

Effect of inhaled steroids on clinical and inflammatory parameters in children with cystic fibrosis

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Background/aim: The effectiveness of inhaled corticosteroids (ICSs) in cystic fibrosis (CF) is controversial. The aim of this study was to investigate the effect of an ICS on bronchial hyperreactivity (BHR), oxidative status, and clinical and inflammatory parameters in CF patients.

Materials and methods: CF patients were randomized to receive either 2 mg/day nebulized budesonide or 0.9% normal saline as placebo for 8 weeks.

Results: Twenty-nine CF patients (mean age: 10.5 ± 2.9 years) were enrolled in the study. There was no statistically significant difference between the two groups at the end of 8 weeks in terms of symptoms, pulmonary function, BHR, oxidative burst, hs-CRP, or ESR. Although there was a significant decrease in malondialdehyde levels in both groups, there was no difference between the two groups. Percentage of neutrophils in the sputum of patients decreased in the budesonide group (P = 0.006). Although sputum IL-8 levels significantly increased in both groups, there was no statistically significant difference between the two groups.

Conclusion: Although there was a significant decrease in the percentage of neutrophils in sputum with budesonide, 8 weeks of 2 mg/day nebulized budesonide was not effective in terms of BHR, oxidative status, or clinical and other inflammatory parameters in children with CF.

Key words: Antiinflammatory treatment, children, cystic fibrosis, glucocorticoids, inhalation

1. Introduction

Cystic fibrosis (CF) is a lung disease characterized by a vicious cycle of airway obstruction, chronic bacterial infection, and neutrophil-dominated airway inflammation (1,2). Local pulmonary inflammation in CF starts early in life and, if untreated, this inflammatory process can irreversibly damage the airways, leading to bronchiectasis and respiratory failure. To control the inflammation at the early stages may limit the destructive effect of inflammation, delay the pulmonary deterioration, and decrease the morbidity and mortality (3). In CF, the antiinflammatory effects of oral corticosteroids, ibuprofen, and macrolide antibiotics have been studied (4–6). However, the efficacy and safety of inhaled corticosteroids (ICSs) are still controversial. Some studies suggest that ICS therapy in CF may improve lung function, reduce bronchial hyperreactivity (BHR), improve symptoms

like cough and dyspnea, and reduce airway inflammation (7–9). In contrast, some studies did not show any improvement in clinical status or lung function (10,11). A recent metaanalysis about ICSs for CF stated that evidence from trials has been insufficient to establish whether ICSs are beneficial in CF, but withdrawal in those already taking them has been shown to be safe (12). Despite the controversies, ICSs are widely used in CF patients as an antiinflammatory medication. They are better tolerated and have less side effects than oral corticosteroids (13). Therefore, new multifaceted studies are needed to investigate the effect of ICSs in the treatment of children with CF. The aim of the present study was to investigate the effect of an ICS on oxidative status and clinical and inflammatory parameters as well as BHR in CF patients. We hypothesized that ICSs are effective for treatment of these parameters in CF.

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2. Materials and methods

2.1. Study population

CF patients with two abnormal sweat tests (chloride concentration > 60 mEq/L) were recruited from the pediatric pulmonology clinic of Marmara University Hospital. Inclusion criteria: All CF patients over 6 years of age attending the CF clinic. Exclusion criteria: systemic steroid use within the previous 6 months, ICS use within the previous 2 months, pulmonary exacerbation within the previous 4 weeks, history of asthma and atopy in the family and the child, and inability to perform spirometry. All of the CF patients were receiving concomitant nebulized bronchodilator and DNase treatment, while those with *Pseudomonas* colonization were also receiving nebulized antibiotics.

2.2. Study design

The study was prospectively designed and the protocol was approved by the Marmara University Medical School Ethics Committee (MAR-YÇ-2006-0034) and parental informed consent was obtained for each patient.

At the first visit, symptoms, physical examination findings and pulmonary function test (PFT) with bronchodilator response were recorded. Blood samples

and induced sputum were collected. One week later, a methacholine challenge test (MCT) was performed. After generating a random number row, patients with even and odd numbers were randomized to receive either 2 mg/day nebulized budesonide (2 × 2 cc) or 0.9% normal saline (2 × 2 cc) as placebo for 8 weeks by a second investigator who was blinded to the clinical status of the patient. The study was designed as a single-blinded study due to nonavailability of 0.9% normal saline in a similar vial as budesonide. At the end of 8 weeks, patients were re-evaluated for clinical and laboratory parameters and the MCT was performed 1 week later (Figure 1).

2.3. Clinical and radiological scores

Clinical status of the patients and the severity of the disease were evaluated by Shwachman–Kulczycki (S-K) (14) and Brasfield scores (15).

Chest radiographs were taken from all patients at the beginning of the study. High resolution computed tomography (HRCT) was obtained for patients who had not undergone it in the previous 6 months. Chest radiographs and HRCTs were scored by a blind radiologist according to the Brasfield clinical scoring system (15) and CT scoring system used by Helbich et al. (16), respectively.

