

Interrelationships among asthma, atopy, rhinitis and exhaled nitric oxide in a population-based sample of children

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Summary

Background Exhaled nitric oxide (eNO) has attracted increasing interest as a non-invasive marker of airway inflammation in asthma. However, little evidence exists on the influences exerted on eNO by the interrelations among atopic status, asthma and rhinitis.

Methods Among the 1156 children who participated in a large-scale epidemiological survey on asthma and allergies (ISAAC II: International Study of Asthma and Allergies in Childhood Phase II) in the city of Clermont-Ferrand, 53 asthmatics without corticosteroid treatment and 96 non-asthmatics were invited to perform eNO and skin prick tests (SPTs) to 12 common allergens.

Results Atopic asthmatic children had higher eNO than non-atopic asthmatic children (28.9 ± 9.1 vs. 17.1 ± 13.1 p.p.b.; $P = 0.0004$) with a significant increase when one SPT or more are positive (26.5 ± 7.8 vs. 17.1 ± 13.1 p.p.b.; $P = 0.03$). Similarly, non-asthmatic, atopic subjects had higher eNO than non-atopic subjects with a significant increase when two SPTs or more are positive (19.4 ± 9.8 vs. 11.7 ± 6.7 p.p.b.; $P = 0.003$). In the case of equal levels of positive SPTs (0, 1, ≥ 2), asthmatic children always had higher eNO than non-asthmatic ones. Furthermore, among non-asthmatic children, the eNO level increased only in atopics who had rhinitis (20.7 ± 13 vs. 12.5 ± 6.4 p.p.b. in atopic controls (subjects without rhinitis and asthma) and 12.3 ± 6.6 p.p.b. in non-atopic controls; $P = 0.001$), whereas among asthmatic children, eNO level increased in atopics independently of rhinitis (28.2 ± 9.5 p.p.b. in those with rhinitis and 30.9 ± 8.1 p.p.b. in those without) as well as in non-atopics with rhinitis (22.5 ± 17.2 p.p.b.).

Conclusions Our data suggest that besides atopy and asthma, allergic rhinitis should also be taken into account in the assessment of eNO.

Keywords asthma, atopy, children, exhaled nitric oxide, rhinitis, skin prick test

Submitted 9 May 2002; revised 13 November 2002; accepted 2 August 2003

Introduction

In pulmonary inflammatory diseases, such as asthma, expression of inducible nitric oxide (NO) synthase [1] is increased in the airways as a consequence of the level of NO being elevated in exhaled breath [2, 3]. Furthermore, inhaled corticosteroids decrease exhaled NO (eNO) [4, 5]. These results support the argument that eNO level is a marker of airway inflammation and suggest that measurement of eNO may be important in the management of asthma [6]. Recently, it has been suggested that atopy affects eNO concentration in asthmatic children as atopic asthmatic children have been reported to have significantly higher eNO levels than non-atopic asthmatics [7]. Furthermore, eNO has been shown to be elevated among non-asthmatic adults in concomitance with allergic rhinitis [8]. The aim of this study was to determine how allergic sensitization (as assessed by skin prick test (SPT) positivity to common allergens) and rhinitis

modulate the relationship between eNO and asthma. To this extent, eNO was performed in the framework of ISAAC II (International Study of Asthma and Allergies in Childhood Phase II) in Clermont-Ferrand (France), which allowed selecting homogeneous population-based samples of asthmatic and non-asthmatic children at school.

Materials and methods

Study subjects and study design

The study was conducted in an urban population including 53 asthmatics and 96 non-asthmatics, who were enrolled in Clermont-Ferrand to participate in ISAAC II aimed at establishing risk factors for allergies. They were drawn from an initial sample of 1156 children aged 10.3 years in mean by taking all asthmatics and almost two non-asthmatics (for each asthmatic, two successive non-asthmatic children were chosen). Children were excluded from the study if they had chronic pulmonary disease (out of asthma), recent upper or lower respiratory tract infection (3 weeks before) and if they were taking inhaled or oral steroids. In order to avoid pollen

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contamination, children seen during pollen season were excluded from the study. According to the ISAAC standardized protocol (International Study of Asthma and Allergies in Childhood Phase II Manual, December 1999; 2nd edn. Auckland (NZ), Munster (Germany)), children underwent skin examination, SPTs to 12 common allergens. Anthropometrical characteristics (height, weight, waist circumference) were also assessed. Parents filled in a standardized questionnaire on respiratory and allergic health of their children and potential risk factors. The medical ethics committee granted approval for the study and written informed consent was obtained from the parents of all children.

