



Inflammatory markers as exacerbation risk factors after asthma therapy switch from inhaled steroids to montelukast



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ABSTRACT

Background: Asthma guidelines allow anti-leukotriene medications to be used as an alternative to inhaled corticosteroids (ICS) in second-step intensity therapy. The aim of the study was to analyze the risk factors of exacerbations, particularly inflammatory markers, during the 12-month period following therapy reduction from an ICS to montelukast in young patients with mild asthma.

Methods: A total of 84 patients (aged 7–18 years old) with mild asthma controlled by low-dose ICS, had their treatment switched to montelukast. Exhaled nitric oxide (eNO), sputum eosinophils (sEos), and bronchial hyperreactivity (BHR) were assessed at the beginning and then every three months throughout the one-year period. The patients with asthma exacerbations (first severe or third mild) were discontinued from the study.

Results: Over the study period, 22 patients (26%) discontinued montelukast due to asthma exacerbations. An increased risk of exacerbations was noted among patients with initial sEos above 2.5% (relative risk, RR 36.6; 95% CI: 7.1–189.3; $p < 0.001$), as well as those with augmented BHR (RR 9.5; 2.8–31.6; $p < 0.001$), or eNO greater than 20 ppb (RR 3.7; 95% CI: 1.3–10.7; $p = 0.013$). An increase in BHR and eNO was observed during the last visit before exclusion.

Conclusions: After switching treatment from a low-dose ICS, montelukast maintained control of asthma symptoms in 75% of patients. High sEos before the treatment change was the strongest exacerbation risk factor. In patients with asthma controlled by low-dose ICS and low inflammatory markers, treatment could be safely switched to montelukast.

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1. Introduction

GINA 2006 and subsequent updates allow the use of montelukast as an alternative drug in the second step of asthma management [1]. According to the last GINA edition, montelukast is suitable for patients not willing or unable to use ICS, experienced important side-effects, or have concomitant allergic rhinitis [1]. Authors of the guidelines base their recommendations on studies comparing the anti-inflammatory potency of the LTRA and low-dose ICS. However, consensus PRACTALL and Australian guidelines recommend anti-leukotrienes as a good equivalent to low-dose inhaled corticosteroids [2,3]. In particular, some reports

suggest a comparable efficacy with both drugs [4,5]. Moreover, GINA experts emphasize the necessity to find lowest ICS dose to maintain control of symptoms, but tapering ICS may increase the risk of uncontrolled asthma symptoms. In recent GINA Guidelines, sputum eosinophilia and airway hyperresponsiveness are mentioned as increasing the risk of future exacerbations, even if asthma is well controlled by current treatment. In patients with persistent eosinophilic inflammation this risk may occur during the reduction of treatment, or after exposure to triggers as allergens or smoking. Other important risk factors are low FEV1, comorbidities and exposure to smoking or allergens. Additionally, complete cessation of ICS therapy is associated with increased risk of exacerbations.

However, only few step-down studies have been performed in children. Moreover, none of the current guidelines formulate specific recommendations for the switch from ICS to LTRA as a possible strategy for inflammatory treatment reduction, and there is a lack

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of data on the possible risk of such modification. Montelukast provides a good control of symptoms in the majority of mild asthma cases [6,7]. However, the data on the control of eosinophilic inflammation by montelukast is scarce and applies mainly to adults [8,9]. In *in vitro* studies, montelukast has a beneficial effect on eosinophilic inflammation in the context of chemotaxis and apoptosis [10,11], but clinical studies have demonstrated only an attenuation of inflammation [8,12,13]. Therefore, is unknown how subclinical eosinophilic inflammation translates into early and late sequelae of asthma, especially exacerbations. Additionally, there is a lack of data assessing the disease progression in the context of risk (or success) factors of exacerbations following treatment reduction in asthmatic children.

The aim of the study was to determine the risk factors of asthma exacerbations after replacing ICS treatment with LTRA in children with mild asthma. In particular, we investigated various inflammatory markers and control of airway hyperresponsiveness during the year-long follow-up period.

2. Material and methods

2.1. Subjects

The study group consisted of children and adolescents diagnosed with mild asthma for at least two years. In that period (i.e. at least one year prior to enrollment to the study), the disease activity was confirmed by the measurement of nonspecific bronchial hyperresponsiveness by a challenge with hypertonic saline after a four-week break in ICS treatment. There were no asthma exacerbation during this short ICS withdrawal. Eligible patients: maintained control of asthma for at least one year using a low dose of inhaled corticosteroid (budesonide 100 mcg or equivalent once a day); had an FEV1 greater than 80% of predicted; no exacerbation requiring systemic steroids within the last year; and no symptoms of respiratory tract infection for one month. They were selected from a pool of 133 patients with mild asthma from the Allergy Clinic of The Regional Public Hospital in Lesko (Poland). There were 14 patients that were not included due to changes in asthma symptom intensity; 7 did not receive ICS; 14 were non-compliant; and 12 refused to participate in the study.

The study was approved by the Bioethics Committee of National Institute of Tuberculosis and Lung Disorders (the approval number KE-3/2009). Parents or guardians of all patients, and patients over the age of 15 provided written consent to participate in the study prior to its commencement.

2.2. Study design and assessment of inflammation

The study consisted of an initial two-week period of clinical observation of the current treatment by low-dose ICS (running in) and 12-month clinical follow-up after the change of treatment (proper period of the study).

During the initial 2-week phase, patients continued their low-dose ICS and kept a diary of daily and nocturnal symptoms, including the use of beta-agonists, medical intervention, and measured PEFR (Personal Best) twice a day.

At the end of the initial period, patients' asthma control on low-dose ICS therapy was evaluated by the physician according to the GINA criteria and using the ACT test (version for adults and older children). Only patients with good asthma control and an ACT result of 20 points or greater were included in the switch to LTRA, and baseline values of monitored parameters were calculated.

