Safety and application of induced sputum analysis in childhood asthma

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Background: The value of sputum induction in pediatric asthma lies in its potential to directly and noninvasively assess airway inflammation in children, because bronchoscopy and biopsy carry some risk. The Childhood Asthma Management Program (CAMP) study was designed to evaluate the long-term effects of budesonide and nedocromil compared with placebo in children with mild to moderate asthma across 8 centers.
Objective: At the Denver CAMP site, we sought to evaluate the safety of sputum induction, to determine differences in airway inflammation between treatment groups by using induced sputum analysis, and to examine correlations between other biomarkers and sputum eosinophils.
Methods: Sputum induction was performed, and exhaled nitric oxide, circulating eosinophil counts, and serum eosinophil cationic protein were obtained at treatment discontinuation and after washout. Spirometry and a methacholine challenge were also performed according to the CAMP protocol.
Results: Ninety of 117 children provided an adequate sputum sample for analysis. In 9 subjects (3 nedocromil and 6 placebo), sputum induction resulted in bronchospasm. These subjects had greater disease severity, as measured by a lower median prebronchodilator FEV_1 percentage predicted (85.0% vs 96.0%; $P = .024$) and FEV_1/FVC ratio (70.0% vs 79.0%; $P = .0008$); greater bronchodilator reversibility (16.5% vs 6.8%; $P = .004$); higher serum IgE (1390.0 vs 495.0 ng/mL; $P = .017$) and circulating eosinophil count (757.0 vs 282.0/mm^3; $P = .04$); greater use of prednisone (1.9 vs 0.9 courses per 100 person-years; $P = .05$); and greater supplemental inhaled steroid doses (85.3 vs 0 mg; $P = .016$). At treatment discontinuation, budesonide-treated patients had a lower median (1st, 3rd quartile) sputum percentage eosinophil (SPEos) (0.2% [0%, 1.2%] vs 0.8% [0.2%, 4.6%]; $P = .03$) compared with those treated with placebo; no significant difference was noted between nedocromil- and placebo-treated patients. Higher SPEos at the time of treatment discontinuation was associated with asthma worsening that required rescue prednisone (n = 23) during the washout period compared with patients who remained stable (3.6% [0.4%, 6.4%] vs 0.6% [0.2%, 3.2%] SPEos; $P = .023$). Finally, greater SPEos was associated with atopy, higher bronchodilator reversibility, lower FEV_1/FVC ratio, higher exhaled nitric oxide levels, circulating eosinophils, sputum and serum eosinophil cationic protein, more prednisone courses during the treatment period, and greater asthma severity.
Conclusions: Sputum induction is a relatively noninvasive and safe procedure that can provide information on eosinophilic inflammation and treatment response and is also associated with several measures of asthma control. However, this procedure still remains a research tool in asthma because of its requirements for technical expertise. (J Allergy Clin Immunol 2004;114:575-82.)

Key words: *Airway inflammation, asthma, eosinophils, exhaled nitric oxide, induced sputum, inflammatory markers, pulmonary function*

Sputum induction has been used as a surrogate measure of airway inflammation. However, only a few studies have evaluated the value of induced sputum (IS) analysis in children with asthma. Higher sputum eosinophils have been reported in children with controlled or uncontrolled asthma compared with healthy subjects, even in the presence of normal lung function.[1,2] The utility of sputum eosinophils as a biomarker for use in adult asthma management was recently demonstrated by Green et al.[3] These investigators sought to determine whether a management strategy that minimized sputum eosinophils would reduce asthma exacerbations compared with a standard management strategy. Fewer severe exacerbations and fewer hospitalizations were found in the sputum management group versus the standard management group, with no increased steroid burden.

In children with mild to moderate asthma who were enrolled in the Denver site of the Childhood Asthma Management Program (CAMP), we recently demonstrated the clinical utility of exhaled nitric oxide (FE_{NO}) on the basis of associations between FE_{NO} and other markers of disease activity, such as total eosinophil count (TEC), bronchodilator reversibility, atopy, and severity.[4] CAMP is a randomized, double-blind, double-dummy, controlled study that was designed to evaluate the long-term efficacy and safety of budesonide (200 μg) and nedocromil (8 mg) twice daily over placebo across 8 centers in the United States and Canada.[5] In this study, we sought to evaluate the safety of sputum induction, to

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Abbreviations used
CAMP: Childhood Asthma Management Program
ECP: Eosinophil cationic protein
FE_{NO}: Exhaled nitric oxide
FVC: Forced vital capacity
IS: Induced sputum
PEF: Peak expiratory flow
SPEos: Sputum percentage eosinophil
TEC: Total eosinophil count

determine differences in airway inflammation between treatment groups by using IS analysis, and to examine correlations between other biomarkers and sputum eosinophils at discontinuation of treatment and after washout.