Outcomes of interest

Asthma and allergic rhinitis were defined according to ISAAC (Beasley in order to allow international comparisons) [9]. Asthma was defined as a positive answer to the classical question: 'Has your child ever had asthma?' Allergic rhinitis was defined as a positive answer to one of the two questions: 'Has your child ever had hayfever?' or 'Has your child ever had another allergic rhinitis?' with the indication of the type of allergen (question not included in ISAAC) [10]. For the purpose of the analysis, the following four groups of children were defined: controls (C, children without asthma and rhinitis), with asthma (A), with rhinitis (R), and with asthma and rhinitis (AR).

SPTs

Skin prick testing was performed using the prick method of the ISAAC study (Stallergène®, Antony, France). The solutions used were positive control (histamine dihydrochloride, 10 mg/mL), negative control, common allergens (*Dermaphagoides pteronyssinus* and *D. farinae*, alternaria, cat, dog, cockroach, grass pollen, tree pollen, ragweed, cypress, cod, egg, peanut). One drop of each was applied to the appropriately marked test site on the volar part of the forearm. The plastic lancet was then pressed through the drop at 90°C to the skin surface, and the drops were carefully blotted off using a tissue. Weal size was read at 15 min and recorded as the mean of the longest and the midpoint orthogonal diameters. A mean weal diameter of ≥ 3 mm the negative control was considered to be positive. Atopy was defined as at least one positive test to one allergen.

eNO measurement

The determination of eNO varies considerably, depending on the techniques and conditions of measurement [11, 12]. In the present study, eNO concentration was measured using exhaled flow and the pressure controlled reservoir collection (Sievers Instruments, Boulder, CO, USA, kit bag collection) [13, 14]. eNO measurement was obtained with the subjects in the standing position. Subjects did not wear nose clips, and were instructed not to hold their breath before exhalation. To eliminate ambient NO levels, subjects inhaled through an ambient air filter to total lung capacity. Then they exhaled through a mouthpiece connected to a T valve that allowed the separation of exhaled air into two fractions: the first portion of exhaled air was discarded (dead space) and the remainder

(lung air) was collected in an NO impermeable Mylar reservoir. In order to keep the soft palate closed, the column of collecting device had an internal restrictor that created a mouth pressure of 18 cm H₂O during exhalation, which was visualized on a manometer. At this mouth pressure, the expiratory flow was kept constant at 100 mL/s. Bags were sealed and analysis for eNO from the bags was completed within 4 h by aspirating the bag into the analyser. eNO was measured using a chemiluminescent analyser (NOA 280; Sievers Instruments, Boulder, CO, USA). The sensitivity of the analyser for measurement of gas-phase NO is less than 1 p.p.b. by volume. The NOA device was calibrated at 0 and 100 p.p.b. Our method has been reported to provide results similar to those obtained by the direct analysis of eNO [13].

Data analysis

Data on eNO were expressed as means $\pm$ SD. Statistical differences between percentages were assessed using the χ^2 test. Comparisons between groups were carried out using ANOVA. Differences indicated by *post hoc* test were analysed using the Fisher test (Statview Software, Abacus Concept Inc., Berkeley, CA, USA). Statistical significance was used at the conventional 5% level ($P < 0.05$).

Results

Characteristics of the groups

Twelve asthmatic children and one non-asthmatic child seen during the pollen season as well as eight asthmatic children taking corticosteroids were excluded from the study. The final sample included 53 asthmatics (27/53 of whom were atopics) and 96 non-asthmatics (31/96 atopics). The four groups (AR, A, R, C) did not differ in age, sex and anthropometrical characteristics (Table 1). The most frequently demonstrated SPT positivity was to house dust mite (81.5% in asthmatics vs. 74.2% in non-asthmatic subjects), followed by grass pollen (29.6% vs. 16.1%), tree pollens (13.2% vs. 12.9%) and cat (11.3% vs. 12.9%). Rhinitis was reported in 56.0% of asthmatic children, and in 22.9% of non-asthmatic subjects. Within the rhinitis group, 59.6% had one or more positive SPT. No difference was found in mean peak flow in the four groups, probably because asthmatic children taking corticosteroids were excluded from the selection (Table 1).