The fraction of nitric oxide in exhaled air (eNO) was measured using an electrochemical-based Medisoft HypAir FE (NO) device (Medisoft SA, Belgium). The measurements were performed at an

expiratory flow rate of 50 mL/s and the duration of exhalation was at least 6 s to ensure a stable eNO level. Three measurements were obtained for all subjects, and the mean value was recorded as the level of eNO [14].

Next, spirometry was performed (Easy One - Medizin Technik Schweiz) according to the ATS/ERS guidelines [15], with at least three correct forced exhalations. FEV1 (forced expiratory volume in 1 s), FVC (forced vital capacity), and MMEF (maximal mid-expiratory flow) were retained for analysis.

Next, the induction of sputum and measurement of airway hyperresponsiveness to hypertonic saline was performed at a single stage using the combined method [16]. The test consisted of 8 cycles of inhalation of 4.5% sodium chloride: 30 s, 30 s, 1 min, 2 min, and then four times for 4 min. FEV1 was measured at 1 min after each cycle and sputum expectoration was attempted. The procedure was terminated after the last inhalation or earlier if the FEV1 fell by more than 15%.

The induced sputum was processed according to the method described by the Polish recommendations in Ref. [17]. The sputum was initially assessed macroscopically for plugs at the time of collection. Total cell counts were determined, and cytopsin slides were prepared. An adequate specimen was defined as one producing countable cytopsin slides for an estimation via differential cell count, with minimal squamous contamination (<50%) and pulmonary macrophages present. A differential cell count was obtained from 200 cells on May-Grunwald-Giemsa stained slides.

The dose-response slope (DRS) was calculated as the ratio of reduction of FEV1 (% of initial value) by volume of the sodium chloride inhaled (mL) [18].

Upon the completion and analysis of these tests, the anti-inflammatory treatment was changed in all patients from 100 mcg/day of budesonide to a montelukast dose of either 5 mg (children under 15) or 10 mg (children over 15).

Patients kept a diary of daily observations of asthma symptoms, use of beta-agonists and PEFR measurements performed during the 12-month follow-up period for the first three months, and then continuously for four weeks straight prior to further testing at 6, 9, and 12 months. Moreover, patients reported to their primary physician every four weeks during the first 12 weeks of the study for the evaluation of asthma control according to the GINA guidelines [1] and the ACT tests. Measurement of nitric oxide in exhaled air and spirometry were performed during these visits.

Further visits conducted every three months included a spirometry measurement, determination of nitric oxide levels, bronchial hyperresponsiveness, and induced sputum collection. During these visits, a question regarding compliance was always asked, and physicians insisted that the patient take all prescribed doses. Monthly phone interviews were also carried out with the patients' parent or guardian to inquire about the child's well-being, results of observations, and compliance.

2.3. Definitions of an exacerbation

Participation of the patient in the study was terminated upon an occurrence of the first severe exacerbation or third mild exacerbation [19]. A severe exacerbation was diagnosed in patients if there was a decreased PEFR of more than 30% of baseline during two consecutive days or need for systemic steroids (oral prednisone 1 mg/kg for seven days) prescribed by a physician in addition to beta-agonists. Patients were diagnosed with a mild exacerbations if their PEFR decrease was in between 20% and 30% of the average baseline for two consecutive days, if they needed to use more than three additional inhaled SABA doses per day, or there was an occurrence of nighttime awakenings (or early in the morning) due to asthma symptoms. Therefore, beta-agonists were given ad-hoc

and for the first two episodes, during which inhaled corticosteroids (2×200 mcg budesonide) were administered for seven days.

The primary study endpoint consisted of the inflammatory markers (sputum eosinophilia, the level of eNO, and airway hyperresponsiveness), analyzed within 12 months after the treatment modification. Secondary endpoints included the following clinical criteria: 1) symptoms score and use of beta-agonists; 2) days with asthma symptoms and medication intervention; 3) the patient's self-ACT test; 4) measurement of PEFR; and 5) spirometry (FEV1, FEV1/FVC, and MMEF).

2.4. Data analysis

Based on the initial two week period (when patients continued treatment with a low dose ICS) baseline values of inflammatory markers and BHR were calculated, as well as spirometry, PEFR, and clinical parameters.

The initial values of the parameters in patients who experienced exacerbations leading to discontinuation from the study were compared with those obtained in patients who completed the study, searching for prognostic factors of exacerbations. The initial values of the clinical parameters measured prior to the treatment reduction and at the final visit were compared in patients with exacerbations. In the group of patients who completed the 12-month study period, we analyzed inflammatory and clinical markers, comparing them to the initial values. Additionally, the change in parameters in both groups was also compared.

2.5. Statistical analysis

The mean values for study groups were compared using a Student's *t*-test or Mann-Whitney *U* test after testing for the normality. Distributions of patients were compared using a Chi-squared test. Repeated measurements were compared using the Friedman test or Wilcoxon test. The correlation of inflammatory markers was evaluated using Spearman's rank order. The ability of each inflammatory marker to discriminate between patients that were discontinued due to exacerbations and those remaining in the study was estimated by plotting receiver operating characteristic curve (ROC). On an ROC curve, the optimal test positivity threshold was determined as the point closest to "ideal point" (i.e., where both sensitivity and specificity are 1) in the upper left corner of the graph [20]. Additionally, positive predictive value (PPV, which corresponds to percent of excluded patients among those with elevated markers) and negative predictive value (NPV, percent of remaining patients among those with low values) of inflammatory markers were calculated.

All analyzes were performed using StatSoft Statistica version, Inc. (2011), version 10.

3. Results

The preliminary study included 86 patients with mild asthma aged 7–18 years (23 girls and 63 boys). There were no asthma exacerbations during the run-in period. One patient was discontinued (during the study period) because of side effects (abdominal pains), and another did not attend the subsequent visits. Table 1 summarizes the characteristics of the remaining 84 patients included for further analysis.

eNO and DRS measurements were feasible for all children at all study points. After inhalation of hypertonic saline, 11 patients (13%) experienced a decline in FEV1 between 20% and 30% of their initial value. None of the patients had a decrease in SpO2 and all recovered quickly after the administration of salbutamol.