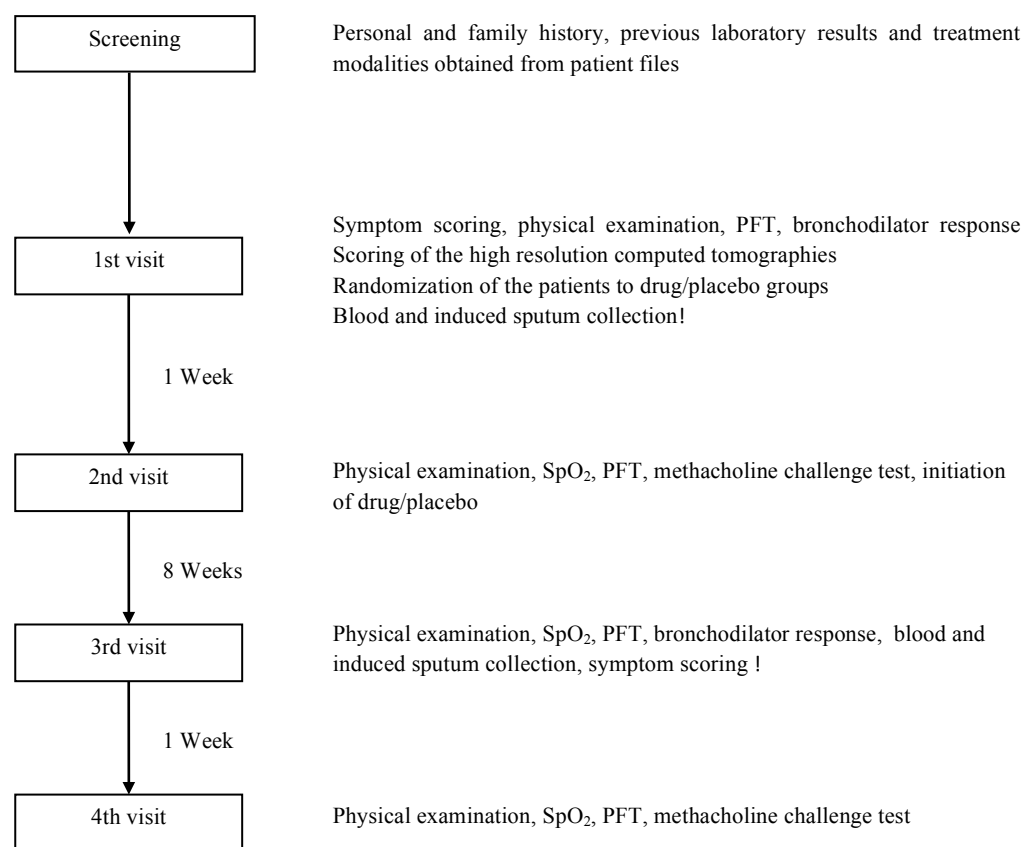


Figure 1. Study plan.

Cough, wheezing, dyspnea, and sputum amount and color were scored by the patients using a subjective 0–3 scoring system, where 0 = none, 1 = mild, 2 = moderate, and 3 = severe. Symptoms were separately scored with presence and increasing intensity, with a possible total score of 0 if there are no symptoms and 15 if patients have severe symptoms and green sputum (17).

2.4. Sputum induction

Sputum samples of the patients were obtained by sputum induction (18) with the inhalation of an aerosol hypertonic saline (3% NaCl) solution through the reservoir of ultrasonic nebulizer (UN - 808 Sigma Medical Supplies Corp. Taipei, Taiwan). The sputum induction procedure was stopped if the patient had symptoms or wheezing was detected on physical examination or $\geq 20\%$ decrease was detected in FEV₁. If any of these occurred, the patient was administered inhaled salbutamol and observed until FEV₁ recovered to 10% of baseline.

Obtained sputum samples were processed within 2 h. After processing, the samples were divided into two. One sample was examined immediately in terms of total white blood cell (WBC) count and percent of neutrophils (LH 750 Coulter, Brea, CA, USA) while the other sample was stored at $-80\text{ }^{\circ}\text{C}$ for TNF- α and IL-8 measurement by chemiluminescence method (DPC Immulite 1000, Siemens, USA).

2.5. Pulmonary function tests (PFTs)

Vital capacity and flow rates were measured by spirometry (MIR Srl Spirobank, Italy, No. 23432) according to the criteria of the American Thoracic Society (ATS) while patients were awake and in the seated position (19). Spirometry results were reported as the ratio of percentage of normal values based on age, sex, and height. The best value of a minimum of the three adequate measurements was taken. Reference values obtained by Knudson et al. (20) were used.

