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## The long-term variability of FeNO in pregnant asthmatic women with controlled asthma

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### Abstract

**Introduction:** Fractional exhaled nitric oxide (FeNO) is considered a useful, noninvasive marker of airway inflammation in asthma and allergic rhinitis. It has also been suggested that anti-inflammatory treatment guided by monitoring of exhaled NO could improve overall asthma control. However, long-term intrasubject variability of this parameter as well as the rate of its change, which can be considered clinically significant, have not been established yet.

The aim of our study was to assess the long-term variability of FeNO in pregnant asthmatic women with controlled asthma

**Material and methods:** Pregnant, non-smoking women with asthma were recruited at between 3 and 6 months of gestation. Exhaled nitric oxide (FeNO) spirometric parameters were measured, and asthma control tests (ACT) were completed at monthly visits up to delivery. The data of 26 subjects with well controlled asthma during pregnancy (ACT values within the range 20–25, normal spirometric parameters, stable treatment) were analysed. The variability of FeNO values was assessed using the variation coefficient CV (standard deviation  $\times$  100%/arithmetic mean).

**Results:** The median level of FeNO coefficient of variation (CV) was: 33.8% (range 11.3 to 121.9) in all subjects with well controlled asthma during pregnancy. There were no statistically significant differences in FeNO variability between groups of patients who had at least one measurement of FeNO higher than 50ppb (39%; 11.8–121.9%) and those with all FeNO values below 50ppb (29.9%; 11.3–71.8%), as well as between atopic (35.7%; 11.8–121.9%) and nonatopic (24.2%; 11.3–71.8%) pregnant asthmatics ( $p = 0.95$  and  $0.11$ , respectively).

**Conclusions:** High long-term variability of fractional exhaled nitric oxide values revealed in pregnant women with well controlled asthma indicates that changes in this parameter should be interpreted with caution when used for asthma treatment monitoring.

**Keywords:** FeNO, long-term variability of FeNO, pregnant asthmatic women, controlled asthma

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### Introduction

Asthma is one of the most frequent chronic diseases occurring in pregnant women, the appropriate treatment is crucial to the health of both — mother and her child. The basic anti-inflammatory drugs used in asthmatic pregnant women are inhaled glycocorticosteroids (GKS-IH). However, establishing the right, optimal dose of the drug can pose some problems. It should be high enough to ensure the control of asthma and to protect the

mother from exacerbation of the disease, but at the same time it should be low enough to produce no side effects in the mother and the foetus [1].

Therefore, an objective parameter, helpful in establishing a dose of corticosteroid, has been researched. Great hopes have been placed in a relatively new method that monitors the inflammatory process in the lungs and evaluates the concentration of exhaled nitric oxide (NO). It has been discovered that the concentration of exhaled nitric oxide in patients with asthma is proportional

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to the degree of inflammation found in the bronchial walls, eosinophilia in induced sputum and the degree of bronchial hyperreactivity. Increased concentration of NO is connected with asthma exacerbation, and its reduction closely correlates with the effectiveness of anti-inflammatory treatment used in asthma [2, 3].

Optimistic observations have been described by Powell et al. [4]. Using a therapeutic model based on the evaluation of FeNO and ACT (Asthma Control Test), researchers have shown a smaller number of asthma exacerbations and lower dose of used corticosteroids in a group of pregnant women who had had determined NO, compared to a group of pregnant women treated only on the basis of clinical symptoms. In this model successive changes in patient treatment were required when nitric oxide concentrations were higher than 16 ppb and 29 ppb. It is possible that the result was influenced by the inhomogeneity of asthma course in the two groups (in the first group 46% of patients were treated with corticosteroids and 19% with long acting beta adrenergic, while in the second group these proportions were 76% and 45%, respectively).

However, the interpretation of the measurement of nitric oxide concentrations has its limitations [5]. The natural, long-term variability of this parameter in patients with asthma is unrevealed. Nevertheless, it is known that the variability of FeNO during a week-long observation in healthy people is on average 13.9%; 4.6%-23.9%. Cut off values for this parameter have not clearly been defined so far, and the discussion about what change of exhaled nitric oxide concentration should be considered as significant continues. Recommendations published in 2011 [6] regarding the use and interpretation of FeNO in the assessment of controlled asthma suggest that the change should be considered as significant when FeNO increases by 10 ppb for the FeNO values below 50 ppb, and by 20% when FeNO exceeds 50 ppb. In view of the lack of information about the natural variability of FeNO in asthma, such arbitrarily defined thresholds may raise doubts.