Adequate sputum was obtained at the initial visit in 72 patients

Table 1

Description of patients included in the study.

Age (years)	14.0 (5.0)
Boys	73.3%
Atopy	97.6%
Dust mite allergy	87.2%
Age of asthma onset (years)	4.4 (3.1)
Asthma duration (years)	9.0 (4.0)
Duration of treatment with ICS (years)	6.0 (3.0)
Symptom score	1.45 $\pm$ 2.82
eNO (ppb)	21.0 (15.0)
Sputum eosinophilia (%) ^a	1.0 (4.0)
DRS	0.27 (0.40)
Rescue medication use (doses/2 weeks)	0.58 $\pm$ 1.42
Days with symptoms (/2 weeks)	0.94 $\pm$ 1.88
Days with rescue medication (/2weeks)	0.48 $\pm$ 1.15
PEFR (% predicted)	104.8 (21.0)
ACT	25.0 (1.0)
FEV1 (% predicted)	97.0 (12.5)
FEV1/FVC (%)	86.0 (13.0)
MMEF (% predicted)	86.5 (27.0)

^a n = 72.

(85.7%), and in 71.8%–90.3% of subjects during control visits.

3.1. Exacerbations and clinical features

Severe exacerbations occurred in 10 patients (11.9%) during the year-long treatment with montelukast, after a median time of 17 weeks after switching from low doses of ICS. The average rate of severe exacerbations was 0.12 of exacerbations per patient/year. There were 81 mild exacerbations in total: 3 exacerbations occurred in 12 patients (leading to discontinuation from the study, after the median time of 23.3 weeks); two exacerbations occurred in 8 patients; and 29 patients had one mild exacerbation. As a consequence, 22 patients were discontinued due to exacerbations (26.1%, 13 boys and 9 girls), according to the study protocol. The median time that elapsed between the last visit and the exclusion was 3 (IQR 2–9.8) weeks.

3.2. Clinical aspects

A total of 22 patients who were discontinued from the study due to exacerbations had a significantly shorter disease duration compared to the group which completed the study [7.7 (3.8) vs. 9.0 (4.8), $p = 0.006$], but treatment period with ICS did not differ significantly [5.0 (2.8) vs. 6.0 (4.0), $p = 0.11$]. The median ACT score at the last visit prior to exacerbation remained in the range of good asthma control [23.5 (3.0)] but was slightly lower than final values of patients who completed the study [25.0 (1.0), $p = 0.02$].

Similarly, the number of days with rescue drugs in the last measurement before the discontinuation was higher than the initial (1.9 ± 2.0 vs. 0.4 ± 0.9 , $p = 0.029$), but not significantly different than the final in patients who completed the study (0.6 ± 2.4 , $p = 0.072$). Consequently, the amount of salbutamol inhalation in the last measurement prior to exacerbation leading to discontinuation was higher than the initial (3.4 ± 3.7 vs. 0.5 ± 1.0 , $p = 0.038$) and final of the patients who completed the study (0.8 ± 2.7 , $p = 0.049$). The initial values of other clinical and spirometric indices did not differ significantly between the groups.

3.3. Inflammatory markers

An adequate amount of sputum at the beginning of the study was obtained in 16 of the 22 patients (73%), who were later discontinued because of exacerbations. Comparisons of the initial and final values between the discontinued patients and the group who

completed the study are shown in Table 2.

According to ROC curves (Fig. 1), optimal cut-off points were 2.5% for sEos, 20 ppb for NO, and 0.25 for DRS. Conferring to established cut-off points, 23 of 72 patients (32%) had eosinophilia values above 2.5% at the initial visit, elevated DRS (above 0.25) was observed in 38 patients (45%), and eNO values above 20 ppb in 42 patients (50.0%). Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and the relative risk (RR) of initial values of inflammatory markers as risk factors of exclusion due to exacerbations are shown in Table 3. Two of the 49 patients with eosinophilia $\leq 2.5\%$ were discontinued, as well as 14 out of 23 with a higher eosinophilia ($p < 0.001$).

Four of the 46 patients with low bronchial responsiveness and 18 out of 38 with a bronchial hyperresponsiveness ($p < 0.001$) were discontinued. We also discontinued 6 of the 42 patients with normal eNO and 16 out of 42 with elevated nitric oxide ($p = 0.013$).

A significant increase in eNO was observed in last measurement prior to the last exacerbation [26.0 (15.0) vs. 39.0 (31.0), $p = 0.013$], as well as in DRS [0.55 (0.53) vs. 2.06 (3.46), $p = 0.01$], but sEos did not increase significantly [5.8 (4.6) vs. 7.3 (5.0), $p = 0.13$]. [Table 2].

3.4. The outcome of inflammatory markers and hyperresponsiveness in patients remaining in the study

In the group of 62 patients (74%) treated during the 12 months on montelukast, significant changes in inflammatory markers were observed in the measurements performed every three months (Table 4 and Fig. 2).

In contrast, clinical or spirometric indices did not differ significantly between the initial and periodic measurements.

Before the reduction treatment (i.e., during treatment with low dose ICS) there was no correlation between sEos, eNO, and DRS, as well as in the exacerbation group during the entire study.

During treatment with montelukast, sEos and eNO correlated at each point of the measurement in the group without exacerbations ($p < 0.05$, Spearman rank order test: r from 0.32 to 0.48). Sputum eosinophilia was also correlated with airway hyperresponsiveness, but only after 6 and 12 months of treatment with montelukast (Spearman: $r = 0.34$, $p = 0.016$, and $r = 0.30$ and $p = 0.023$ respectively), but not after 3 and 9 months ($r = 0.16$, $p = 0.24$ and $r = 0.09$, $p = 0.46$ respectively). Airway hyperresponsiveness did not correlate with eNO at any stage of the study.