2.6. Methacholine challenge test

The MCT was performed by using the five-breath dosimeter protocol (21) at least 12 h after the last dose of inhaled β_2 agonist. Solution of methacholine chloride (Provocholine; Methapharm Inc, Brantford, ON, Canada) was used. Aerosols were generated by a nebulizer (DeVilbiss 646 nebulizer, automatic inspirium-sensored dosimeter zAn - 200 ProVAir, Spiromed). After inhalation of an isotonic saline solution, doubling concentrations (0.0125–32 mg/mL) of methacholine were administered. The response to methacholine was measured as the change in the percentage of FEV₁ of the initial value. The concentration of methacholine solution at which FEV₁ fell by 20% of postsaline value (PC₂₀) was obtained by interpolation of the log dose-response curve. BHR was defined as a PC₂₀ of ≤ 8 mg/mL (22).

2.7. Blood samples

Blood samples were obtained between 0800 and 0930 after 8 h fasting and immediately centrifuged at $3500 \times g$ for 10 min and the serum was stored at $-80\text{ }^{\circ}\text{C}$ until assayed. Blood samples were obtained for high sensitive C-reactive protein (hs-CRP), erythrocyte sedimentation rate (ESR), total blood count, malondialdehyde (MDA), and monocyte oxidative burst measurement. To evaluate inflammatory status, hs-CRP, ESR, and oxidative burst of monocytes were measured while to evaluate oxidative damage intensity, plasma concentrations of MDA were measured.

MDA measurements were done with high-performance liquid chromatography (HPLC) (Agilent 1100) and hs-CRP immunoturbidometrically (Modular P, Roche Diagnostics, Mannheim, Germany). Respiratory burst of monocytes was measured by luminal-enhanced chemiluminescence (Minilumat, Berthold LB, Bad Wildbad, Germany) before and after phorbol-myristate acetate (PMA) induction.

2.8. Statistical analysis

The statistical analysis was carried out with Statistical Package for the Social Sciences (SPSS) 11.0 for Windows. The data were not distributed normally and described as median and interquartile ranges. While comparing the two groups receiving drug or placebo, categorical variables were analyzed by chi-square test and continuous variables were analyzed using Wilcoxon's test. A $P < 0.05$ was regarded as statistically significant.

3. Results

Thirty-two CF patients were enrolled in the study. Three patients were excluded during the study: two patients were diagnosed and treated for allergic bronchopulmonary aspergillosis and the other patient was excluded up on her parents' request. Therefore, the study was concluded with 29 patients and statistical analysis was carried out on the results of these 29 patients. All three patients who were excluded were in the placebo group.

3.1. Comparison of baseline data

Mean age was 10.5 ± 2.9 years and mean follow-up duration was 4.9 ± 3.4 years. There was no statistically significant difference between the two groups in terms of demographic features, clinical status, symptom scores, inflammatory markers, pulmonary function tests, bronchodilator response, or MCT results at baseline; however, the rate of *Pseudomonas* colonization and total WBC count in the sputum were significantly different (Table 1).

3.2. Comparison of budesonide and placebo treatments

There were no statistically significant differences between the two groups in terms of clinical symptoms or PFT measurements at the end of 8 weeks (Table 2). Twenty-four

Table 1. Demographic features, clinical status, PFT, and laboratory test results of the patients at baseline (mean \pm SD/median (25th–75th percentiles)).

	Budesonide (n = 17)	0.9% normal saline (n = 12)	P
Female/male (n)	12/5	8/4	0.82
Age (years)	11.3 \pm 3.0	9.5 \pm 2.74	0.10
S-K score	76.15 \pm 15.83	85.63 \pm 14.25	0.18
Brasfield score	13.31 \pm 6.61	12.25 \pm 9.68	0.77
HRCT score	14 (6.5–19.5)	6 (4–15)	0.10
Symptom score	2.5 (0.3–5.0)	3.0 (1.0–4.0)	0.95
MDA (ng/dL)	1.7 (1.6–1.9)	1.8 (1.4–1.9)	0.92
Δ AU/mL	109,399 (64,908–520,201)	74,967 (14,494–648,110)	0.59
hs-CRP	1.6 (0.5–7.4)	0.6 (0.2–1.9)	0.11
ESR	20.5 (12.5–34.5)	21 (7–22.7)	0.41
Blood WBC count	8576.92 \pm 4095.76	7228.57 \pm 1285.45	0.55
Sputum WBC count ($10^3/\mu$ L)	0.8 (0.6–4)	0.3 (0.2–0.7)	0.01
FVC (%)	81.66 \pm 15.37	87.84 \pm 18.79	0.34
FEV ₁ (%)	84.83 \pm 18.16	92.94 \pm 18.18	0.25
FEF _{25–75} (%)	91.47 \pm 32.01	98.03 \pm 24.62	0.56
Pseudomonas colonization n (%)	8 (47)	1 (8.3)	0.01

patients were able to perform the methacholine challenge test both at the beginning and at the end of the study. The number of patients with BHR decreased following budesonide treatment in contrast to the placebo group (Table 2); however, there was no concomitant decrease in symptoms or inflammatory mediators except MDA, which decreased in both groups at the end of 8 weeks without a significant difference between the two groups. There was no statistically significant difference in PC₂₀FEV₁ values before and after budesonide/placebo treatment (Table 2).

Following treatment, there were no statistically significant differences in each group or between the two groups in terms of oxidative burst of monocytes, hs-CRP, ESR results, or sputum TNF- α levels (Table 2).