The aim of the study was the prospective assessment of the long-term variability of fractional exhaled nitric oxide in pregnant asthmatic women with controlled asthma.

## Material and methods

Pregnant (between the 2<sup>nd</sup> and 6<sup>th</sup> months of gestation), adult, non-smoking women with bronchial asthma diagnosed and treated by specialists

for at least 12 months were qualified for the study. The patients were referred to the Department of Internal Medicine, Geriatrics and Allergology (Wroclaw Medical University) by specialist clinics in the years 2007–2010.

During the first visit (V1):

1. The patient's consent to the treatment program was obtained.
2. Diagnosis was verified based on medical history, physical examination and spirometry results.
3. The presence of atopy was evaluated on the basis of past sIgE testing (skin prick test) — the vesicle equal to or larger than 3 mm or positive serum sIgE tests for inhaled allergens (> 0.35 kUI/l).
4. ACT test was performed.
5. Spirometry was performed.
6. FeNO was determined.
7. Pharmacological treatment of asthma in accordance with current consensus and the rules of pharmacotherapy for pregnant women [7] was established; and the ability to use inhalers was checked.

Subsequent, planned visits took place every 4 weeks until delivery. During each visit the following factors were evaluated:

1. Degree of asthma control during the previous 4 weeks.
2. The results of the asthma control tests (ACT) performed by the patient every 7 days (ACT mean value of the month).
3. Spirometry was performed and exhaled nitric oxide was measured.

Only women with controlled asthma during the whole period of observation were taken for analysis, i.e.

- symptoms during the day occurred not more than twice a week,
- patients did not suffer any impediment in daily life due to asthma,
- there were no nocturnal symptoms,
- there was no need to use bronchodilating drugs more often than twice a week,
- there was no decrease in spirometric parameters below the predicted values [7].

Asthma control test (ACT) was performed using a standardized form including 5 questions with five-grade scale answers [8]. It is usually recommended that this test be performed once a month; in our study it was performed every week. This was due to the presence of questions concerning symptoms occurring during the week. In this way the precise evaluation of the level of asthma control during the following weeks was performed.

In order to assess minor differences in asthma control, in the studied group of patients, according to suggestions included in the GINA report [7], classification of treatment based on the dose of inhaled corticosteroid expressed as budesonide dose, was created (none of the patients was treated with long-acting b2-agonist).

The following classifications were assumed:

- 1<sup>st</sup> degree: GKS-IH < 200 mcg
- 2<sup>nd</sup> degree: 400 > GKS-IH ≥ 200 mcg
- 3<sup>rd</sup> degree: 800 > GKS-IH ≥ 400 mcg
- 4<sup>th</sup> degree: GKS-IH ≥ 800 mcg

For FeNO measurement, the set Niox (Aerocrine, Sweden) operating on the basis of on-line chemiluminescence method was used. Measurements were conducted in accordance with recommendations of the American Thoracic Society (ATS) [2], expiratory flow was maintained at the range of 0.045–0.055 l/s, and a mean value of three reproducible measurements (not differing from each other by more than 10%) was assumed as a final result. Measurement was presented in ppb (parts per billion).

According to guidelines, patients did not have any food or make any physical effort at least 4 hours before determination. The tests were run before midday (10 a.m.–12 p.m.), and spirometry was performed after determination of exhaled nitric oxide. None of the women included in the study smoked cigarettes.

According to ATS/ERS guidelines, spirometry was performed with a Master Scope spirometer manufactured by Jaeger; special attention was paid to repeatability and correctness criteria whilst running the test [9].

The test results were analysed using the Statistica 9 program. The normality of distribution was checked with the help of Shapiro-Wilk test. Due to the lack of normal distribution of the parameters studied, non-parametric tests were used in comparisons. The median and the lowest and highest value of the parameters were used to describe the data (Me; Min-Max). The comparison of two independent samples was made using the Kolmogorov-Smirnov test, and that of dependent samples, by using the Wilcoxon signed rank test.  $P < 0.05$  was assumed as statistically significant probability ( $p$ ). The variability of the FeNO parameter was assessed with the help of the variability coefficient (standard deviation  $\times$  100%/arithmetic mean). Correlation between variables was analysed using the Spearman correlation test.  $P = 0.05$  was assumed as the level of significance.