Factors influencing eNO (univariate analysis)

eNO was not related to age, sex or anthropometrical characteristics. Compared to non-asthmatic children, asthmatic children presented a higher eNO level (23.1 ± 12.6 vs. 13 ± 8 p.p.b.; $P < 0.0001$). Similarly, atopic children had a more elevated eNO level than non-atopic children (21.9 ± 11.4 vs. 13.3 ± 9.3 p.p.b.; $P < 0.0001$). For the clinical factors, analysis shows that the eNO level was higher in children with rhinitis compared with non-rhinitis children (21 ± 12.8 vs. 14.5 ± 9.9 p.p.b.; $P = 0.002$).

Table 1. Subject characteristics by group

	<i>n</i>	Sex (F/M)	Age (years)	Weight (kg)	Height (cm)	PEF (L/min)
Asthmatics with rhinitis						
Atopics	20	7/13	10.3 ± 0.6	35.7 ± 6.6	143.6 ± 7.5	320 ± 40
Non-atopics	10	9/1	10.8 ± 0.9	37.7 ± 7.1	145.3 ± 8.7	325 ± 53
Asthmatics without rhinitis						
Atopics	7	3/4	10.6 ± 0.3	37 ± 9.3	144.4 ± 10.7	314 ± 53
Non-atopics	16	8/8	10.7 ± 0.7	39.1 ± 12.2	143.4 ± 8.4	307 ± 41
Non-asthmatics with rhinitis						
Atopics	11	4/7	10.4 ± 0.7	37.1 ± 8.4	141.6 ± 9.3	296 ± 43
Non-atopics	11	6/5	10.7 ± 0.8	37.9 ± 11.3	143.2 ± 7.3	344 ± 54
Controls						
Atopics	20	12/8	9.9 ± 0.5	33.3 ± 5.1	138.3 ± 6.1	298 ± 50.8
Non-atopics	54	34/20	10.2 ± 0.6	36.2 ± 9.1	138.8 ± 18.8	322 ± 50

Data are presented as absolute number or as means ± SD. Subject number (*n*). PEF, peak expiratory flow.

eNO level, asthma and atopic status

Atopic asthmatic children had higher eNO than non-atopic asthmatic children (28.9 ± 9.1 vs. 17.1 ± 13.1 ; $P < 0.0001$) (Fig. 1). Likewise, in non-asthmatic children, atopics had higher eNO than non-atopics (15.8 ± 9.6 vs. 11.7 ± 6.6 ; $P = 0.019$).

eNO level and SPT positivity

Exhaled NO increased with the number of positive SPT in both asthmatics and non-asthmatics. In asthmatic children, the presence of at least one positive prick test was sufficient to make eNO increase significantly (SPT −: eNO = 17.1 ± 13.1 ; 1 SPT +: eNO = 26.5 ± 7.9 ; 2 SPT + or more: eNO = 30.5 ± 9.8 p.p.b.; $P = 0.03$), whereas in non-asthmatic subjects at least two prick tests were needed (SPT −: eNO = 11.7 ± 6.7 ; 1 SPT +: eNO = 14.3 ± 9.5 ; 2 SPT + or more: eNO = 19.4 ± 9.8 p.p.b.; $P = 0.003$). (Fig. 2).

eNO, atopic status and rhinitis

Among non-asthmatic children, only the atopics with rhinitis had an elevated eNO compared with controls (20.7 ± 13 vs. 13 ± 6.1 p.p.b. in atopics and 12.3 ± 6.6 in non-atopics; $P = 0.001$) (Fig. 3). Asthmatic children who were not atopic and who did not suffer from rhinitis had a lower eNO (13.7 ± 8.7 p.p.b.), close to the eNO value found in controls, whereas asthmatics with atopy but without rhinitis had a twofold increase in eNO level (30.9 ± 8.1 p.p.b.; $P = 0.0003$) (Fig. 3). Finally, children with both asthma and rhinitis had a significantly higher eNO independently of atopic status (28.2 ± 9.5 and 22.5 ± 17.3 p.p.b., respectively).