4. Discussion

In our study, inflammatory markers were analyzed in a group of young patients with mild asthma after switching the treatment from low-dose ICS to LTRA, and divided into subgroups with and

Table 2
Comparisons of the initial and final values between the discontinued patients and those who completed the study.

	Excluded	Completers	p
sEos – baseline	5.8 (4.6)	0.70 (2.0)	<0.001
final sEos	7.3 (5.0) ^a	2.0 (3.5)	<0.001
Change in sEos	2.6 (7.3)	0.5 (4.0)	0.426
DRS – baseline	0.55 (0.53)	0.22 (0.29)	<0.001
Final DRS	2.06 (3.46) ^{b*}	0.29 (0.38)	<0.001
Change in DRS	1.55 (3.33)	–0.02 (0.47)	<0.001
eNO – baseline	26.0 (15.0)	18.5 (12.0)	0.03
Final eNO	39.0 (31.0)*	24.5 (15.0)	0.03
Change in eNO	7.5 (20.0)	5.0 (21.0)	0.28

* $p < 0.05$ compared to baseline.

^a 8 patients.

^b 11 patients (excluded after 3 months).

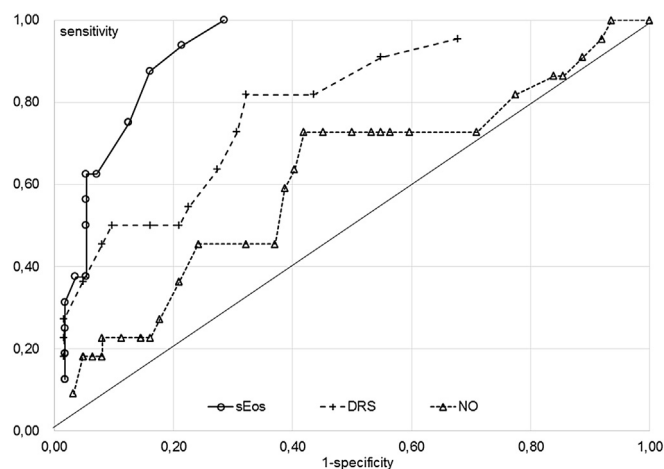


Fig. 1. Receiver operating characteristic (ROC) curves showing the relationship between specificity and sensitivity for different cut-off points of inflammatory markers. sEos – sputum eosinophils, DRS – dose – response slope, eNO – exhaled nitric oxide. Point with the greatest distance from the “chance” diagonal line represents the maximum combined value of sensitivity and specificity achieved with the marker.

Table 3
Sensitivity, specificity, positive and negative predictive values, relative risk and area under ROC for calculated cut – off points.

	Sensitivity	Specificity	PPV	NPV	RR	Area under ROC
sEos > 2.5	87.5	83.9	60.9	95.9	36.6	0.92
DRS > 0.25	81.8	67.7	47.4	91.3	9.5	0.80
eNO > 20 ppb	72.7	58.1	38.1	85.7	3.7	0.61

without asthma exacerbations. The main objective of the study was to determine the risk factors for the failure of such a change in treatment. An initial sputum eosinophilia above 2.5% and airway hyperresponsiveness (DRS > 0.25) prior the reduction of the treatment were major risk factors for exacerbations. They had high both specificity and a negative predictive value, which suggests that children on low-dose ICS with low values of the inflammatory markers can be safely subjected to a change in treatment.

Exacerbations leading to exclusion from the study were reported in a quarter of our patients; half of which occurred within the first three months [21]. This suggests that the first months after treatment reduction is the period of greatest exacerbation risk, requiring particular attention. The average rate of severe exacerbations was 0.12 per patient/year and is similar to the data of children and adults in other yearly studies on the treatment of montelukast [6,22]. However, the proportion of severe exacerbations in our study (11.9%) was about 50% lower than in 74 subjects with the placebo group in the annual study of mild asthma in children (TREXA Study, 23%) [23]. This can be explained by both the anti-inflammatory activity of montelukast received by our patients, as well as slight differences in initial asthma severity or patient exposure. In our group of patients, exacerbations were mostly experienced by patients with a shorter duration of illness. However, the initial clinical and spirometric data were not predictive of exacerbation or the age of asthma onset, contrary to that observed in adults [24].

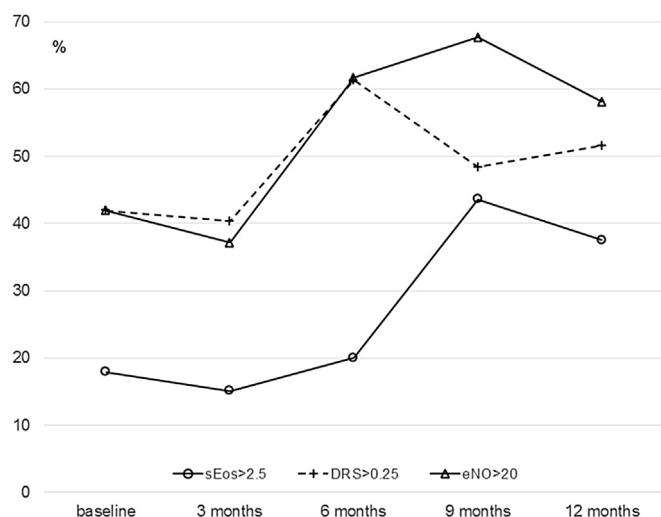
In our study, induced sputum eosinophilia before the change in treatment was a major predictor of the risk of exacerbations. High eosinophilia (above 2.5%) increased the risk of failure after the change of therapy from low-dose ICS to montelukast 36.6-fold. In addition, the percentage of eosinophils in the sputum cytology related to an increased risk of exacerbations (>2.5%), was similar to

Table 4

Changes in inflammatory markers in patients remaining in the study.