Both WBC and percent of neutrophils (Figure 2) in sputum decreased in the budesonide group, while there was no decrease in the placebo group (Table 2). At the end of 8 weeks, sputum IL-8 levels increased in both groups, only the increase in the budesonide group was statistically significant, and there was no statistically significant difference between the two groups (Figure 3).

4. Discussion

In this study, we evaluated the efficacy of 8 weeks of nebulized budesonide in CF patients and found that this treatment was not effective for improving symptoms, PFT, BHR, and inflammatory parameters, except the WBC count and percent of neutrophils in sputum, which were significantly decreased in the budesonide group.

ICSs are widely used in CF patients in many countries. Konstan et al. reported that the use of ICSs in CF patients significantly increased from 16% to 49.3% from 1995 to 2005 (23). The use of ICSs may be justified as a form of symptomatic prophylaxis for those with recurrent wheezing or CF asthma (24), but they are also prescribed by many clinicians purely for their perceived benefit as an antiinflammatory agent (25). In this study, we aimed to investigate the effect of an ICS on BHR, oxidative status, and clinical and inflammatory parameters in CF patients.

FEV₁ measurement is the most widely used method to evaluate the efficacy of antiinflammatory drugs in CF (26). The PFT results were not significantly different following budesonide treatment in our study. This result was similar

Table 2. Blood and sputum laboratory test and PFT results of the patients before and after treatment (mean ± SD/median(25th–75th percentiles)).

	Budesonide (n = 17)		P	0.9% normal saline (n = 12)		P	P ¹
	Before	After		Before	After		
Symptom score	2.5 (0.3–5.0)	3.0 (2.0–4.0)	0.88	3.0 (1.0–4.0)	2.5 (0.0–4.0)	0.43	0.59
MDA (ng/dL)	1.7 (1.6–1.9)	1.1 (1.0–1.4)	<0.001	1.8 (1.4–1.9)	1.1 (0.9–1.2)	0.01	0.68
ΔAUC/mL	109,399.0 (64,908.5–520,201.2)	90,714.2 (33,323.6715,955.2)	0.83	74,967.0 (14,494.2–648,110.2)	176,867.8 (31,732.6–639,216.2)	1.00	0.91
hs-CRP	1.6 (0.5–7.4)	1.0 (0.1–4.6)	0.23	0.6 (0.2–1.9)	0.3 (0.2–4.3)	0.93	0.84
ESR	20.5 (12.2–34.5)	15.5 (6.7–30.7)	0.08	21 (7.0–22.7)	20 (7.7–32.7)	0.40	0.84
Sputum WBC count (10 ³ /μL)	0.8 (0.6–4.0)	0.6 (0.4–0.8)	0.02	0.3 (0.2–0.7)	0.4 (0.1–0.5)	0.47	0.06
Percentage of neutrophils in the sputum	68.9 (19.9–87.6)	18.6 (13.9–36.7)	0.01	37.4 (17.6–51.1)	28.2 (10.9–47.9)	0.50	0.59
Sputum TNF-α	27.7 (21.8–30.2)	26.4 (24.0–28.2)	0.96	26.7 (20.6–27.6)	26.5 (22.6–44.2)	0.10	0.96
Sputum IL-8	2263.0 (149.6–4169.0)	6261.5 (767.0–13440)	0.04	23.3 (14.9–6853.0)	3565.5 (755.0–6081.2)	0.35	0.44
FVC (%)	81.6 ± 15.3	82.2 ± 21.6	0.84	87.8 ± 18.7	84.7 ± 19.4	0.36	0.75
FEV ₁ (%)	84.8 ± 18.1	81.9 ± 23.3	0.32	92.9 ± 28.1	88.6 ± 19.8	0.23	0.43
FEF ₂₅₋₇₅ (%)	91.4 ± 32.0	79.8 ± 31.8	0.03	98.0 ± 24.6	91.6 ± 29.8	0.47	0.32
BHR n (%)	8 (47)	6 (35)	0.73	6 (50)	8 (67)	0.68	0.70
PC ₂₀ FEV ₁	3.5 (2.4–6.8)	2.9 (1.1–11.6)	1.00	1.5 (0.7–8.3)	1.6 (0.4–5.8)	0.40	0.23