The study obtained a positive review from the Commission of Bioethics at Wroclaw Medical University, no. KB-436/2007.

## Results

Seventy-two pregnant (between the 2<sup>nd</sup> and 6<sup>th</sup> months of gestation), asthmatic, non-smoking women were qualified for the study. During observation 46 women were excluded due to periods of asthma exacerbation or changes in treatment. Finally, in accordance with the aim of this study, the following subjects were qualified for statistical analysis: 26 women mean age 30 (19–36) years, with asthma (duration of the disease 5.5; 1.0–22 years), treated with a fixed dose of inhaled corticosteroids, with a stable course of the disease (ACT result — 20–25 points), and without impaired spirometric parameters during the whole period of observation. Demographic data of the study subjects are presented in Table 1. In the study group, in accordance with the assumed criterion, based on the fixed dose of the inhaled corticosteroid used, the 1st degree of treatment was diagnosed in 5 women (19.2%), 2<sup>nd</sup> degree in 11 women (42.3%), 3rd degree in 4 patients (15.4%) and 4th degree in 6 patients (23.1%).

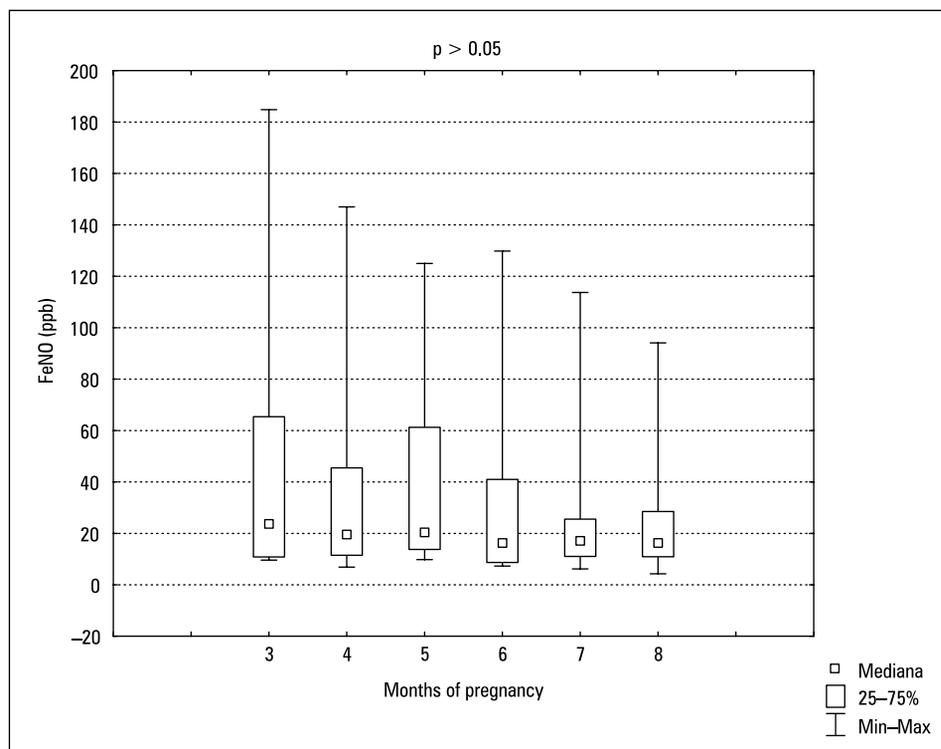
Allergic asthma (IgE-dependent) was diagnosed in 20 (76.9%) patients and allergic rhinitis in 19 (73.0%) patients. Nitric oxide concentrations during visit V1 were within a wide range of values (7.3 ppb to 184.8 ppb). Consecutive follow-up examinations were performed every 4 weeks until delivery for 4; 3-6 months.