METHODS

Patients at the Denver site of the CAMP study were invited to participate in this ancillary study that included the end-of-treatment visit and a second visit 2 to 4 months after discontinuation of their study medication. The full study report contains further details about the trial design.[5] Procedures for this ancillary study were performed in the following sequence: FE_{NO} determinations, phlebotomy for TEC and serum eosinophil cationic protein (ECP), baseline spirometry with prebronchodilator and postbronchodilator measurements, and sputum induction. A methacholine challenge test was performed at a separate visit during the washout period as part of the scheduled CAMP visits. All these procedures were completed before the study medication was unblinded. Exclusion criteria included pregnancy and children unable to come for both visits. The protocol was approved by the National Jewish Medical and Research Center Institutional Review Board and the CAMP Steering Committee. Written, informed consent was obtained from all patients, the parent, or both.

Clinical features

Clinical indices such as demographic data, skin prick test results, current symptoms, pulmonary function measurements, physician assessment, and additional treatment were derived from the CAMP database. On the basis of the CAMP questionnaire, the patients/parents reported on β_2-agonist use and nocturnal symptoms since the last follow-up visit as follows: A = daily or nightly; B = at least once a week, but not daily or nightly; C = at least once a month, but not weekly; D = at least once, but not monthly; or E = never. Physician assessment was reported as follows: A = active, mild; B = active, moderate; C = active, severe; D = in remission; E = others. Adjacent visits were scheduled at a minimum of 61 days and averaged every 4 months. The patients' original study medication and medication history during the CAMP study period were also noted.

Procedures

A full description of the FE_{NO} measurements at a constant expiratory flow rate of 50 mL/s, TEC, and serum ECP has been previously reported.[4] Procedures for spirometry using the Collins Stead-Wells dry-seal Survey III device (S&M Instrument, Doylestown, Pa) interfaced to a computer were performed according to American Thoracic Society standards.[6] Spirometry was performed at least 4 hours after the last use of a short-acting bronchodilator and 24 hours after the last use of a long-acting bronchodilator. Prebronchodilator and postbronchodilator readings after 15 minutes (using 2 inhalations or 180 μg of albuterol by metered dose inhaler) were expressed in both liters and as percentage predicted FEV_1 and FEV_1/forced vital capacity (FVC) with corrections for age, sex, height, and race. Bronchodilator reversibility was calculated [(postbronchodilator FEV_1 − prebronchodilator FEV_1)/prebronchodilator FEV_1 × 100)]. Methacholine challenges were performed by administering doubling concentrations of methacholine by using the Wright nebulizer/tidal breathing technique.[6] The dose that induced a 20% decrease from baseline FEV_1 was obtained by linear interpolation of the logarithmic dose-response curve (PC_{20}). The PC_{20} results obtained at the fourth year of treatment (4-20 months before discontinuation of the study treatment) and during washout were used in this study.

Sputum induction

Subjects undergoing sputum induction received an additional 2 inhalations (90 μg per puff) of albuterol after receiving 2 inhalations for the CAMP study's spirometric procedure. Only subjects with at least 70% predicted FEV_1 after 360 μg of albuterol were allowed to undergo sputum induction.

A 12-minute sputum induction was then started. Nebulized sterile 3% saline was delivered via a Devilbiss ultrasonic nebulizer (Devilbiss Health Care, Somerset, Pa) at 2.5 mL/min. Inhalation of hypertonic saline was interrupted every 2 minutes so that the subjects could expectorate all secretions into clean plastic containers: one for saliva and another for sputum. To monitor for possible bronchospasm, peak expiratory flow (PEF) measurements with a Wright peak flowmeter were performed every 2 minutes after collection of the sputum. If the PEF was <80% of the postalbuterol baseline PEF value, the subject underwent spirometry, and if FEV_1 was also <80% of the postalbuterol baseline value, the procedure was terminated. The subject then received at least 2 inhalations of albuterol, and spirometry was again performed 15 minutes later.

After collection, an equal volume of dithiothreitol 10% (Sputolysin, Behring Diagnostic Inc, Somerville, NJ) was added to the IS. The samples were then gently vortexed and placed in a shaking water bath at 37°C for 15 minutes to ensure adequate homogenization. A differential cell count was performed with 1 mL of sputum.[7] The remainder was then spun at 1000 rpm for 5 minutes, aliquoted in 1-mL volumes, and frozen at −70°C. ECP in IS was measured by using the Pharmacia CAP system, a fluorescence enzyme immunoassay (Pharmacia Diagnostics AB, Uppsala, Sweden).

An IS sample was considered inadequate if sputum induction was tolerated for less than 4 minutes, IS volume was <1 mL, or the squamous cell percentage was >80%.[8] Patients who did not produce an adequate sputum sample or those who did not tolerate the procedure during the first visit did not undergo a second sputum induction. If a patient tolerated sputum induction for more than 4 min but less than 12 min at the first closeout visit, then the duration of sputum induction at the second visit was identical to that at visit 1.