P¹: P values of the comparison of the second measurements

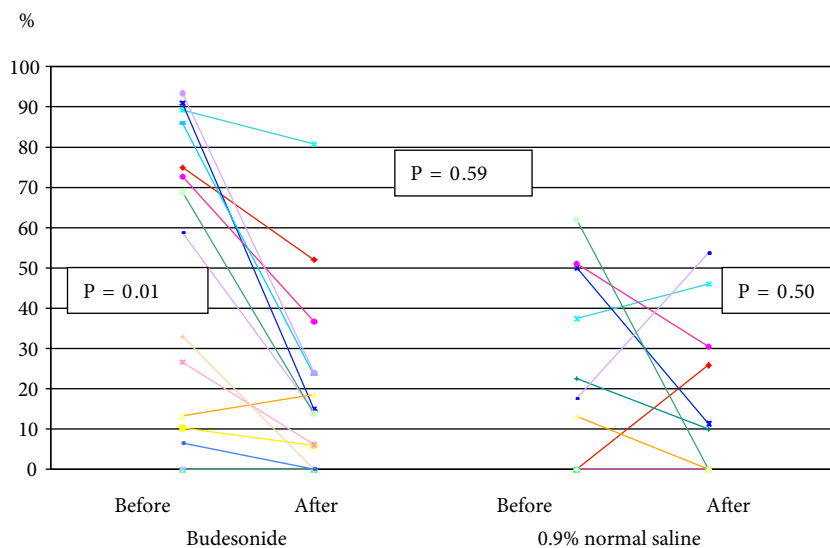


Figure 2. Percent of neutrophils in the sputum of cystic fibrosis patients before and after treatment.

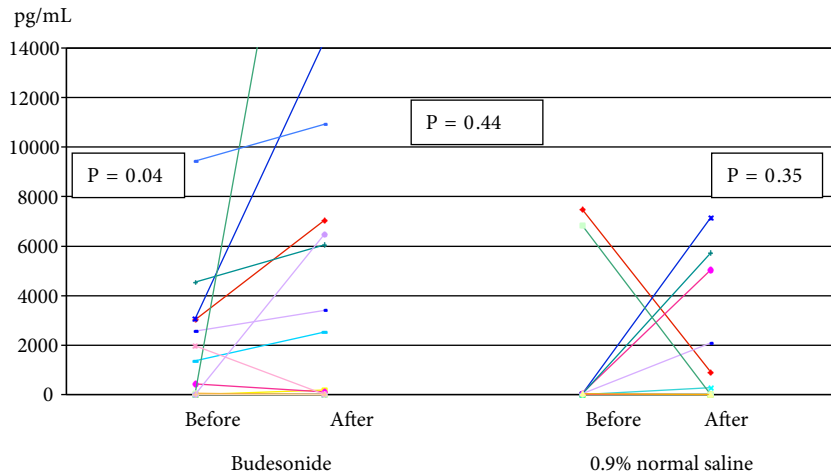


Figure 3. Sputum IL-8 results of cystic fibrosis patients before and after treatment.

to the results of a metaanalysis in which FEV₁ results of the patients were compared at the end of 6 weeks, 3 months, or 6 months of inhaled budesonide, fluticasone, or placebo use and no statistically significant difference was found between the drug and placebo groups (25). However, there are also some retrospective studies showing a reduction in the rate of FEV₁ decline with the use of ICSs in CF patients (27,28).

In CF, the dominant pathology in the lung is inflammation generated primarily by failure to clear microorganisms and the generation of a toxic pro-inflammatory local microenvironment (29). There is chronic neutrophilic airway inflammation, typified by increased membrane-bound and free neutrophil elastase activity. Airway infection is a predominant and persistent cause of neutrophilic inflammation. However, inflammation can also be seen in asymptomatic children with CF without lower airway colonization, and the pathogenesis of noninfectious inflammation in these cases is poorly understood (30). Airway inflammation in asthma consists of mucosal, submucosal, and adventitial edema; cellular infiltration, particularly by eosinophils, activated helper T cells, mast cells, and sometimes neutrophils; increased airway secretions, including secreted mucus, desquamated lining cells, and intraluminal eosinophils; capillary engorgement; hyperplasia of smooth muscle; and deposition of excess collagen, particularly immediately beneath the basement membrane of the epithelium (31). BHR, which may be an indicator of airway inflammation, has a well-known relationship with asthma. It can also be seen in CF patients. Presence of BHR has been reported in 40% of children with CF at the ages of 4–7 years and in 77% of patients at the ages of 8–18 years (32). ICSs are first line anti-inflammatory medications in asthma patients. The use of an ICS for 2 months has been shown to cause

a permanent improvement in BHR (33). ICSs treat BHR both by suppressing inflammation and also by preventing persistent airway changes. Therefore, it can be proposed that CF patients may benefit from treatment with ICSs. van Haren et al. showed that BHR and symptoms in adult CF patients benefit from budesonide (8). In our study, although the number of the patients with BHR was decreased following budesonide treatment, there was no statistically significant difference in PC₂₀FEV₁ values of patients before and after treatment. Our results were also different from the results reported by Bisgaard et al., who showed a decrease in BHR in response to budesonide in CF patients. However, their patients were older than ours, had chronic *P. aeruginosa* infection, and used ICS for a longer duration (33). The response to indirect stimuli such as hypertonic saline or exercise has been shown to be different in CF from that in asthma. CF patients usually bronchodilate after such challenges (34). In the light of these findings, the mechanism leading to BHR in CF may be different from that in asthma (24).

In terms of the symptom scores, there was no statistically significant difference between the two groups at the end of 8 weeks. Only one study showed an improvement in cough and dyspnea at rest with the use of budesonide for 6 weeks in adult CF patients (8). However, no statistically significant change in symptoms after ICS use has been seen in other studies, just like in our study (25).