Discussion

In agreement with the literature, we observed higher eNO values in asthmatic than in non-asthmatic subjects [2, 3]. In addition, our results suggest that the eNO level of atopic asthmatic children is greater than that of non-atopic asthmatic children. These findings are consistent with

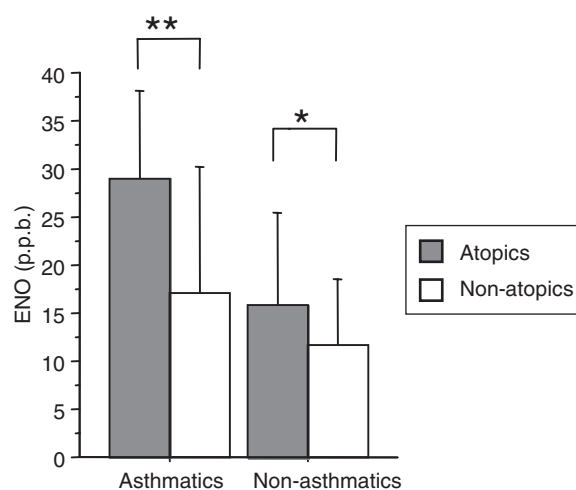


Fig 1. Exhaled NO and atopy in asthmatic and non-asthmatic subjects. Values are means ± SD. * $P < 0.05$; ** $P < 0.001$.

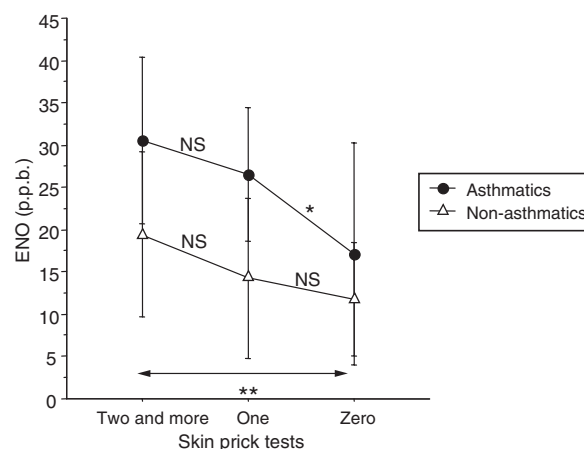


Fig 2. Exhaled nitric oxide and number of positive skin prick test (SPT) in non-asthmatic and asthmatic children. Values are means ± SD. *Significant difference in asthmatic between zero and one SPT + ($P = 0.03$); **significant difference in non-asthmatic between zero and two SPT + ($P = 0.003$); NS, not significant.

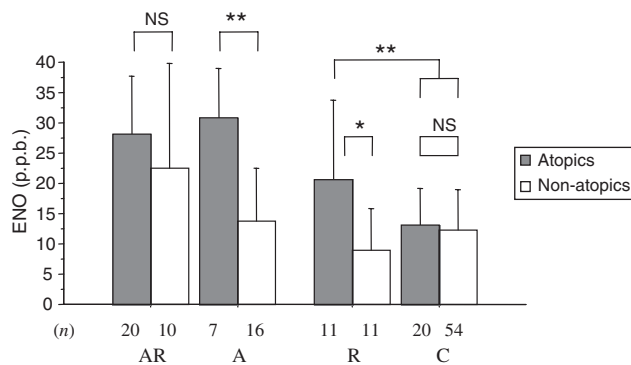


Fig. 3. Exhaled NO and atopy in different groups; healthy (control: C), non-asthmatic children with allergic rhinitis (R), asthmatic (A), asthmatic with allergic rhinitis (AR). Values are means $\pm$ SD. Subject number (n). * $P < 0.05$; ** $P < 0.001$; NS, not significant.

previous studies, in which eNO levels from two to four times higher than normal were found in atopic asthmatic children [7], teenagers [15] and adults [16]. Among non-asthmatic children, atopic subjects had higher eNO concentrations than non-atopic subjects, which contrasts with previous results showing no difference in eNO levels between atopic and non-atopic individuals either in children [7] or in adults [15, 16]. This might be due to the use in these studies of a high (250 mL/s) expiratory flow rate, which has been shown to have poor sensitivity [11, 12, 17]. Indeed, our results were in agreement with studies using a reduced-flow rate technique for measuring eNO in both children [18] and adults [19].