Statistical analysis

Analyses to determine significant differences between groups were based on intention to treat (placebo vs nedocromil and placebo vs budesonide) by using Wilcoxon rank-sum and χ^2 tests. Data from the budesonide placebo and nedocromil placebo groups were combined after preliminary analysis showed comparable baseline characteristics and outcomes. Multivariate analysis was performed with the Spearman rank correlation. Data were analyzed with JMP software version 5.0 and SAS software version 6.12 (SAS Institute, Cary, NC). The data are presented as median (1st, 3rd quartiles)

TABLE I. Characteristics of patients in the Denver CAMP ancillary study with and without bronchospasm during sputum induction

Variable	Without bronchospasm (n = 108)	With bronchospasm (n = 9)	*P* value*
Male (%)	61/106 (58%)	4/9 (44%)	.5†
Age (y)	13.00 (11.00, 15.00)	14.00 (12.00, 15.00)	.3
Treatment, n (%)			.16†
Budesonide	30 (27.8)	0	
Nedocromil	33 (30.6)	3 (33%)	
Placebo	45 (41.7)	6 (67%)	
Physician assessment of asthma severity (% moderate)	23 (21.3%)	6 (66.7%)	.002†
Bronchodilator rescue at least once weekly, %	39 (36.1%)	6 (66.7%)	.07†
Nocturnal symptoms at least once weekly, %	3 (2.8%)	0 (0)	.6†
Age at asthma diagnosis (y)	4.00 (2.00, 6.00)	4.00 (2.50, 7.25)	.4
Duration of asthma (y)	4.75 (3.00, 7.00)	5.40 (3.50, 7.00)	.65
Number of positive skin prick tests	3.00 (1.00, 4.00)	4.00 (2.50, 6.50)	.09
Emergency room visits/100 person-years	0 (0, 0.20)	0.19 (0, 0.47)	.2
Hospitalizations/100 person-years	0 (0, 0)	0 (0, 0.08)	.9
Prednisone courses/100 person-years	0.91 (0.35, 1.97)	1.92 (1.18, 2.18)	.05
Total prednisone dose during trial (mg)	810.00 (275.00, 1747.50)	1710.00 (1067.50, 2032.50)	.04
Total days on prednisone during trial	21.00 (8.00, 48.00)	50.00 (26.50, 62.50)	.04
Total study drug budesonide dose in trial (mg)	0 (0, 571.30)	0 (0, 0)	.07
Total supplemental (inhaled corticosteroid) dose during trial (mg)	0 (0, 151.45)	85.34 (31.75, 323.82)	.016
Prebronchodilator FEV_1 (% predicted)	96.00 (89.00, 105.00)	85.00 (78.50, 96.00)	.024
Prebronchodilator FVC (% predicted)	108.00 (99.25, 114.75)	116.00 (97.50, 122.00)	.38
Prebronchodilator FEV_1/FVC (%)	79.00 (74.00, 84.00)	70.00 (66.50, 73.00)	.0008
Bronchodilator reversibility (%)‡	6.77 (4.30, 10.71)	16.50 (10.49, 24.28)	.004
FEV_1 PC_{20} (mg/mL)‡	2.05 (0.75, 5.19)	1.00 (0.39, 2.06)	.12
Serum IgE (ng/mL)‡	495.00 (183.00, 8897.00)	1390.00 (598.00, 3703.00)	.017
Total circulating eosinophil count (/mm3)	282.00 (158.00, 458.00)	757.00 (246.50, 871.50)	.04
Exhaled nitric oxide (ppb)	42.95 (17.75, 101.00)	115.00 (43.15, 142.50)	.07
Serum eosinophil cationic protein (μg/L)	21.45 (13.05, 31.08)	33.00 (16.30, 43.65)	.3
Sputum neutrophil count (%)	35.2 (19.2, 52.2)	33.4 (13.0, 42.6)	.45
Sputum eosinophil count (%)	0.8 (0.2, 3.6)	5.6 (5.2, 6.4)	.02
Sputum eosinophil cationic protein (μg/L)	60.80 (28.80, 117.50)	217.90 (19.90, 333.10)	.3

Data are expressed as medians (1st, 3rd quartile) values unless indicated otherwise.
**P* value with the Wilcoxon rank sum test, unless indicated as †Fisher exact test (2 tailed; test of proportions).
‡β-Agonist reversibility = (postbronchodilator FEV_1 − prebronchodilator FEV_1) × 100/prebronchodilator FEV_1.
‡Obtained after 4 years of treatment.

unless specified otherwise. *P* values are 2 sided and were considered to be significant if ≤.05.