In order to assess the dependence of exhaled NO concentration on the degree of asthma course, retrospective evaluation of the mean dose of inhaled corticosteroid (expressed as budesonide dose), applied during the last year to achieve a good asthma control, was made [7]. No statistically significant correlation between

**Table 1. Demographic data, spirometry parameters, corticosteroids doses and ACT on visit V1 (n = 26)**

Parameter	Me (min–max)
Age (years)	30 (19–36)
Duration of asthma (years)	5.5 (1–22)
FVC (% normal)	110.3 (85–130)
FEV1 (% normal)	100.3 (82–120)
FeNO (ppb)	22.7 (7.3–184.8)
Corticosteroid doses as converted to budesonide (mcg)	400 (0.0–1200.0)
Asthma Control Test — ACT	24.5 (20–25)

Abbreviations in the text



**Figure 1.** The concentration of nitric oxide in particular months of pregnancy

exhaled NO concentration and the degree of asthma treatment assessed in the above manner ( $p > 0.05$ ) was noted.

No statistically significant difference between measurements of FeNO in individual months of pregnancy was discovered; the results are presented in Figure 1. A distinct, inter-subject difference in FeNO values was observed, which was maintained in the range of 4.3 ppb to 184.8 ppb during the whole period of observation.

After analysis of the data from all visits (26 patients observed for 3–6 months during 116 visits), during which asthma control test and spirometry were carried out and exhaled nitric oxide was assessed, it was proven that exhaled nitric oxide concentration correlated barely ( $r = -0.24$ ) but statistically significantly with the asthma control test result ( $p = 0.017$ ) (Fig. 2). No significant correlation between  $FEV_1$  and FeNO ( $p = 0.2$ ) was noted.

During the observations, in all the patients studied, high variability of exhaled nitric oxide concentration was discovered. The coefficient of variability of FeNO calculated for the whole period of observation in 26 patients ranged from 11.3% (Min) to 121.9% (Max), with median (Me) = 33.8%.

The coefficient of variability did not correlate significantly with the age of the patients studied, asthma control or the dose of corticosteroids applied ( $p > 0.05$ ).

The variability of FeNO in the group of women with at least one measurement of FeNO  $> 50$  ppb was compared to the variability of FeNO in the group of women who did not exceed the threshold of 50 ppb. In the group of 9 women with at least one FeNO value above 50 ppb, the coefficient of variability of (Me; Min–Max) 39.2%; 11.8–121.9% was noted, and in the 17 remaining patients it was 29.9%; 11.3–71.8%. No statistically significant difference in the two populations studied was diagnosed ( $p = 0.95$ ).

The comparison of the coefficient of variability in the group of patients with IgE-dependent and IgE-independent asthma did not reveal statistically significant differences either (35.7%; 11.8–121.9% and 24.2%; 11.3–71.8%, respectively;  $p = 0.11$ ). These results are presented in Figure 3.

## Discussion

Despite the fact that the measurement of nitric oxide concentration has been accepted in the diagnosis and the monitoring of the course of asthma, this method still provokes lively discussion. Even the position on the interpretation of FeNO published by ATS [6] did not solve the majority of the problems. On the contrary, the arbitrariness of the majority of the proposed recommendations only encouraged further discussion.

