43.7; 165.0)	3.9 (1.1; 15.7)
	No	10.5 (8.3; 15.0)	6.07 (5.93; 6.20)	11.9 (9.4; 15.0)	297 (103; 515)	74.9 (24.3; 133.0)	1.6 (1.0; 5.3)
Nasal sIgE	Yes	25.7 (9.2; 63.9)	6.20 (5.89; 6.30)	12.3 (11.5; 15.3)	473 (129; 951)	129.5 (51.1; 158.3)	11.4 (2.5; 33.2)
	No	10.0 (8.3; 13.3)	6.05 (5.97; 6.19)	12.0 (9.4; 14.8)	260 (99; 486)	70.8 (31.4; 133.0)	1.6 (1.0; 6.3)

Table 3. Associations between symptoms and biomarkers.

	FeNO		EBC pH		EBC 8-isoprostane		EBC IL-1β		Nasal ECP		MC activation ^a	
	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)	OR	(95% CI)
Asthma ever	1.02	(0.80; 1.29)	1.41	(0.66; 3.02)	0.87	(0.39; 1.94)	1.03	(0.61; 1.75)	1.67	(0.61; 4.61)	2.55	(0.77; 8.46)
Wheeze ever	1.25	(1.05; 1.50) [†]	1.14	(0.66; 1.96)	0.98	(0.56; 1.74)	1.13	(0.76; 1.66)	1.18	(0.55; 2.50)	1.13	(0.44; 2.88)
Current wheeze	1.43	(1.13; 1.82) [‡]	1.20	(0.46; 3.14)	0.71	(0.24; 2.08)	1.35	(0.77; 2.36)	2.76	(0.71; 10.75)	4.14	(0.87; 19.82)
Rhinitis ever	1.12	(0.96; 1.29)	0.95	(0.59; 1.54)	0.87	(0.53; 1.45)	1.07	(0.74; 1.55)	1.85	(0.92; 3.72)	4.18	(1.64; 10.64) [‡]
Current rhinitis	1.15	(0.99; 1.33)	1.03	(0.63; 1.69)	0.88	(0.52; 1.49)	1.08	(0.74; 1.57)	1.73	(0.85; 3.53)	3.78	(1.51; 9.50) [‡]
Nasal sige	1.85	(1.28; 2.68) [‡]	1.38	(0.68; 2.79)	1.18	(0.60; 2.34)	1.51	(0.98; 2.32)	2.05	(0.78; 5.40)	4.66	(1.45; 15.03) [‡]

Results from logistic regression analysis adjusted for sex and age. Unless otherwise stated, estimated odds ratios (OR) (95% CI) are calculated per IQR increase in biomarker. IQR FeNO = 7.3 ppb, IQR EBC pH = 0.25, IQR EBC 8-isoprostane = 7.0 pg/mL, IQR EBC IL-1β = 443 fg/mL, IQR ECP = 111.2 ng/mL.

^aDefined as nasal tryptase ≥ 5 ng/mL.

[†]p < 0.05.

[‡]p < 0.01.

estimated ORs (95% CI) were 3.78 (1.51; 9.50) and 4.18 (1.64; 10.64) for current rhinitis and rhinitis ever, respectively. Both FeNO and nasal MC activation were associated with nasal allergy, with an OR of 1.85 (1.28; 2.68) per IQR increase in FeNO and an OR of 4.66 (1.45; 15.03) for nasal mast cell activation.

As a sensitivity analysis, the logistic regression models were repeated with exclusion of five children who took anti-asthma or anti-allergy medication during the sampling period. The association between FeNO and wheeze ever was no longer significant with an OR (95% CI) of 1.19 (0.98; 1.43). Other associations remained the same. Furthermore, to test whether biomarkers were associated with rhinitis symptoms in subjects without nasal allergy, regression analyses were performed with exclusion of the 16 children with detectable nasal sIgE. This did not alter the observed associations between rhinitis symptoms and nasal mast cell activation.