	Baseline	3 months	6 months	9 months	12 months
Patients with successful sputum induction	56 (90.3%)	53 (85.5%)	45 (72.6%)	55 (88.7%)	56 (90.3%)
Median of eosinophilia (IQR)	0.7 (2.0)	0.5 (2.0)	1.0 (2.0)	2.0 (3.8)*	2.0 (3.5)*
% of patients with sEos>2.5	17.9	15.1	20.0	43.6	37.5
Median of DRS (IQR)	0.22 (0.29)	0.19 (0.34)	0.30 (0.27)	0.23 (0.45)	0.29 (0.37)
% of patients with DRS>0.25	41.9	40.3	61.3	48.4	51.6
Median of eNO (IQR)	18.5 (12.0)	16.5 (12.8)	29.0 (24.5)**	28.0 (21.0)**	24.5 (14.5)**
% of patients with eNO>20	41.9	37.1	61.7	67.7	58.1

*p < 0.05, **p < 0.01 compared to baseline; Wilcoxon test.

**Fig. 2.** Percent of subjects with increased inflammatory markers in patients on montelukast remaining in the study (n = 62).

other studies [25]. In a group of 72 healthy children, the upper confidence limit of sputum eosinophils was 2.52% [25] and sputum eosinophils were higher in asthmatic patients than in normal children. In other reports, initial eosinophilia was also a risk factor for exacerbations after a dose reduction or discontinuation of ICS [26–29]. In a small group of 15 adults who were the subjects of an eight-week observation after ICS dose reduction, Jatakonon et al. [26] found increased baseline sputum eosinophils in the subgroup with subsequent asthma exacerbations. Similarly, DeMore et al. [27] noted that a high sputum eosinophilia promoted more severe infective symptoms after experimental rhinovirus inoculation in 20 adult asthmatics not receiving ICS. Furthermore, Giannini et al. [28] in well-controlled adult asthmatics on low-dose ICS demonstrated higher sputum eosinophilia in patients who developed asthma symptom recurrence after withdrawal of therapy for three months than in those who remained asymptomatic. In another study in adults, an increase in eosinophilia greater than 0.8% in the sputum tested two weeks after discontinuation of ICS was a highly sensitive predictor of subsequent asthma exacerbation [29].

In our study, there was no significant increase in sputum eosinophilia in the last measurement before exacerbation (Table 2). However, the observed values were significantly higher than the final sEos of patients remaining in the study. The lack of significant change in sEos before exclusion from the study can be explained by the time elapsed between the measurement and the final exacerbation. In this period, an increase in bronchial inflammation could occur, but this change was not evaluated.

In the present study, initial airway hyperresponsiveness was another important predictor of exacerbation, increasing the risk 9.5-fold. Higher DRS values (relative to baseline) were also

observed before the exacerbation. In the patients remaining in the study, montelukast protected against increases in bronchial hyperresponsiveness (Table 2), and this finding is also supported by data from the literature [30]. This means that a simple, cheap and safe provocation test with hypertonic saline can be used when deciding on the reduction of anti-inflammatory therapy (alternatively to sputum induction). Only 13% of our patients experienced a drop in FEV1 exceeding 20%, and all recovered quickly after salbutamol. Combined method of sputum induction allows to gather information about BHR and sputum cells in a single step. Additionally, repeated measurements during long-term observation can be used to monitor the risk of future asthma exacerbations.

The lack of a correlation between the initial values of airway hyperresponsiveness and sputum eosinophilia confirms they are different measures of inflammation. However, each of these indicators was an important exacerbation risk factor. Bronchial hyperresponsiveness is considered a distinct pathophysiological feature of asthma, associated to a greater extent with the infiltration of smooth muscle with mastocytes than mucosal eosinophilia [30]. It is believed that hypertonic saline challenge is a better evaluation of the impact of montelukast on the bronchial hyperresponsiveness than other indirect challenge tests (adenosine, mannitol), as the main mediators triggered in this challenge are leukotrienes [31].

The optimal cut-off point for eNO as a predictor of exacerbation was a value of 20 ppb, which is slightly lower than commonly accepted a level of 25 ppb [32]. However, 20 ppb is a cut-off level recommended by ATS for children [14], as eosinophilic inflammation is less probable in this case [33]. In our study, eNO values above 20 ppb had a relatively low positive predictive value for predicting exacerbations (38%). In the only other published study available concerning the annual montelukast treatment in children, baseline eNO was not a risk factor for exacerbation [22]. In other research models (reduction or discontinuation of ICS), the initial measurement of eNO had no value in predicting exacerbations in children [33,34] or adults [26]. However, in our study, we noted an increase of eNO before exacerbation in relation to the initial value, and this observation is in agreement with other published literature [33–35].

No correlation was found between the initial values of sEos and eNO during ICS treatment, which may mean that a single, simple measurement of eNO has much less practical value and cannot replace the measurement of sputum eosinophilia during ICS dose reduction. In our study, 62% of patients with initial values of eNO greater than 20 ppb did not develop exacerbations. A similar observation was carried out by Zacharasiewicz et al. in more than 30% of a group of 40 children, observed during the ICS dose reduction [34]. Furthermore, they found that low levels of eNO (contrary to the absence of sputum eosinophilia) were also not always a guarantee of the successful treatment reduction as 20% of their patients with low eNO lost control of asthma. Therefore, 14% of asthmatics with low initial eNO were excluded from our study. Similarly, in a prospective 1.5-year study by Gelb et al. [36], 9 of 27

adults with moderate to severe asthma (33%) receiving ICS developed asthma exacerbation despite the low eNO levels (<28 ppb).

In the majority of our patients (3/4 of the whole group), montelukast provided good control of asthma symptoms during the one-year observation period. In the same way, the initial and final clinical and spirometric parameters did not undergo major changes in the patients remaining in the study. Despite this, during the one year of observation after switching treatment to montelukast, a gradual increase of inflammatory markers was observed (eNO, sEos) after the first three months. In particular, induced sputum eosinophilia increased significantly in the third and fourth quarter, which may constitute a potential risk of loss of control and/or exacerbations in the future and requires further observation.