In the present study, we also found that the eNO level increased with the number of positive SPTs in asthmatic as well as in non-asthmatic subjects. For the same number of positive SPTs (three categories: none, one, two or more), the asthmatic children always had a higher level of eNO than the healthy children. It was also noteworthy that the non-asthmatic atopic subjects who had two or more positive SPTs had eNO levels close to those of the non-atopic asthmatic children (18.9 and 17.8 p.p.b., respectively). To our knowledge, no study has yet compared eNO and SPTs of asthmatic and healthy children taking monosensitization vs. polysensitization into account. Nevertheless, our results are consistent with a study [18] involving only healthy children, which found a significant increase in eNO level when two or more SPTs were positive. In a study of asthmatic adults [20], a high correlation between eNO level and the weal diameter of positive SPT ($r = 0.73$) was observed. These different results provide evidence of a relationship between eNO and atopy. The atopic status appears to be a cofactor of eNO variation in asthmatic children as well as in healthy subjects.

In the perspective of clinical applications for eNO, we also studied the influence of a symptomatic criterion (rhinitis) on the interrelationships among eNO, asthma and atopy. As previously noted in adults [16] and in children [21], we found a low level of eNO that did not vary depending on the subjects' atopic or non-atopic status in the control group. This result was consistent with studies [22, 23] that found no inflammatory modification of the bronchoalveolar lining in atopic or non-atopic healthy subjects. In the light of these findings, a subject's atopic nature might simply reflect a state of immune sensitization to allergens. In contrast, as already

noted in adults [16], a significant increase in eNO level was observed in the subgroup of atopic children with rhinitis. This finding lends support to the hypothesis of a relationship between the concentration of eNO and atopy, which is manifested in a certain number of cases by rhinitis. Also consistent with this hypothesis are histochemical studies that showed an inflammation of all the airways [24–26], from the nasal mucous membrane [27–29] to the bronchoalveolar lining [30–33] in atopic subjects with rhinitis. These atopic subjects with rhinitis had probably progressed to a state of symptomatic allergy. This beginning stage of airway inflammation is manifested by increased bronchial hyper-reactivity [8, 34, 35], which has previously been related to rhinitis [36]. Consequently, such subjects have a high risk of developing asthma [37].

Among the asthmatic children without rhinitis, there was still a relationship between atopy and eNO, i.e. there was a substantial increase in eNO level in atopic asthmatics, whereas non-atopic asthmatics had a low eNO, similar to that of the control group. This result was also consistent with previous findings [16, 21]. This difference between the two subgroups raises the question of whether the pathophysiological mechanisms of atopic asthma and non-atopic asthma are different. In the literature, this subject remains controversial. Certain authors have found in atopic asthma, a bronchial inflammation richer in eosinophils [38, 39], basophils [40] and T lymphocytes [38, 39], with a predominant increase in IL-4 [38, 41], IL-5 [38] and greater bronchial wall thickening [38]. In contrast, in non-atopic asthma, inflammatory cell recruitment is thought to involve a greater proportion of neutrophils [38], with higher levels of IL-8. [42]. These results support the hypothesis of a difference in eNO concentration mediated by cell recruitment, in particular that of eosinophils with a T helper 2 pattern of cytokine activation (IL-4, IL-5) [43, 44]. Nevertheless, other studies [45–47] have found a close pathophysiologic resemblance between atopic and non-atopic asthma in terms of both bronchial inflammatory cell recruitment and circulating factors. Consequently, along with the notion of inflammatory cell recruitment, it would appear important, as well, to consider cell activity that is known to produce NO [48]. A low production of eNO in non-atopic asthmatics might be explained either by variations in inflammatory cell activity mediated by intermittent exposure to unknown viral or immune stimuli [45, 49], or by a pathophysiologic mechanism of non-atopic asthma that differs from the mechanism of atopic asthma.

In contrast with previous findings [16], we observed no significant difference in the eNO level according to the atopic status in the group of asthmatics with rhinitis. However, our results are consistent with those of Berlyne et al. [50] comparing eNO to induced sputum, and are in accordance with the hypothesis of a similar physiological process between atopic and non-atopic asthma. Furthermore, the eNO discrepancy between non-atopic asthma with rhinitis and non-atopic asthma without rhinitis could be due to a difference of frequency and intensity of stimulation to an unknown allergen [51]. An intermittent stimulus could act in non-atopic asthma without rhinitis, while in non-atopic asthma with rhinitis a more prolonged exposition during the year could result in more marked symptoms associated

with asthma and rhinitis. Consequently, it would be interesting to identify these undefined allergens in a specific group of non-atopic asthmatics who exhibit rhinitis associated with high NO level.