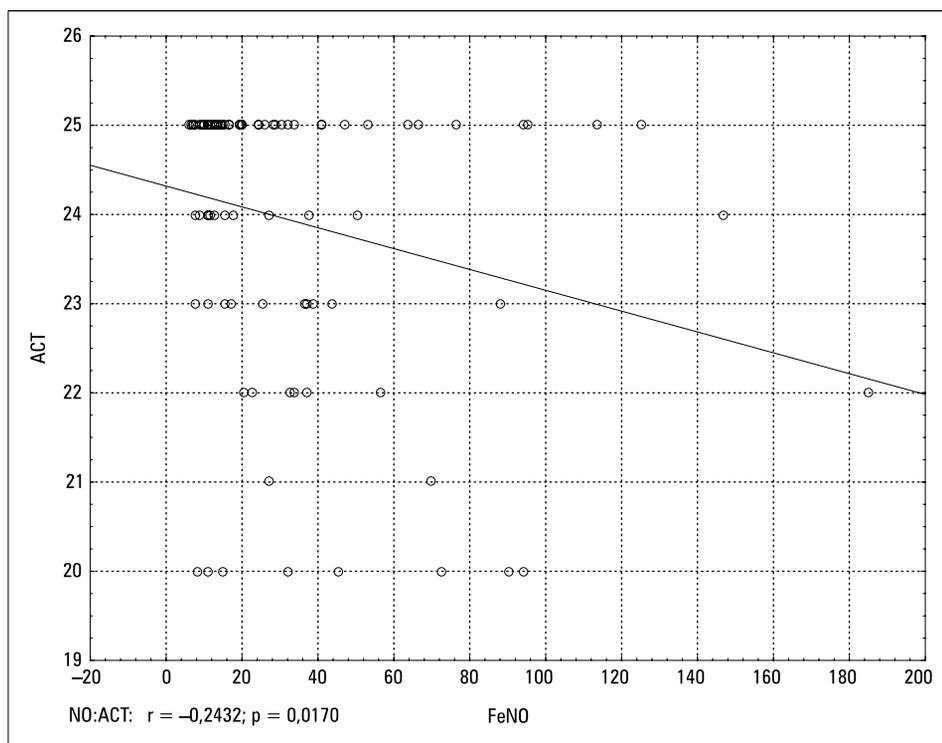


Figure 2. Rang correlation between FeNO levels and ACT

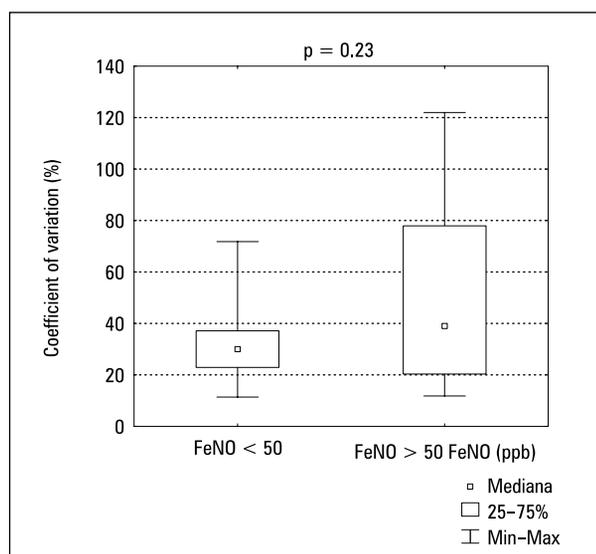


Figure 3. FeNO variability in patients with FeNO < 50 ppb i FeNO > 50 ppb

This study attempted to assess the long-term variability of exhaled nitric oxide concentration in the group of pregnant women with controlled asthma. The research to date has not proven that pregnancy itself influences exhaled nitric oxide concentration [10].

It has been proven so far that FeNO is a sensitive marker of eosinophilic inflammatory process

in the airways, with the help of which, response to corticosteroid therapy may be predicted and monitored. Therefore, we expected correlation between the degree of control and/or degree of asthma treatment with corticosteroids and exhaled NO concentration. An attempt to find the dependence of the ACT result and FeNO has shown the presence of a statistically significant but weak correlation between these parameters ( $r = -0.24$ ). Similar observations were published in 2010, and the authors also found the correlation between ACT result and exhaled nitric oxide concentration at a comparable level ( $r = -0.16$ ) [11]. Although Bernstein et al. discovered a stronger correlation between FeNO and ACT ( $r = 0.48$ ), it concerned only the group which was not treated with corticosteroids, and only in one of the two centres participating in the research [12]. Quaedvlieg et al. did not find statistically significant differences in nitric oxide concentration between asthmatic patients with different levels of asthma control [13]. Similar observations were published by Leung et al. on the basis of research conducted among a group of 113 children [14].

In the present study we did not find any relationship between the degree of asthma treatment based on the amount of the used long-term inhaled corticosteroid and exhaled NO concentration. Comparable results have been presented by Mahut et al. [15].

In this study we attempted to assess the natural variability of exhaled NO concentration during 3–6-months' observation, in patients with satisfactory asthma control, defined by physical examination, ACT and spirometry. In 26 women studied, the variability of FeNO was 33% (11.3–121.9%). Such a high variability of this parameter in controlled asthma demands caution while assuming FeNO to be an exclusive reason to change therapeutic modalities. The ninety-fifth percentile of variability is on the level of 96.5%, so merely twofold growth of nitric oxide concentration can probably be recognized as not accidental. The observed long-term variability of FeNO did not differ significantly in the group of patients with FeNO < 50 ppb and FeNO > 50 ppb; atopy did not influence it either.