The ICS had no significant effect on inflammation and oxidative damage intensity compared to the placebo in our study. In a previous study performed by Dauletbaev et al., 3 weeks of fluticasone therapy had no evident effect on oxidative burst of sputum cells in adult CF patients (35). There is conflicting evidence on the in vitro effect of steroids on the oxidative burst of cells. In some studies, the use of steroids has been reported leading to suppress the formation

of oxygen radicals; however, some studies have found no difference in the production of oxygen radicals (36,37). Both our results and the results reported by Dauletbaev et al. suggest that the use of ICSs in CF patients who are known to be sensitive and prone to oxidative stress should be approached cautiously. The significant decrease in MDA levels in both groups in our study may point to an effect of 0.9% normal saline on MDA. Similarly, an increase in the levels of epithelial lining fluid glutathione, which is a protective CFTR-dependent thiol against oxidative injury, has been seen with 0.9% normal saline in an animal study (38).

Induced sputum is a noninvasive technique to collect cellular and soluble material from airways and its results are similar to those of spontaneously expectorated sputum in inflammatory measures (26). Osika et al. demonstrated increased levels of neutrophil, IL-8, and TNF- α in the sputum of CF patients and a reduction in TNF- α levels with inhaled steroids (39). However, Balfour-Lynn et al. found no significant effect of inhaled fluticasone on sputum IL-8, TNF- α and neutrophil elastase levels (10). Similar to the results reported by Wotzac et al., who showed a decrease in BAL neutrophil counts with 2 months of beclomethasone dipropionate treatment in 12 children with CF (9), the percent of neutrophils in sputum decreased significantly in the budesonide group in our study. Patients in the budesonide group had higher WBC counts in sputum at baseline and following treatment it significantly decreased in the same group. IL-8 level in the sputum samples increased significantly in the budesonide group. We found a small but significant increase in an inflammatory cytokine level; however, sputum is not homogeneous and each cough brings sputum from a different part of the lung. It is also difficult to analyze the sputum of CF patients due to its viscosity (10). Furthermore, cytokine concentrations in the sputum may be variable over time even in the same patient without a change in the clinical status or pulmonary function (40). IL-8 can also be produced by many different cells and IL-8 level in the sputum can increase with the increased number of epithelial cells. Therefore, caution must be exercised while evaluating sputum IL-8 as an inflammatory cytokine in CF (10). Furthermore, epithelial cells exposed to *P. aeruginosa* produce IL-8, leading to increased amounts of IL-8 in the lungs of patients with *P. aeruginosa* infection (41). The increased number of patients colonized with *Pseudomonas* in the budesonide group in our study may also be a reason for the significant increase in IL-8 levels in this group.

In addition to studies investigating the effect of ICSs in CF patients, Balfour-Lynn et al. evaluated the safety of withdrawal of ICS and found no difference in terms of time to first exacerbation, lung function, or antibiotic or rescue bronchodilator use. They concluded that it appears safe to consider stopping ICS in CF patients (42).

Since the inflammation in the lungs plays an important role in the pathogenesis of CF, ICS treatment seems like a good idea theoretically. However, adequate evidence is needed before prescribing this therapy to CF patients. In this study, we could not find any significant effect of the ICS in any clinical or laboratory parameters. The reason for this may be the inability of the ICS to pass through the viscous mucus barrier found in the CF airways. The ability of the inhaled drugs to reach the lungs depends on the severity of the lung disease and the device and the technique used to administer the drug. Different results may be obtained by administering ICS from different ways with different devices in different amounts. The nature of the airway inflammation in CF also may play an important role in terms of treatment response. In CF, inflammation is predominantly neutrophilic and corticosteroids are not found to be effective in conditions with neutrophilic inflammation such as acute bronchiolitis (43). Steroids have been shown to prolong neutrophil survival by inhibiting apoptosis, and so they might even promote inflammation in CF airways (25,44).

According to the results of this study and the others in the literature, there is not enough data to suggest the routine use of ICS in the treatment of CF; however, withdrawal in those already taking them has been shown to be safe. The authors of a recent review recommended that the use of ICSs should be restricted to those with symptomatic wheezing and in whom benefit has been proven (45).

Lack of double-blinding is a limitation of our study. We performed a single-blinded study due to nonavailability of 0.9% normal saline in a similar vial as budesonide. The increased number of patients colonized with *Pseudomonas* in the budesonide group compared to the placebo group can also be a confounding factor while comparing the results, especially the IL-8 levels. Inclusion of a small number of subjects may also limit the generalization of the results.

Although there was a significant decrease in the percentage of neutrophils in sputum with nebulized budesonide in children with CF, 8 weeks of 2 mg/day nebulized budesonide was not effective in improving BHR, oxidative status, or clinical or other inflammatory parameters in children with CF.