Discussion

In primary school children, FeNO and nasal MC activation were positively associated with symptoms of wheeze and rhinitis, respectively. Both FeNO and nasal MC activation were also strongly linked with nasal allergy. For the other biomarkers, no significant associations with symptoms were observed. This may be because EBC pH, 8-isoprostane and IL-1β are less specific for allergic airway inflammation than

FeNO and tryptase, or because there was a higher variability of these markers in our population due to other factors which were not investigated in this study.

In paediatric asthma diagnosis and control, FeNO is often used in combination with lung function tests (Piacentini et al., 2012). In our study, we did not observe higher FeNO values in children reporting ever having asthma. Since the study population consisted of a random sample of a general population rather than case-control subjects, the number of asthmatic children was relatively low. Moreover, FeNO mainly reflects T-helper cell 2-mediated airway inflammation (Bjerner et al., 2014), which means FeNO is not necessarily elevated in all asthma patients. In contrast, FeNO was significantly associated with both current and ever wheezing. Current wheeze was previously linked with FeNO in children 12–14 years of age (Nordvall et al., 2005; Zhao et al., 2013). Recently, FeNO was shown to be associated with wheeze in a general population including both children and adults (Malinovschi et al., 2013). In addition, our study demonstrated that FeNO was associated with the detectability of nasal sIgE. FeNO is indeed linked with eosinophilic i.e. allergic inflammation and its most valuable clinical application lies in the detection of allergic airway inflammation (Dweik et al., 2011). In our study, there was a non-significant trend of higher FeNO in children with rhinitis. Many studies previously reported a link between FeNO and rhinitis (Henriksen et al., 1999; Jouaville et al., 2003; Nordvall et al., 2005; Zhao et al., 2013).

In our study including children between 6 and 12 years old, FeNO was positively associated with age. The American Thoracic Society (ATS) recommends to account for age when analysing FeNO in children younger than 12 years (Dweik et al., 2011). FeNO measurements taken at two time points, on average six months apart, were available in our study. In both periods, the associations between FeNO, wheeze and nasal allergy were similar. This indicates that FeNO reflected chronic airway disease in our population, and that FeNO can be used as a reliable stable marker for airway inflammation.

Nasal tryptase, reflecting mast cell activity, originates from mast cells in the mucosal tissue of the nose. Mast cells migrate from the blood stream into mucosal or connective tissues where they mature into long-lived cells. Mast cells are classically known to play a central role in allergic inflammation (Amin, 2012). This is in line with our study results which showed that nasal MC activation was linked with nasal allergies. We furthermore observed that nasal MC activation was associated with symptoms of current rhinitis. Marcucci et al. (2007) previously described higher nasal tryptase levels in children with rhinoconjunctivitis and asthma compared to controls (Marcucci et al., 2007). Besides allergy, mast cells are involved in innate and adaptive immunity (Weissler et al., 2008). In innate immunity, mast cells can be activated by host-derived signals or directly by pathogens such as bacteria and viruses through Toll-like receptors (TLRs). In adaptive immunity, mast cells can function as antigen-presenting cells. In this context, mast cells could also be involved in non-allergic rhinitis. In our study, the majority of children with recent rhinitis symptoms had no detectable nasal sIgE levels. In this subpopulation of 101 subjects with non-allergic rhinitis, nasal MC activation was still associated with both current and ever rhinitis. This is in contrast to another study which recently described that, compared to controls, both tryptase and mast cell numbers were not higher in subjects with non-allergic chronic rhinosinusitis, but were elevated in patients with allergic rhinitis (Groger et al., 2013). The aetiology of non-allergic rhinitis is diverse and often unknown. Moreover, Since symptoms are often very similar to allergic rhinitis it is difficult to distinguish between different subtypes of rhinitis (Sin & Togias, 2011). In epidemiological studies, it is therefore useful to objectively measure atopy, as was done in our study. Contrary to the classical skin prick test, the Rhinostick method used in this study is a non-conventional way to assess airway allergies. Nevertheless, it was previously shown that the Rhinostick test had a higher specificity and sensitivity for the diagnosis of airway allergies than the skin prick test (Marcucci et al., 2004), making it an ideal tool to non-invasively assess nasal allergies in epidemiological settings.