Previous studies evaluating the effect of montelukast on eosinophilic inflammation are short-term observations in select groups of adults with initially elevated eosinophilia, and not previously treated with ICS. In such patients, only a reduction in eosinophilic inflammation was observed, without normalization of sEos [8,12,13]. In contrast to these observations, 84% of the patients remaining in our study had an initial eosinophilia below the established cut-off value. It seems that only the accumulation of pro-inflammatory factors (i.e., allergens or infections) caused that selective and relatively weak anti-inflammatory activity of montelukast, became insufficient after a few months. This suggests that the eosinophilic airway inflammation after switching therapy from ICS to montelukast increases more slowly than in other test models (e.g., cessation or rapid reduction in the dose of ICS). The earliest, clear and reproducible increase was observed for eNO, another common marker of inflammation. Following the treatment with montelukast, sEos correlated with nitric oxide. Therefore, an early increase in eNO may be a more rapid method of detecting uncontrolled inflammation than the rise in sputum eosinophilia. This may indicate that in the long-term follow-up of patients treated with LTRA, serial measurement of eNO could replace the time-consuming study of sEos. The reasons for the relatively low correlation coefficient (below 0.50) can be complex. First, sputum eosinophilia originates from the central airways, contrary to eNO reflecting inflammation also in the distal airways, which is the location related to mild asthma [37]. Secondly, some studies indicate that patients with increased eNO in exhaled air represent a distinct Th2-dependent subphenotype involving IL-4 and IL-13 in the inflammatory pathway [38]. Moreover, the blood and sputum eosinophilia are associated with increased IL-5 activity [39]. Interestingly, elevated eNO was also observed in non-eosinophilic asthma responding to ICS [40]. Furthermore, elevated values of eNO, unlike sEos, can also depend on constitutional factors not related directly to asthma activity. However, weak effects of anti-leukotriene drugs on the measured values of eNO were found in two annual studies in both children and adults [22,41] compared to ICS.

The following were notable limitations of this study: 1) this was an open, prospective observational study, carried out in a single center; 2) the patients' compliance was determined using patient self-report only; 3) a power calculation before the study was not possible because of the lack of previous relevant data in children; 4) we initially included 84 patients to the study, but our analysis was based on only 22 exacerbation; and 5) although the primary endpoint of the study was a comparison of inflammatory markers in the subgroups of patients with and without exacerbations, the lack of a placebo group made it difficult to interpret the changes.

5. Conclusion

Taken together, successful switching from low doses of ICS to montelukast in mild asthma required both a good control of clinical

symptoms as well as overall asthmatic inflammation. Subclinical eosinophilic inflammation was a risk factor for future exacerbations, with induced sputum eosinophilia of 3% or more before the treatment switch had the greatest predictive value. A similar but smaller value was noted in the measurement of airway hyper-responsiveness by an indirect method. Montelukast only partially and selectively controlled the airway inflammation that extends from the second quarter after a change of treatment. In addition, nitric oxide was an early marker of subclinical inflammation, which correlated with sEos in the course of treatment with montelukast. Therefore, our findings indicate that young patients with asthma controlled by low-dose ICS and negative results of inflammatory marker measurements (i.e., sEos, BHR, and NO), treatment can be safely reduced from ICS to montelukast.

Competing interest

H.Mazurek received honoraria and travel grant from Chiesi, and honoraria from AstraZeneca, GSK Commercial, Sandoz Polska, Teva, LEK-AM and MSD.

J.Ciótkowski, B.Stasiowska and P.Hydzyk declared no conflict of interest.

Author contributions

JC: conception and design of the study, acquisition and interpretation of data, drafting the article, final approval of the version.

HM: conception and design of the study, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, final approval of the version.

PH: analysis and interpretation of data, revising text for important intellectual content, final approval of the version.

BS: acquisition of data, revising text for important intellectual content, final approval of the version.

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References

- [1] Global Strategy for Asthma Management and Prevention www.ginasthma.org.
- [2] L.B. Bacharier, A. Boner, K.H. Carlsen, P.A. Eigenmann, T. Frischer, M. Gotz, P.J. Helms, L. Hunt, A. Liu, N. Papadopoulos, T. Platts – Mills, P. Pohunek, F.E. Simons, E. Valovirta, U. Wahn, J. Wildhaber, Diagnosis and treatment of asthma in childhood: a PRACTALL consensus report, *Allergy* 63 (2008) 5–34.
- [3] Asthma Management Handbook, National Asthma Council Australia, Melbourne, 2006. www.nationalasthma.org.au/hanbook.
- [4] D. Price, S.D. Musgrave, L. Shephstone, E.V. Hillyer, E.J. Sims, R.F. Gilbert, E.F. Juniper, J.G. Ayres, L. Kemp, A. Blyth, E.C. Wilson, S. Wolfe, D. Freeman, H.M. Mugford, J. Murdoch, I. Harvey, Leukotriene antagonists as first-line or add-on asthma-controller therapy, *N. Engl. J. Med.* 364 (18) (2011) 1695–1707.
- [5] R.A. McIvor, A. Kaplan, C. Koch, J.S. Sampalis, Montelukast as an alternative to low-dose inhaled corticosteroids in the management of mild asthma (the SIMPLE trial): an open-label effectiveness trial, *Can. Respir. J.* 16 (Suppl. A) (2009) 11A–21A.
- [6] M.L. Garcia Garcia, U. Wahn, L. Gilles, A. Swern, C.A. Tozzi, P. Polos, Montelukast, compared with fluticasone, for control asthma among 6- to 14-year old patients with mild asthma: the MOSAIC study, *Pediatrics* 116 (2) (2005) 360–369.
- [7] S.P. Peters, N. Anthonisen, M. Castro, J.T. Holbrook, C.G. Irvin, L.J. Smith, R.A. Wise, Randomized comparison of strategies for reducing treatment in mild persistent asthma, *N. Engl. J. Med.* 356 (2007) 2027–2039.
- [8] E. Pizzichini, J.A. Leff, T.F. Reiss, L. Hendele, L.P. Boulet, L.X. Wei, A.E. Efthimiadis, J. Zhang, F.E. Hargreave, Montelukast reduces airway eosinophilic inflammation in asthma: a randomized, controlled trial, *Eur. Respir. J.*