RESULTS

Patient enrollment and safety

One hundred seventeen patients underwent sputum induction in this ancillary study. Sputum induction resulted in bronchospasm (ie, a decrease in FEV_1 to <80% of the baseline value) in 9 children (7.7%). No patient in the budesonide group developed bronchospasm, whereas 3 and 6, respectively, in the nedocromil and placebo groups did (budesonide vs placebo, $P = .08$; nedocromil vs placebo, $P = .73$). Although the FEV_1 percentage predicted values after 360 μg of albuterol were not different between the children who did and did not develop bronchospasm (96.0% [84%, 116%] and 99.0% [91.0%, 109.0%], respectively; $P = .6$), those who developed bronchospasm had significantly lower prebronchodilator FEV_1 percentage predicted and FEV_1/FVC ratios and higher bronchodilator reversibility before the procedure, serum IgE, TEC, and sputum percentage eosinophils (SPEos) (Table I). Their FE_{NO} measurements and airway reactivity tended to be greater, although statistical significance was not attained. In addition, they had more severe disease as assessed by the medical staff, greater use of prednisone, and greater supplemental inhaled corticosteroid doses. The mean decrease in percentage predicted FEV_1 and PEF rate for those who had bronchospasm was 30.4% ± 13.1% and 75.0 ± 19.7 L/min, respectively; mean time at which bronchospasm occurred was 8.5 ± 3.5 minutes. These patients received at least 180 μg of albuterol as rescue, and the mean improvement in FEV_1 percentage predicted was 16.1% ± 3.9% ($P = .0043$).

Aside from bronchospasm, there were only minor adverse events, including gastrointestinal discomfort, nausea, or belching (n = 4); sore throat (n = 2); tingling sensation in the hands and face (n = 1); and generalized

TABLE II. Markers of inflammation, clinical measures of asthma severity, and pulmonary function by treatment group at the end of the treatment period in the Denver CAMP participants with available induced sputum data

Variable	Budesonide (n = 21), median (1st, 3rd quartiles)	Nedocromil (n = 31), median (1st, 3rd quartiles)	Placebo (n = 38), median (1st, 3rd quartiles)	*P* value, budesonide vs placebo*	*P* value, nedocromil vs placebo†
Sputum percentage eosinophils	0.2 (0, 1.2)	1.4 (0.4, 5.2)	0.8 (0.2, 4.6)	.03	.6
Sputum percentage neutrophils	30.8 (15.1, 48.9)	35.2 (24.8, 54.0)	35.6 (18.9, 50.4)	.51	.8
Sputum eosinophil cationic protein (μg/L)	54.8 (24.2, 86.4)	108.0 (44.8, 318.8)	74.4 (32.1, 122.7)	.38	.09
Exhaled nitric oxide (ppb)	19.4 (13.2, 44.3)	60.3 (16.9, 114.0)	63.0 (26.5, 115.0)	.002	.6
Total blood eosinophil count (/mm^3)	334.0 (114.5, 572.0)	299.0 (149.2, 444.5)	299.5 (171.5, 563.0)	.67	.5
Serum eosinophil cationic protein (μg/L)	17.0 (10.6, 25.2)	25.9 (15.2, 36.7)	24.5 (15.8, 36.9)	.06	.86
Prebronchodilator FEV_1 (% predicted)	100.0 (95.5, 116.5)	92.0 (81.0, 100.0)	95.0 (88.2, 103.2)	.04	.2
Prebronchodilator FVC (% predicted)	112.0 (103.5, 121.5)	106.0 (100.0, 116.0)	108.0 (97.0, 116.2)	.1	.7
Prebronchodilator FEV_1/FVC (%)	79.0 (74.5, 84.0)	77.0 (71.0, 81.0)	78.5 (72.0, 85.2)	.6	.23
Postbronchodilator FEV_1 (% predicted)	111.0 (101.5, 121.5)	100.0 (93.0, 108.0)	107.0 (98.0, 113.2)	.8	.1
Postbronchodilator FVC (% predicted)	113.0 (101.5, 124.0)	107.0 (101.0, 116.0)	107.5 (99.8, 117.0)	.24	.77
β-Agonist reversibility‡	5.3 (3.9, 9.9)	7.0 (4.8, 14.6)	7.8 (4.3, 13.0)	.2	.9
FEV_1 PC_{20} (mg/mL)§	2.25 (1.27, 6.39)	1.17 (0.73, 4.45)	2.40 (0.83, 5.75)	.78	.24
Age (y)	13.0 (11.0, 15.0)	12.0 (11.0, 14.0)	14.0 (11.0, 15.0)	.4	.12
Duration of asthma (y)	9.3 (7.4, 11.5)	8.6 (6.8, 10.9)	9.0 (7.0, 10.7)	.6	.7
Serum IgE (ng/mL)§	301.5 (71.0, 662.5)	341.0 (155.0, 1428.0)	734.0 (379.0, 1647.0)	.004	.07
Prednisone days over last year	0 (0, 4.0)	4.0 (0, 8.0)	0 (0, 8.0)	.4	.6
Urgent care visits due to asthma(n/100 person-years)	9.60	15.77	20.03	.3	.4
Hospitalizations due to asthma (n/100 person-years)	1.43	11.64	7.91	.005	.6
Prednisone course (n/100 person-years)	100.04	160.65	168.15	.06	.7
Physician assessment of severity, No. mild (%)	19 (90.5)	18 (58.1)	29 (76.3)	.3	.13
β-Agonist use at least once weekly, No. patients (%)	5 (24)	12 (38.7)	18 (47.4)	.1‖	.6¶
Nocturnal symptoms at least once weekly, no. patients (%)	0	2 (6.4)	0 (0)	1.0‖	.2¶