In conclusion, eNO measured in the presented groups of children is thought to reflect the allergic inflammatory activity of airways, depending on the degree of atopy. As a consequence, the interpretation of eNO in clinical practice should take into account the notions of asthma, atopy and rhinitis. Other studies will be necessary to determine the pathophysiological contribution of each of these processes to eNO variations.

Acknowledgements

We thank Prof. R. Mercier, J. C. Calart, G. Dollat, O. Lajoinie and C. Martin for technical assistance, D. Moreau for data management, and ATMO-Auvergne for financial support.

References

- Koarai A, Ichinose M, Sugiura H, Yamagata S, Hattori T, Shirato K. Allergic airway hyperresponsiveness and eosinophil infiltration is reduced by a selective iNOS inhibitor, 1400W, in mice. *Pulm Pharmacol Ther* 2000; 13:267–75.
- Alving K, Weitzberg E, Lundberg JM. Increased amount of nitric oxide in exhaled air of asthmatics. *Eur Respir J* 1993; 6:1368–70.
- Kharitonov SA, Yates D, Robbins RA, Logan-Sinclair R, Shinebourne EA, Barnes PJ. Increased nitric oxide in exhaled air of asthmatic patients. *Lancet* 1994; 343:133–5.
- Kharitonov SA, Yates DH, Barnes PJ. Inhaled glucocorticoids decrease nitric oxide in exhaled air of asthmatic patients. *Am J Respir Crit Care Med* 1996; 153:454–7.
- Kharitonov SA, Yates DH, Chung KF, Barnes PJ. Changes in the dose of inhaled steroid affect exhaled nitric oxide levels in asthmatic patients. *Eur Respir J* 1996; 9:196–201.
- Kharitonov SA, Barnes PJ. Nitric oxide in exhaled air is a new marker of airway inflammation. *Monaldi Arch Chest Dis* 1996; 51:533–7.
- Frank TL, Adisesh A, Pickering AC et al. Relationship between exhaled nitric oxide and childhood asthma. *Am J Respir Crit Care Med* 1998; 158:1032–6.
- Henriksen AH, Sue-Chu M, Lingaas Holmen T, Langhammer A, Bjermer L. Exhaled and nasal NO levels in allergic rhinitis: relation to sensitization, pollen season and bronchial hyperresponsiveness. *Eur Respir J* 1999; 13:301–6.
- The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis and atopic eczema: ISAAC. *Lancet* 1998; 351:1225–32.
- Annesi-Maesano I, Didier A, Klossek M, Chanal I, Moreau D, Bousquet J. The score for allergic rhinitis (SFAR) – a simple and valid assessment in population. *Allergy* 2002; 57:107–14.
- Kharitonov S, Alving K, Barnes PJ. Exhaled and nasal nitric oxide measurements: recommendations. The European Respiratory Society Task Force. *Eur Respir J* 1997; 10:1683–93.
- Silkoff PE, Stevens A, Pak J, Bucher-Bartelson B, Martin RJ. A method for the standardized offline collection of exhaled nitric oxide. *Chest* 1999; 116:754–9.
- Kissoon N, Duckworth LJ, Blake KV, Murphy SP, Taylor CL, Silkoff PE. FE(NO): relationship to exhalation rates and online versus bag collection in healthy adolescents. *Am J Respir Crit Care Med* 2000; 162:539–45.
- Byrnes CA, Dinarevic S, Busst CA, Shinebourne EA, Bush A. Effect of measurement conditions on measured levels of peak exhaled nitric oxide. *Thorax* 1997; 52:697–701.
- Henriksen AH, Lingaas-Holmen T, Sue-Chu M, Bjermer L. Combined use of exhaled nitric oxide and airway hyperresponsiveness in characterizing asthma in a large population survey. *Eur Respir J* 2000; 15:849–55.