- 14 (1) (1999) 12–18.
- [9] C.F. Ramsay, P. Sullivan, M. Gizycki, D. Wang, A.S. Swern, N.C. Barnes, T.F. Reiss, P.K. Jeffery, Montelukast and bronchial inflammation in asthma: a randomised, double-blind placebo-controlled trial, *Respir. Med.* 103 (7) (2009) 995–1003.
 - [10] L. Hemelaers, M. Henket, J. Sele, F. Bureau, R. Louis, Cysteinyll-leukotrienes contribute to sputum eosinophil chemotactic activity in asthmatics, *Allergy* 61 (1) (2006) 136–139.
 - [11] O. Abadoglu, D. Mungan, O. Aksu, S. Ereku, Z. Misirligil, The effect of montelukast on eosinophil apoptosis: induced sputum findings of patients with mild persistent asthma, *Allergol. Immunopathol. Madr.* 33 (2) (2005) 105–111.
 - [12] L. Jayaram, E. Pizzichini, C. Lemi re, S.F. Man, A. Cartier, F.E. Hargreave, M.M. Pizzichini, Steroid naive eosinophilic asthma: anti-inflammatory effects of fluticasone and montelukast, *Thorax* 60 (2) (2005) 100–105.
 - [13] K. Minoguchi, Y. Kohno, H. Minoguchi, N. Kihara, Y. Sano, H. Yasuhara, M. Adachi, Reduction of eosinophilic inflammation in the airways of patients with asthma using montelukast, *Chest* 121 (3) (2002) 732–738.
 - [14] ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, *Am. J. Respir. Crit. Care Med.* 171 (2005) 912–930.
 - [15] M.R. Miller, J. Hankinson, V. Brusasco, F. Burgos, R. Casaburi, A. Coates, R. Crapo, P. Enright, C.P.M. van den Gr nten, P. Gustaffson, R. Jensen, D.C. Johnson, N. ManIntyre, R. McKay, D. Navajas, O.F. Pedersen, R. Pellegrino, G. Viegi, J. Wagner, Standardisation of spirometry, *Eur. Respir. J.* 26 (2005) 319–338.
 - [16] P.G. Gibson, J.L. Simpson, R. Hankin, H. Powell, R.L. Henry, Relationship between induced sputum eosinophils and the clinical pattern of childhood asthma, *Thorax* 58 (2003) 116–121.
 - [17] B. Chmielewicz, A. Obojski, A. Barczyk, E. Sozańska, Z. Kycler, U. Demkow, Methodology of induced sputum induction and processing – recommendation of polish respiratory society, *Pneumonol. Alergol. Pol.* 76 (2008) 378–395.
 - [18] J.D. Leuppi, S.D. Anderson, J.D. Brannan, E. Belousova, H.K. Reddel, L.T. Rodwell, Questionnaire responses that predict airway response to hypertonic saline, *Respiration* 72 (2005) 52–60.
 - [19] R.A. Pauwels, C.G. Lofdahl, D.S. Postma, A.E. Tattersfield, P. O’Byrne, P.J. Barnes, A. Ullman, Effect of inhaled formoterol and budesonide on exacerbations of asthma. formoterol and corticosteroids establishing therapy (FACET) international study group, *N. Engl. J. Med.* 337 (1997) 1405–1411.
 - [20] S. Ollier, J. Osman, D.A. Hordle, M. Amin, B. Overell, R.J. Davies, Skin-prick test preparation of *Dermatophagoides pteronyssinus* for prediction of a positive response to provocation testing, *Clin. Exp. Allergy* 19 (4) (1989) 457–462.
 - [21] J. C  łkowski, H. Mazurek, B. Stasiowska, Evaluation of step-down therapy from an inhaled steroid to montelukast in childhood asthma, *Allergologia Immunopathol.* 42 (2014) 282–288.
 - [22] C.A. Sorkness, R.F. Lemanske Jr., D.T. Mauger, S.J. Boehmer, V.M. Chinchilli, F.D. Martinez, R.C. Strunk, S.J. Szeffler, R.S. Zeiger, L.B. Bacharier, G.R. Bloomberg, R.A. Covar, T.W. Guilbert, G. Heldt, G. Larsen, M.H. Mellon, W.J. Morgan, M.H. Moss, J.D. Spahn, L.M. Taussig, Long-term comparison of 3 controller regimens for mild-moderate persistent childhood asthma: the pediatric asthma controller trial, *J. Allergy Clin. Immunol.* 119 (1) (2007) 64–72.
 - [23] F.D. Martinez, V.M. Chinchilli, W.J. Morgan, S.J. Boehmer, F.R. Lemanske, D.T. Mauger, R.C. Strunk, S.J. Szeffler, R.S. Zeiger, L.B. Bacharier, E. Bade, R.A. Covar, N.J. Friedman, T.W. Guilbert, H. Heidarian – Raissy, H.W. Kelly, J. Malka – Rais, M.H. Mellon, C.A. Sorkness, L. Taussig, Use of beclomethasone dipropionate as rescue treatment for children with mild persistent asthma (TREXA): a randomised, double-blind, placebo-controlled trial, *Lancet* 377 (2011) 650–657.
 - [24] M.B. Drummond, S.P. Peters, M. Castro, J.T. Holbrook, C.G. Irvin, L.J. Smith, R.A. Wise, E.A. Sugar, Risk factors for montelukast treatment failure in step-down therapy for controlled asthma, *J. Asthma* 48 (10) (2011) 1051–1057.