*Wilcoxon rank sum test comparing budesonide versus placebo.
†Wilcoxon rank sum test comparing nedocromil versus placebo.
‡β-Agonist reversibility = (postbronchodilator FEV_1 − prebronchodilator FEV_1) × 100/prebronchodilator FEV_1.
§Obtained after 4 years of treatment.
‖Fisher exact test, 2 tailed: test of proportions between budesonide and placebo.
¶Fisher exact test, 2-tailed: test of proportions between nedocromil and placebo.

discomfort (n = 1). Twenty-seven of the 117 who underwent sputum induction (23%) had unavailable or inadequate sputum samples: in 15 participants, the percentage of squamous cells was >80%, in 11 children the sample produced was <1 mL, and in 1 child the duration of sputum induction was <4 minutes.

At the second visit, of the 90 patients eligible, 74 had adequate sputum for analysis. Failure to complete the

TABLE III. Pulmonary function and markers of inflammation by treatment group during the washout period for Denver CAMP participants with available induced sputum data

Variable	Budesonide (n = 17), median (1st, 3rd quartiles)	Nedocromil (n = 24), median (1st, 3rd quartiles)	Placebo (n = 33), median (1st, 3rd quartiles)	*P* value, budesonide vs placebo*	*P* value, nedocromil vs placebo†
Sputum percentage eosinophils	1.6 (0, 4.1)	1.9 (0.2, 4.8)	0.8 (0.1, 3.6)	.97	.4
Sputum percentage neutrophils	32.0 (17.5, 42.3)	40.4 (21.2, 55.8)	32.6 (21.2, 55.8)	.36	.36
Sputum eosinophil cationic protein (μg/L)	55.8 (24.2, 104.2)	111.2 (68.4, 153.6)	98.8 (45.2, 279.6)	.16	.7
Exhaled nitric oxide (ppb)	45.8 (20.2, 94.8)	67.9 (21.9, 107.5)	44.9 (12.7, 81.8)	.7	.32
Total eosinophil count (/mm^3)	493.0 (273.0, 677.5)	325.5 (175.8, 449.0)	273.0 (180.5, 668.8)	.2	.9
Serum eosinophil cationic protein (μg/L)	22.9 (14.8, 35.3)	28.4 (13.7, 58.4)	26.5 (11.1, 43.4)	.8	.6
Prebronchodilator FEV_1 (% predicted)	98.0 (87.0, 112.0)	91.0 (81.2, 99.2)	96.0 (87.0, 105.0)	.34	.15
Prebronchodilator FVC (% predicted)	110.0 (97.5, 121.0)	104.0 (98.5, 113.0)	108.0 (102.5, 116.5)	.7	.3
Prebronchodilator FEV_1/FVC (%)	78.0 (73.0, 88.0)	76.0 (69.8, 82.2)	77.0 (73.0, 83.0)	.6	.4
Postbronchodilator FEV_1 (% predicted)	109.0 (103.0, 117.8)	97.0 (91.0, 106.5)	106.0 (97.0, 113.5)	.13	.06
Postbronchodilator FVC (% predicted)	111.0 (102.5, 120.8)	104.0 (100.0, 117.5)	107.0 (98.5, 117.0)	.2	.8
β-Agonist reversibility‡	7.5 (4.6, 16.8)	6.6 (2.8, 18.5)	8.2 (4.5, 11.1)	.8	.95
FEV_1 PC_{20} (mg/mL)	1.72 (0.62, 10.56)	2.37 (0.88, 4.68)	3.80 (0.87, 7.82)	.85	.7

Data are presented as median (1st, 3rd quartile) values.
*Wilcoxon rank sum test comparing budesonide versus placebo.
†Wilcoxon rank sum test comparing nedocromil versus placebo.
‡β-Agonist reversibility = (postbronchodilator FEV_1 − prebronchodilator FEV_1) × 100/prebronchodilator FEV_1.

second sputum induction was due to inadequate sputum volume (<1 mL; n = 3), >80% squamous cells (n = 7), not available for the washout visit (n = 4), acute asthma exacerbation requiring a prednisone burst (n = 1), and withdrawal of consent for sputum induction (n = 1).