References

1. Konstan MW, Berger M. Current understanding of the inflammatory process in cystic fibrosis: onset and etiology. *Pediatr Pulmonol* 1997; 24: 137-142.
2. Chmiel JF, Konstan MW. Inflammation and anti-inflammatory therapies for cystic fibrosis. *Clin Chest Med* 2007; 28: 331-346.
3. Kennedy MJ. Inflammation and cystic fibrosis pulmonary disease. *Pharmacotherapy* 2001; 21: 593-603.
4. Rosenstein BJ, Eigen H. Risks of alternate day prednisone in patients with cystic fibrosis. *Pediatrics* 1991; 87: 245-246.
5. Kotsan MW, Byard PJ, Hoppel CL, Davis PB. Effects of high dose ibuprofen in patients with cystic fibrosis. *N Engl J Med* 1995; 332: 848-854.
6. Jaffe A, Francis J, Rosenthal M, Bush A. Long-term azithromycin may improve lung function in children with cystic fibrosis. *Lancet* 1998; 351: 420.
7. Nikolaizik WH, Schoni MH. Pilot study to assess the effect of inhaled corticosteroids on lung function in patients with cystic fibrosis. *J Pediatr* 1996; 128: 271-274.
8. van Haren EH, Lammers JW, Festen J, Heijerman HG, Groot CA, van Herwaarden CL. The effects of the inhaled corticosteroid budesonide on lung function and bronchial hyperresponsiveness in adult patients with cystic fibrosis. *Respir Med* 1995; 89: 209-214.
9. Wojtczak HA, Kerby GS, Wagener JS, Copenhaver SC, Gotlin RW, Riches DW, Accurso FJ. Beclomethasone dipropionate reduced airway inflammation without adrenal suppression in young children with cystic fibrosis: a pilot study. *Pediatr Pulmonol* 2001; 32: 293-302.
10. Balfour-Lynn IM, Klein NJ, Dinwiddie R. Randomised controlled trial of inhaled corticosteroids (fluticasone propionate) in cystic fibrosis. *Arch Dis Child* 1997; 77: 124-130.
11. Prescott WA, Johnson CE. Antiinflammatory therapies for cystic fibrosis: past, present and future. *Pharmacotherapy* 2005; 25: 555-573.
12. Balfour-Lynn IM, Welch K. Inhaled corticosteroids for cystic fibrosis (review). *Cochrane Database Syst Rev* 2014; 10: CD001915. doi: 10.1002/14651858.CD001915.
13. Allen DB, Bielory L, Derendorf H, Dluhy R, Colice GL, Szelfer SJ. Inhaled corticosteroids: past lessons and future issues. *J Allergy Clin Immunol* 2003; 112: S1-40.
14. Shwachman H, Kulczycki LL. Long-term study of one hundred five patients with cystic fibrosis; studies made over a five- to fourteen-year period. *AMA J Dis Child* 1958; 96: 6-15.
15. Brasfield D, Hicks G, Soong S, Tiller RE. The chest roentgenogram in cystic fibrosis: a new scoring system. *Pediatrics* 1979; 63: 24-29.
16. Helbich TH, Heinz-Peer G, Eichler I, Wunderbaldinger P, Götz M, Wojnarowski C, Brasch RC, Herold CJ. Cystic fibrosis: CT assessment of lung involvement in children and adults. *Radiology* 1999; 213: 537-544.
17. Guran T, Ersu R, Karadag B, Karakoc F, Demirel GY, Hekim N, Dagli E. Withdrawal of inhaled steroids in children with non-cystic fibrosis bronchiectasis. *J Clin Pharm Ther* 2008; 33: 603-611.
18. Wong HH, Fahy JV. Safety of one method of sputum induction in asthmatic subjects. *Am J Respir Crit Care Med* 1997; 156: 299-303.
19. American Thoracic Society. Standardization of spirometry, 1994 update. *Am J Respir Crit Care Med* 1995; 152: 1107-1136.
20. Knudson RJ, Lebowitz MD, Holberg GJ, Burrows B. Changes in the normal maximal expiratory flow-volume curve with growth and aging. *Am Rev Respir Dis* 1983; 127: 725-734.
21. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, MacIntyre NR, McKay RT, Wanger JS, Anderson SD et al. Guidelines for metacholine and exercise challenge testing - 1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 2000; 161: 309-329.
22. Hargreave FE, Ryan G, Thomson NC, O'Byrne PM, Latimer K, Juniper EF, Dolovich J. Bronchial responsiveness to histamine or metacholine in asthma: measurement and clinical significance. *J Allergy Clin Immunol* 1981; 68: 347-355.
23. Konstan MW, VanDevanter DR, Rasouliyan L, Pasta DJ, Yegin A, Morgan WJ, Wagener JS, for the Scientific Advisory Group and Investigators and Coordinators of the Epidemiologic Study of Cystic Fibrosis. Trends in the use of routine therapies in cystic fibrosis. *Pediatr Pulmonol* 2010; 45: 1167-1172.
24. Balfour-Lynn IM, Elborn JS. "CF asthma": what is it and what do we do about it? *Thorax* 2002; 57: 742-748.
25. Balfour-Lynn IM, Welch K. Inhaled corticosteroids for cystic fibrosis. *Cochrane Database Syst Rev* 2009 Jan 21; 1: CD001915. doi: 10.1002/14651858.CD001915.
26. Elizur A, Cannon C, Ferkol TW. Airway inflammation in cystic fibrosis. *Chest* 2008; 133: 489-495.
27. Ren CL, Pasta DJ, Rasouliyan L, Wagener JS, Konstan MW, Morgan WJ: Scientific Advisory Group and the Investigators and Coordinators of the Epidemiologic Study of Cystic Fibrosis. Relationship between inhaled corticosteroid therapy and rate of lung function decline in children with cystic fibrosis. *J Pediatr* 2008; 153: 746-751.
28. De Boeck K, Vermeulen F, Wanyama S, Thoms M, on behalf of the members of the Belgian CF Registry. Inhaled corticosteroids and lower lung function decline in young children with cystic fibrosis. *Eur Respir J* 2011; 37: 1091-1095.
29. Elborn JS. Cystic fibrosis. *Lancet* 2016; 388: 2519-2531.
30. Montgomery ST, Mall MA, Kicic A, Stick SM on behalf of AREST CF. Hypoxia and sterile inflammation in cystic fibrosis airways: mechanisms and potential therapies. *Eur Respir J* 2017; 49. pii: 1600903.
31. Sawicki G, Haver K. Asthma in children younger than 12 years: epidemiology and pathophysiology. *UpToDate* 2016.