 - [25] Y. Cai, K. Carty, R.L. Henry, P.G. Gibson, Persistence of sputum eosinophilia in children with controlled asthma when compared with healthy children, *Eur. Respir. J.* 11 (1998) 848–853.
 - [26] A. Jatakanon, S. Lim, P.J. Barnes, Changes in sputum eosinophils predict loss of asthma control, *Am. J. Respir. Crit. Care Med.* 161 (2000) 64–72.
 - [27] J.P. DeMore, E.H. Weisshaar, R.F. Vrtis, C.A. Swenson, M.D. Evans, A. Morin, E. Hazel, J.A. Bork, S. Kakumanu, R. Sorkness, W.W. Busse, J.E. Gern, Similar colds in subjects with allergic asthma and nonatopic subjects after inoculation with rhinovirus-16, *J. Allergy Clin. Immunol.* 124 (2) (2009) 245–252.
 - [28] D. Giannini, A. Di Franco, S. Cianchetti, E. Bacci, F.L. Dente, B. Vagaggini, P.L. Paggiaro, Analysis of induced sputum before and after withdrawal of treatment with inhaled corticosteroids in asthmatic patients, *Clin. Exp. Allergy* 30 (12) (2000) 1777–1784.
 - [29] A. Deykin, S.C. Lazarus, J.V. Fahy, M.E. Wechsler, H.A. Boushey, V.M. Chinchilli, T.J. Craig, E. Dimango, M. Kraft, F. Leone, R.F. Lemanske, R.J. Martin, G.R. Pesola, S.P. Peters, C.A. Sorkness, S.J. Szeffler, E. Israel, Sputum eosinophil counts predict asthma control after discontinuation of inhaled corticosteroids, *J. Allergy Clin. Immunol.* 115 (4) (2005) 720–727.
 - [30] P. Bradding, A.F. Walls, S.T. Holgate, The role of the mast cell in the pathophysiology of asthma, *J. Allergy Clin. Immunol.* 117 (6) (2006) 1277–1284.
 - [31] S. Kazani, J. Sadeh, S. Bunga, M.E. Wechsler, E. Israel, Cysteinyll leukotriene antagonism inhibits bronchoconstriction in response to hypertonic saline inhalation in asthma, *Respir. Med.* 105 (2011) 667–673.
 - [32] J.E. Knuffman, C.A. Sorkness, R.F. Lemanske Jr., D.T. Mauger, S.J. Boehmer, F.D. Martinez, L.B. Bacharier, R.C. Strunk, S.J. Szeffler, R.S. Zeiger, L.M. Taussig, Phenotypic predictors of long-term response to inhaled corticosteroid and leukotriene modifier therapies in pediatric asthma, *J. Allergy Clin. Immunol.* 123 (2009) 411–416.
 - [33] M.W. Pijnenburg, W. Hoffhuis, W.C. Hop, J.C. De Jongste, Exhaled nitric oxide predicts asthma relapse in children with clinical asthma remission, *Thorax* 60 (3) (2005) 215–218.
 - [34] A. Zacharasiewicz, N. Wilson, C. Lex, E.M. Erin, A.M. Li, T. Hansel, A. Bush, Clinical use of noninvasive measurements of airway inflammation in steroid reduction in children, *Am. J. Respir. Crit. Care Med.* 171 (2005) 1077–1082.
 - [35] R.J. van der Valk, E. Baraldi, G. Stern, U. Frey, J.C. de Jongste, Daily exhaled nitric oxide measurements and asthma exacerbations in children, *Allergy* 67 (2) (2012) 265–271.
 - [36] A.F. Gelb, C. Flynn Taylor, C.M. Shinar, C. Gutierrez, N. Zamel, Role of spirometry and exhaled nitric oxide to predict exacerbations in treated asthmatics, *Chest* 129 (6) (2006) 1492–1499.
 - [37] J.H. Van Veen, P.J. Sterk, R. Schot, S.A. Gauw, K.F. Rabe, E.H. Bel, Alveolar nitric oxide versus measures of peripheral airway dysfunction in severe asthma, *Eur. Respir. J.* 27 (2006), 961–956.
 - [38] J. Corren, R.F. Lemanske, N.A. Hanania, P.E. Korenblat, M.V. Parsey, J.R. Arron, J.M. Harris, H. Scheerens, L.C. Wu, Z. Su, S. Mosesova, M.D. Eisner, S.P. Bohen, J.G. Matthews, Leukotriene treatment in adults with asthma, *N. Engl. J. Med.* 365 (12) (2011) 1088–1098.
 - [39] P. Haldar, C.E. Brightling, B. Hargadon, S. Gupta, W. Monteiro, A. Sousa, R.P. Marshall, P. Bradding, R.H. Green, A.J. Wardlaw, I.D. Pavord, Mepolizumab and exacerbations of refractory eosinophilic asthma, *N. Engl. J. Med.* 360 (10) (2009) 973–984.
 - [40] D.C. Cowan, J.O. Cowan, R. Palmay, A. Williamson, D.R. Taylor, Effects of steroid therapy on inflammatory cell subtypes in asthma, *Thorax* 65 (5) (2010) 384–390.
 - [41] H.A. Boushey, C.A. Sorkness, T.S. King, S.D. Sullivan, J.V. Fahy, S.C. Lazarus, V.M. Chinchilli, T.J. Craig, E.A. Dimango, A. Deykin, J.K. Fagan, J.E. Fish, J.G. Ford, M. Kraft, R.F. Lemanske Jr., F.T. Leone, R.J. Martin, E.A. Mauger, G.R. Pesola, S.P. Peters, N.J. Rollings, S.J. Szeffler, M.E. Wechsler, E. Israel, Daily versus as-needed corticosteroids for mild persistent asthma, *N. Engl. J. Med.* 352 (2005) 1519–1528.