Treatment and washout effects

At the end of the CAMP treatment period, children in the budesonide group (compared with placebo) had lower median SPEos (0.2% [0%, 1.2%] vs 0.8% [0.2%, 4.6%]; $P = .03$) and FE_{NO} (19.4 ppb [13.2, 44.3 ppb] vs 63.0 ppb [26.5, 115.0 ppb]; $P = .002$), whereas no significant difference in either marker was noted between nedocromil- and placebo-treated patients (Table II). Children in the budesonide group also had a higher prebronchodilator FEV_1 percentage predicted (100.0% [95.5%, 116.5%] vs 95.0% [88.2%, 103.2%]; $P = .04$) and fewer hospitalizations due to asthma (1.43 vs 7.91 per 100 person-years; $P = .005$) compared with those in the placebo group. In contrast, there were no differences in the markers of inflammation and pulmonary function or in clinical characteristics between nedocromil- and placebo-treated children at the end of treatment.

Two to 4 months after study medication was discontinued, markers of inflammation and lung function measurements were comparable in children previously taking budesonide and placebo (Table III). Mean changes in SPEos, FE_{NO}, and prebronchodilator FEV_1 percentage predicted within the treatment groups from the end of treatment to the washout period were statistically significant only for FE_{NO} in the budesonide group (mean increase of 20.3 ppb; $P = .03$) and the placebo group (mean decrease of 14.6 ppb; $P = .04$).

Of the 90 patients who had a successful sputum induction procedure at the end of treatment, 23 had an asthma exacerbation needing rescue prednisone during the washout phase. Participants who needed a prednisone course had higher SPE_{OS} at the end of treatment (3.6% [0.4%, 6.4%] vs 0.6% [0.2%, 3.2%]; $P = .023$) and lower FEV_1 percentage predicted (94.0% [81.0%, 100.0%] vs 97.0% [89.0%, 107.0%]; $P = .05$) than those who did not. At the end of treatment, there were no statistically significant differences in TEC (370.0/mm^3 [202.5, 774.0/mm^3] vs 282/mm^3 [141.0, 458.0/mm^3]), serum ECP (19.0 μg/L [11.4, 24.8 μg/L] vs 23.6 μg/L [14.4, 33.9 μg/L]), sputum ECP (128.8 μg/L [27.2, 360.0 μg/L] vs 58.8 μg/L [28.0, 108.0 μg/L]), or FE_{NO} (57.2 ppb [23.5, 112.8 ppb] vs 45.8 ppb [17.6, 101.2 ppb]) between groups.

Relationship of SPEos to other markers of inflammation, pulmonary function, and clinical asthma features

The SPEos was found to correlate significantly with TEC and FE_{NO} levels (Fig 1) at the end of treatment and during the washout period. The changes in SPEos and FE_{NO} from the end of treatment to the washout period

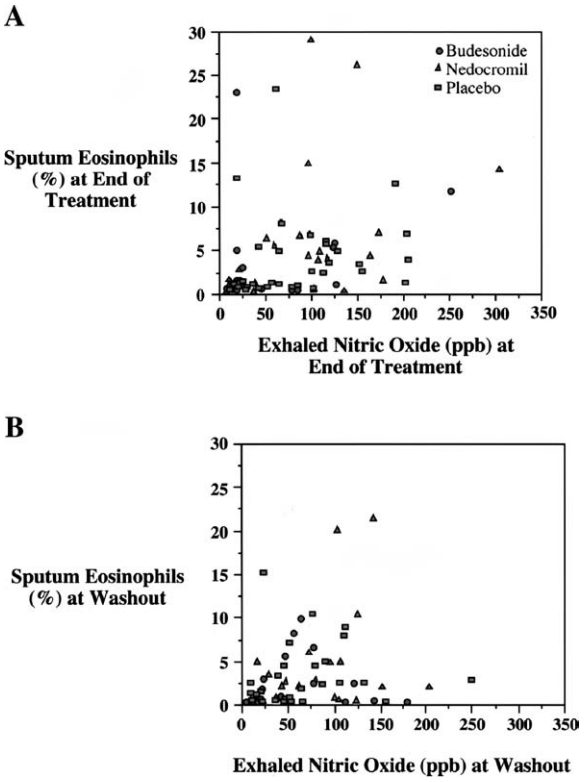

FIG 1. Sputum percentage eosinophils correlate with exhaled nitric oxide levels (ppb) at the end of the treatment phase ($\rho = 0.51$; $P < .0001$) **(A)** and at the washout period ($\rho = 0.42$; $P = .0002$) **(B)**.