- Gratziau C, Lignos M, Dassiou M, Roussos C. Influence of atopy on exhaled nitric oxide in patients with stable asthma and rhinitis. *Eur Respir J* 1999; 14:897–901.
- Silkoff PE, McClean PA, Slutsky AS et al. Marked flow-dependence of exhaled nitric oxide using a new technique to exclude nasal nitric oxide. *Am J Respir Crit Care Med* 1997; 155:260–7.
- Franklin PJ, Taplin R, Stick SM. A community study of exhaled nitric oxide in healthy children. *Am J Respir Crit Care Med* 1999; 159:69–73.
- Horvath I, Barnes PJ. Exhaled monoxides in asymptomatic atopic subjects. *Clin Exp Allergy* 1999; 29:1276–80.
- Ho LP, Wood FT, Robson A, Innes JA, Greening AP. Atopy influences exhaled nitric oxide levels in adult asthmatics. *Chest* 2000; 118:1327–31.
- Barreto M, Villa MP, Martella S et al. Exhaled nitric oxide in asthmatic and non-asthmatic children: influence of type of allergen sensitization and exposure to tobacco smoke. *Pediatr Allergy Immunol* 2001; 12:247–56.
- Ying S, Humbert M, Meng Q et al. Local expression of epsilon germline gene transcripts and RNA for the epsilon heavy chain of IgE in the bronchial mucosa in atopic and nonatopic asthma. *J Allergy Clin Immunol* 2001; 107:686–92.
- Ying S, Meng Q, Zeibecoglou K et al. Eosinophil chemotactic chemokines (eotaxin, eotaxin-2, RANTES, monocyte chemoattractant protein-3 (MCP-3), and MCP-4), and C-C chemokine receptor 3 expression in bronchial biopsies from atopic and nonatopic (intrinsic) asthmatics. *J Immunol* 1999; 163:6321–9.
- Foresi A, Leone C, Pelucchi A et al. Eosinophils, mast cells, and basophils in induced sputum from patients with seasonal allergic rhinitis and perennial asthma: relationship to methacholine responsiveness. *J Allergy Clin Immunol* 1997; 100:58–64.
- Braunstaal GJ, Kleinjan A, Overbeek SE, Prins JB, Hoogsteden HC, Fokkens WJ. Segmental bronchial provocation induces nasal inflammation in allergic rhinitis patients. *Am J Respir Crit Care Med* 2000; 161:2051–7.
- Vignola AM, Bousquet J. Rhinitis and asthma: a continuum of disease? *Clin Exp Allergy* 2001; 31:674–7.
- Rapp J, Kosa L, Halasz A, Kereki E, Borzsonyi L. Levels of interleukin-4, interleukin-5, tryptase and eosinophil cationic protein of nasal lavage fluid in pollen allergic rhinitis. *Orv Hetil* 2000; 141:1919–22.
- Godthelp T, Holm AF, Fokkens WJ et al. Dynamics of nasal eosinophils in response to a nonnatural allergen challenge in patients with allergic rhinitis and control subjects: a biopsy and brush study. *J Allergy Clin Immunol* 1996; 97:800–11.
- Karlsson MG, Hellquist HB. Phenotype switch and activation of T lymphocytes in patients with allergic rhinitis. *ORL J Otorhinolaryngol Relat Spec* 1994; 56:166–72.
- Sihra BS, Kon OM, Grant JA, Kay AB. Expression of high-affinity IgE receptors (Fc epsilon RI) on peripheral blood basophils, monocytes, and eosinophils in atopic and nonatopic subjects: relationship to total serum IgE concentrations. *J Allergy Clin Immunol* 1997; 99:699–706.
- Djukanovic R, Lai CK, Wilson JW et al. Bronchial mucosal manifestations of atopy: a comparison of markers of inflammation between atopic asthmatics, atopic nonasthmatics and healthy controls. *Eur Respir J* 1992; 5:538–44.