ECP, released by eosinophils, has been shown to have cytotoxic effects against bacteria, parasites viruses and respiratory cells (Bloemen et al., 2007). In our study, nasal ECP was higher in allergic subjects although this difference was not statistically significant. This is in line with earlier observations also describing a positive non-significant elevation of ECP and eosinophils in patients with allergic rhinitis compared to controls (Groger et al., 2013). Some authors suggested that ECP is not exclusively produced by eosinophils but also by neutrophils (Monteseirin & Vega, 2008; Sur et al.,

1998). This may explain why the association with allergy was not as strong as observed for tryptase. Moreover, it is suggested that eosinophil-derived neurotoxin (EDN), another eosinophil degranulation product, is a better marker for eosinophilic inflammation than ECP (Kim, 2013). Serum levels of EDN were more strongly correlated with asthma (Kim et al., 2007) and atopic dermatitis (Taniuchi et al., 2001) in children. Whether this is also true for nasal samples remains to be established.

In our study, we did not find differences in both EBC pH and 8-isoprostane between children with or without asthma or wheeze. EBC pH is often reported to be lower in asthmatic subjects compared to healthy controls while 8-isoprostane in EBC is often higher in asthmatics (Thomas et al., 2013). Lower EBC pH values were also reported in wheezing children compared to healthy controls (Vogelberg et al., 2007; von Jagwitz et al., 2011). In contrast, 8-isoprostane concentrations in EBC from wheezing children were not higher than those in EBC samples from healthy children (Caballero et al., 2013; Latzin et al., 2007).

IL-1 β is a pro-inflammatory cytokine involved in host defence and local and systemic inflammation (Dinarello, 2009). In the airways, IL-1 β is produced by epithelial cells (Hirota et al., 2012; Sousa et al., 1996) and macrophages (Ishii et al., 2004). We did not find differences in IL-1 β between asthmatic and non-asthmatic subjects, or any other symptoms. This is in accordance with a previous case-control study in which IL-1 β levels in EBC of asthmatic compared to healthy children were similar (Robroeks et al., 2010). Nevertheless, a role was recently suggested for IL-1 β in allergy-related disorders, such as bronchial asthma (Krause et al., 2012).

There are some limitations to our study. First, we did not have a case-control study design, leading to relatively small groups of children with symptoms or nasal allergy. This may explain why we were not able to confirm known associations between biomarkers and disease such as asthma, which is a heterogeneous disease with different endotypes. Another limitation is the seasonal difference in timing of sIgE measurements in spring and tryptase and ECP analysis in autumn. Practical issues concerning field work timing did not allow simultaneous analysis of all nasal markers. Another drawback is that we only tested sIgE for five aeroallergens, probably leading to some non-diagnosed allergic subjects. A final limitation is that no bronchial samples were available such as bronchoalveolar lavage fluid, induced sputum or bronchial biopsies to study lower airway disease. These methods require specialized clinical settings, which is not always feasible in studies with healthy subjects. However, some studies suggest that secretions from nasal epithelial cells might serve as a surrogate for bronchial epithelial cells (McDougall et al., 2008; Pringle et al., 2012; Thavagnanam et al., 2014), and that this may be useful in epidemiological studies.

Conclusions

In conclusion, rhinitis symptoms were associated with nasal MC activation while wheeze was associated with an increase in FeNO levels, indicating that these biomarkers reflect

ongoing allergic inflammation in the airways. Both biomarkers were furthermore strongly linked with nasal allergy status, reflected by nasal sIgE.

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