32. Sanchez I, Powell RE, Pasterkamp H. Wheezing and airflow obstruction during methacholine challenge in children with cystic fibrosis and in normal children. *Am Rev Respir Dis* 1993; 147: 705-709.
33. Bisgaard H, Pedersen SS, Nielsen KG, Skov M, Laursen EM, Kronborg G, Reimert CM, Høiby N, Koch C. Controlled trial of inhaled budesonide in patients with cystic fibrosis and chronic bronchopulmonary *Pseudomonas aeruginosa* infection. *Am J Respir Crit Care Med* 1997; 156: 1190-1196.
34. Brand PL. Bronchodilators in cystic fibrosis. *J R Soc Med* 2000; 93: 37-39.
35. Dauletbaev N, Viel K, Behr J, Loitsch S, Buhl R, Wagner TO, Bargon J. Effects of short-term inhaled fluticasone on oxidative burst of sputum cells in cystic fibrosis patients. *Eur Respir J* 1999; 14: 1150-1155.
36. Leech M, Hutchinson P, Holdsworth SR, Morand EF. Endogenous glucocorticoids modulate neutrophil migration and synovial P-selectin but not neutrophil phagocytic or oxidative function in experimental arthritis. *Clin Exp Immunol* 1998; 112: 383-388.
37. Llewellyn-Jones CG, Hill SL, Stockley RA. Effect of fluticasone propionate on neutrophil chemotaxis, superoxide generation, and extracellular proteolytic activity in vitro. *Thorax* 1994; 49: 207-212.
38. Gould NS, Gauthier S, Kariya CT, Min E, Huang J, Brian DJ. Hypertonic saline increases lung epithelial lining fluid glutathione and thiocyanate: two protective CFTR-dependent thiols against oxidative injury. *Respir Res* 2010; 11: 119-129.
39. Osika E, Cavaillon JM, Chadelat K, Boule M, Fitting C, Tournier G, Clement A. Distinct sputum cytokine profiles in cystic fibrosis and other chronic inflammatory airway disease. *Eur Respir J* 1999; 14: 339-346.
40. Salva PS, Doyle NA, Graham L, Eigen H, Doerschuk CM. TNF- α , soluble ICAM-1 and neutrophils in sputum of cystic fibrosis patients. *Pediatr Pulmonol* 1996; 21: 11-19.
41. Shanks KK, Guang W, Kim KC, Lillehoj EP. Interleukin-8 production by human airway epithelial cells in response to *Pseudomonas aeruginosa* clinical isolates expressing type a or type b flagellins. *Clin Vaccine Immunol* 2010; 17: 1196-1202.
42. Balfour-Lynn IM, Lees B, Hall P, Phillips G, Khan M, Flather M, Elborn JS, on behalf of the CF WISE Investigators. Multicenter randomized controlled trial of withdrawal of inhaled corticosteroids in cystic fibrosis. *Am J Respir Crit Care Med* 2006; 173: 1356-1362.
43. Patel H, Platt R, Lozano JM, Wang EEL. Glucocorticoids for acute viral bronchiolitis in infants and young children. *Cochrane Database of Systematic Reviews* 2004, Issue 3. [DOI: 10.1002/14651858].
44. Zhang X, Moilanen E, Kankaanranta H. Beclomethasone, budesonide and fluticasone propionate inhibit human neutrophil apoptosis. *Eur J Pharmacol* 2001; 431: 365-371.
45. Balfour-Lynn IM, Welch K. Inhaled corticosteroids for cystic fibrosis (review). *Cochrane Database Syst Rev* 2016; 10: CD001915. doi: 10.1002/14651858.CD001915.