As suggested in new recommendations and considered as significant, the change of exhaled NO concentration (20% for the values above 50 ppb, and 10% for the values below 50 ppb [6]) seems to be within the range of natural variability of this parameter.

### Conclusions

Concentration of FeNO is characterized by a high longitudinal variability in pregnant asthmatic women with controlled asthma, irrespective of the long-term dose of inhaled corticosteroid.

Correlation of FeNO and ACT is significant but weak.

On the basis of the above results, it seems that in the treatment of asthma in pregnant women ATS/ERS criteria concerning FeNO concentration should be taken with caution, as they may lead in some patients to decisions that are unjustified by clinical conditions.

There is a need to continue research on a bigger group of pregnant females in order to establish appropriate critical values.

### Conflict of interest

The authors declare no conflict of interests

### References:

- Namazy J.A., Schatz M. Asthma and pregnancy. *J. Allergy. Clin. Immunol.* 2011; 128: 1384–1385.
- American Thoracic Society; European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am. J. Respir. Crit. Care. Med.* 2005; 171: 912–30.
- Smith A.D., Cowan J.O., Brassett K.P. et al. Use of exhaled nitric oxide measurements to guide treatment in chronic asthma. *N. Engl. J. Med.* 2005; 352: 2163–2173.
- Powell H., Murphy V.E., Taylor D.R. et al. Management of asthma in pregnancy guided by measurement of fraction of exhaled nitric oxide: a double-blind, randomised controlled trial. *Lancet* 2011; 378: 983–990.
- Kumór M., Przybyłowski T., Maskey-Warzęchowska M. et al. Powtarzalność pomiaru stężenia tlenku azotu w powietrzu wydechowym (FeNO) przeprowadzonego z wykorzystaniem zestawu NIOX u zdrowych osób. *Pneumonol. Alergol. Pol.* 2004; 72: 283–285.
- Dweik R.A., Boggs P.B., Erzurum S.C. et al. American Thoracic Society Committee on Interpretation of Exhaled Nitric Oxide Levels (FeNO) for Clinical Applications. An official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FeNO) for clinical applications. *Am. J. Respir. Crit. Care. Med.* 2011; 184: 602–1518.
- GINA Report, Global Strategy for Asthma Management and Prevention, From the Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA) 2010. Available from: <http://www.ginasthma.org/>
- Nathan R.A., Sorkness C.A., Kosinski M. et al. Development of the asthma control test: a survey for assessing asthma control. *J. Allergy. Clin. Immunol.* 2004; 113: 59–65.
- Miller M.R., Hankinson J., Brusasco V. et al. ATS/ERS Task Force. Standardisation of spirometry. *Eur. Respir. J.* 2005 26: 319–338.
- Tamási L., Bohács A., Bikov A. et al. Exhaled nitric oxide in pregnant healthy and asthmatic women. *J. Asthma* 2009; 46: 786–791.
- Alvarez-Gutiérrez F.J., Medina-Gallardo J.F., Pérez-Navarro P. et al. Comparison of the Asthma Control Test (ACT) with lung function, levels of exhaled nitric oxide and control according to the Global Initiative for Asthma (GINA). *Arch. Bronconeumol.* 2010; 46: 370–377.
- Bernstein J.A., Davis B., Alvarez-Puebla M.J., Nguyen D., Levin L., Olaguibel J.M. Is exhaled nitric oxide a useful adjunctive test for assessing asthma? *J. Asthma* 2009; 46: 955–960.
- Quaedvlieg V., Sele J., Henket M., Louis R. Association between asthma control and bronchial hyperresponsiveness and airways inflammation: a cross-sectional study in daily practice. *Clin. Exp. Allergy* 2009; 39: 1822–1929.
- Leung T.F., Ko F.W., Sy H.Y. et al. Identifying uncontrolled asthma in young children: clinical scores or objective variables? *J. Asthma* 2009; 46: 130–156.
- Mahut B., Trinquart L., Le Bourgeois M. et al. Multicentre trial evaluating alveolar NO fraction as a marker of asthma control and severity. *Allergy* 2010; 65: 636–644.