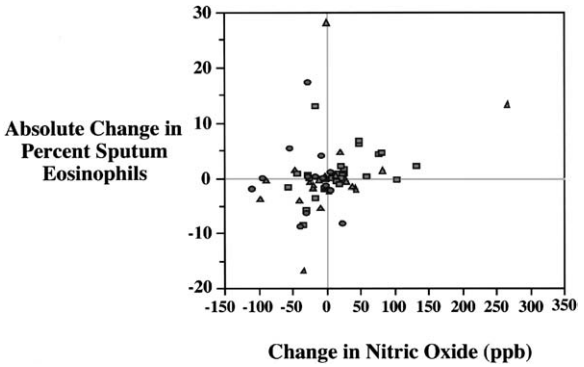

FIG 2. The absolute change in sputum percentage eosinophils (SPEos) from the end of treatment to the washout period (SPEos at end of treatment minus SPEos in the washout period) correlates with the absolute change in exhaled nitric oxide levels (ppb) ($\rho = 0.40$; $P = .0007$).

were also significantly correlated ($\rho = 0.40$; $P = .0007$; Fig 2). SPEos modestly correlated with serum ECP only during the washout period. In addition, SPEos correlated with measures of atopy obtained at the fourth year of treatment, ie, serum IgE and number of positive skin prick tests to a standard battery of inhalant allergens. Significant correlations were found of SPEos with bronchodilator reversibility at both visits, and inverse correlations were found between SPEos and prebronchodilator FEV_1 percentage predicted at the end of treatment and FEV_1/FVC or PC_{20} values at both visits (Table IV). No significant correlations were found between the SPEos and postbronchodilator FEV_1 and FVC percentage predicted.

The SPEos was increased in patients who reported β-agonist use at least once a week at the end of treatment (2.0% [0.4%, 5.0%] vs 0.6% [0%, 3.0%]; $P = .033$) and during washout (2.2% [0.6%, 5.4%] vs 0.2% [0%, 2.5%]; $P = .01$) compared with those who had less frequent symptoms. The 2 patients in this cohort who had nocturnal symptoms at least once weekly at the end of treatment had SPEos of 14% and 26%. During the washout period, 5 patients had nocturnal symptoms at least once weekly, and their median SPEos was higher than that in those who had less frequent nighttime symptoms (2.4% [1.0%, 11.2%] vs 1.2% [0.2%, 4.2%]), but this did not reach statistical significance. Patients with moderate to severe asthma at the end of treatment had significantly increased SPEos compared with those who had mild asthma (4.5% [0.8%, 7.4%] vs 0.6% [0%, 2.4%]; $P = .0002$). The number of prednisone courses throughout the treatment phase in CAMP correlated significantly, although weakly, with the SPEos (Table IV). With analysis by treatment group, the correlations with SPEos that remained significant in both the budesonide- and placebo-treated groups were TEC, sputum ECP, PC_{20}, serum IgE, and number of positive skin prick test (as shown in Table E1 in the Journal's Online Repository at www.mosby.com/jaci).

DISCUSSION

Although numerous studies in adults have shown IS analysis to be a useful and safe tool in the evaluation of asthma, very few studies have evaluated this tool in children. Bronchoscopy with or without biopsy, considered to be the ''gold standard'' in the assessment of airway inflammation, is generally safe and tolerable under the hands of experienced clinicians[9-11]; however, the procedure is unlikely to be widely performed in children with mild to moderate asthma because of its inherent technical difficulties and sedation requirements. IS analysis is a less invasive alternative to bronchoscopy in children with mild to moderate asthma.

From a well-characterized cohort of more than 100 children with mild to moderate asthma, we are able to demonstrate the safety and tolerability of sputum induction. From our data, several clinically important observations can be made. First, sputum induction with 3% saline was successful in three fourths of the subjects. Other published studies have reported success rates ranging from 68% to 100%.[12] The variability in these rates could be due to differences in the methods used, such as induction with or without a bronchodilator pretreatment, or differences in criteria for defining a satisfactory sample. Second, sputum induction resulted in significant bronchospasm in 8% of patients despite pretreatment with 360 μg of albuterol. This was, however, easily reversed with albuterol. Those who developed bronchospasm had greater airflow limitation; inflammation, as measured by

TABLE IV. Correlations of asthma features with sputum percentage eosinophil at the end of treatment and in the washout period

	End of treatment			Washout		
Variable	**n**	**ρ**	***P* value***	**n**	**ρ**	***P* value***
Total blood eosinophil count (/mm^3)	89	0.53	<.0001	74	0.45	<.0001
Serum eosinophil cationic protein (μg/L)	90	0.07	.51	74	0.37	.002
Sputum eosinophil cationic protein (μg/L)	86	0.50	<.0001	72	0.31	.007
Serum IgE (ng/mL)†	81	0.55	<.0001		NA	NA
Exhaled nitric oxide (ppb)	85	0.51	<.0001	74	0.41	.0002
Prebronchodilator FEV_1 (% predicted)	90	−0.20	.05	73	–0.23	.052
Prebronchodilator FVC (% predicted)	90	−0.08	.50	73	–0.02	.86
Postbronchodilator FEV_1 (% predicted)	90	−0.02	.82	73	0.05	.7
Postbronchodilator FVC (% predicted)	90	−0.01	.89	73	0.07	.57
Prebronchodilator FEV_1/FVC (%)	90	−0.23	.03	73	–0.28	.02
β-Agonist reversibility (%)‡	90	0.40	.0001	73	0.47	<.0001
FEV_1 PC_{20} (mg/mL)†	90	−0.46	<.0001	61	–0.42	.0007
Prednisone days over last year	90	0.19	.065		NA	NA
Urgent care visits due to asthma(n/100 person-years)	90	0.13	.2		NA	NA
Hospitalizations due to asthma (n/100 person-years)	90	0.19	.08		NA	NA
Prednisone courses (n/100 person-years)	90	0.25	.02		NA	NA
Number of positive skin prick tests†	90	0.27	.01		NA	NA