- 32 Chakir J, Laviolette M, Turcotte H, Boutet M, Boulet LP. Cytokine expression in the lower airways of nonasthmatic subjects with allergic rhinitis: influence of natural allergen exposure. *J Allergy Clin Immunol* 2000; 106:904–10.
- 33 Polosa R, Ciamarra I, Mangano G et al. Bronchial hyperresponsiveness and airway inflammation markers in nonasthmatics with allergic rhinitis. *Eur Respir J* 2000; 15:30–5.
- 34 Boulet LP, Morin D, Milot J, Turcotte H. Bronchial responsiveness increases after seasonal antigen exposure in non-asthmatic subjects with pollen-induced rhinitis. *Ann Allergy* 1989; 63:114–9.
- 35 Townley RG, Ryo UY, Kolotkin BM, Kang B. Bronchial sensitivity to methacholine in current and former asthmatic and allergic rhinitis patients and control subjects. *J Allergy Clin Immunol* 1975; 56:429–42.
- 36 Annesi I, Oryszczyn MP, Neukirch F, Orvoen-Frija E, Korobaef M, Kauffmann F. Relationship of upper airways disorders to FEV1 and bronchial hyperresponsiveness in an epidemiological study. *Eur Respir J* 1992; 5:1104–10.
- 37 Laprise C, Laviolette M, Boutet M, Boulet LP. Asymptomatic airway hyperresponsiveness: relationships with airway inflammation and remodelling. *Eur Respir J* 1999; 14:63–73.
- 38 Amin K, Ludviksdottir D, Janson C et al. Inflammation and structural changes in the airways of patients with atopic and nonatopic asthma. BHR Group. *Am J Respir Crit Care Med* 2000; 162:2295–301.
- 39 Mitsunobu F, Mifune T, Hosaki Y et al. Different roles of histamine and leukotriene C4 in the airways between patients with atopic and nonatopic asthma. *J Asthma* 1998; 35:367–72.
- 40 Kotsimbos AT, Humbert M, Minshall E et al. Upregulation of alpha GM-CSF-receptor in nonatopic asthma but not in atopic asthma. *J Allergy Clin Immunol* 1997; 99:666–72.
- 41 Walker C, Bode E, Boer L, Hansel TT, Blaser K, Virchow JC Jr. Allergic and nonallergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage. *Am Rev Respir Dis* 1992; 146:109–15.
- 42 Gelder CM, Thomas PS, Yates DH, Adcock IM, Morrison JF, Barnes PJ. Cytokine expression in normal, atopic, and asthmatic subjects using the combination of sputum induction and the polymerase chain reaction. *Thorax* 1995; 50:1033–7.
- 43 Iijima H, Duguet A, Eum SY, Hamid Q, Eidelman DH. Nitric oxide and protein nitration are eosinophil dependent in allergen-challenged mice. *Am J Respir Crit Care Med* 2001; 163:1233–40.
- 44 MacPherson JC, Comhair SA, Erzurum SC et al. Eosinophils are a major source of nitric oxide-derived oxidants in severe asthma: characterization of pathways available to eosinophils for generating reactive nitrogen species. *J Immunol* 2001; 166:5763–72.
- 45 Humbert M. Does 'intrinsic' asthma exist? *Rev Mal Respir* 2000; 17:245–54.
- 46 Humbert M, Durham SR, Ying S et al. IL-4 and IL-5 mRNA and protein in bronchial biopsies from patients with atopic and nonatopic asthma: evidence against 'intrinsic' asthma being a distinct immunopathologic entity. *Am J Respir Crit Care Med* 1996; 154:1497–1504.
- 47 Humbert M, Menz G, Ying S et al. The immunopathology of extrinsic (atopic) and intrinsic (non-atopic) asthma: more similarities than differences. *Immunol Today* 1999; 20:528–33.
- 48 Thomassen MJ, Raychaudhuri B, Dweik RA et al. Nitric oxide regulation of asthmatic airway inflammation with segmental allergen challenge. *J Allergy Clin Immunol* 1999; 104:1174–82.
- 49 Menz G, Ying S, Durham SR et al. Molecular concepts of IgE-initiated inflammation in atopic and nonatopic asthma. *Allergy* 1998; 53:15–21.
- 50 Berlyne GS, Parameswaran K, Kamada D, Efthimiadis A, Hargreave FE. A comparison of exhaled nitric oxide and induced sputum as markers of airway inflammation. *J Allergy Clin Immunol* 2000; 106:638–44.
- 51 Gratziau C, Rovina N, Lignos M, Vogiatzis I, Roussos C. Exhaled nitric oxide in seasonal allergic rhinitis: influence of pollen season and therapy. *Clin Exp Allergy* 2001; 31:409–16.