NA, Not applicable.
**P* value with the Spearman rank correlation.
†Obtained after 4 years of treatment.
‡β-Agonist reversibility = (postbronchodilator FEV_1 − prebronchodilator FEV_1) × 100/prebronchodilator FEV_1.

higher sputum and circulating eosinophil counts, serum IgE, and FE_{NO} values; and nonspecific bronchial hyperresponsiveness. Of note, none of the subjects in the budesonide-treated group developed bronchospasm. Other adverse effects were mild and still tolerable. Therefore, in the hands of well-trained and experienced staff, this is a safe procedure that can yield useful information.

This study provides compelling evidence supporting the relationship between eosinophilic airway inflammation and asthma severity in children with asthma. SPEos correlated with various measures of chronic and current asthma control, such as prednisone use during the trial, β_2-agonist rescue use, and frequency of nocturnal symptoms. In addition, SPEos positively correlated with the degree of atopy, bronchial hyperresponsiveness, bronchodilator reversibility, and markers of inflammation (including FE_{NO}) and inversely correlated with airflow obstruction, as measured by the FEV_1/FVC ratio. When analysis by treatment group was performed, SPEos correlated consistently only with atopy, bronchial reactivity, sputum ECP, and peripheral eosinophilia in both the budesonide- and placebo-treated groups. This suggests that these relationships are less likely to be affected by treatment and that monitoring these parameters may provide information on airway inflammation independent of inhaled steroid therapy. Such is the case in the correlation between FE_{NO} and SPEos, which was documented only in the groups not treated with inhaled steroids.[13,14] This observation suggests that FE_{NO} levels may be more sensitive to the effects of inhaled steroids than SPEos and that FE_{NO} likely reflects aspects of the inflammatory processes that are not dependent on eosinophils. In patients who require significant inhaled or oral corticosteroids, diminished FE_{NO} levels can be found. In such patients, mechanisms that do not necessarily involve inducible nitric oxide synthase activity and yet can elucidate the poor clinical response to anti-inflammatory therapy may be obtained from IS by using not only cellular, but also immunohistochemical, flow cytometry, or polymerase chain reaction, analysis.

Of importance, children who required a steroid burst because of acute asthma exacerbation after treatment discontinuation had increased SPEos compared with those who did not require a steroid burst. This finding in children mirrors those seen in adult patients with asthma, who have demonstrated a greater risk for exacerbations after inhaled corticosteroid withdrawal in those with increased sputum eosinophils.[15] Jones et al[16] evaluated the ability of FE_{NO} and SPEos to predict loss of asthma control in adult patients with asthma after discontinuation of inhaled corticosteroid therapy. They found that the prognostic accuracy of FE_{NO} was comparable to that of SPEos in predicting loss of control. These studies demonstrate that SPEos can be a potential marker for predicting loss of asthma control. Green et al[3] extended these observations by evaluating the applicability of monitoring sputum eosinophils to reduce asthma exacerbations. They showed that treatment designed to ''normalize'' the percentage of sputum eosinophils reduced the number of asthma exacerbations, thus precluding the need for additional

anti-inflammatory therapy. Their results indicate that the current practice guidelines would be enhanced by additional measurements, such as biomarker assessment. Therefore, inflammatory markers offer complementary information to currently available clinical tools available in the treatment of children with asthma.

Sputum induction is a relatively safe, noninvasive procedure that allows a direct assessment of airway inflammation. It can provide information regarding cellular and molecular mechanisms (ie, cytokines and mediators) that may account for suboptimal responses to treatment. Despite the relative safety and tolerability of sputum induction in children, it has several potential limitations. First, the procedure requires a substantial amount of time to perform and process (average of 3 hours). Second, significant technical support and expertise are required to process, stain, and interpret the samples. Third, a significant minority (23%) of our cohort were not able to provide technically adequate or acceptable samples. Finally, 8% developed bronchospasm despite pretreatment with 360 μg of albuterol. Patients who developed bronchospasm, in general, had more severe disease and represent those who would most likely benefit from applying this additional measure of asthma control. Hence, this procedure will likely remain a research tool for the evaluation of asthma in children, because of strict requirements for standardization in addition to logistical and technical considerations.

Because we found relationships of SPEos with other biomarkers, such as FE_{NO}, and measures of asthma control/severity, the use of these noninvasive and more easily obtainable tools should be tested in controlled trials. Hence, further investigation is required into the use of other biomarkers that are easier to obtain and will complement or provide information similar to that gleaned from IS.

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