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Nebulized fluticasone propionate, a viable alternative to systemic route in the management of childhood moderate asthma attack: A double-blind, double-dummy study

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ABSTRACT

Background: In this study, we compared the clinical and immunological efficacy of nebulized corticosteroid (CS) to systemic route during treatment of moderate asthma attack in children.
Methods: In this randomized, placebo-controlled, double-blind, double-dummy, prospective study, 81 children aged 12 months to 16 years experiencing asthma attack randomized into two treatment groups to receive, either; nebulized fluticasone propionate (n = 39, 2000 mcg/day) or oral methylprednisolone (n = 41, 1 mg/kg/day). Pulmonary index scores (PIS) were assessed at admission and at 1st, 4th, 8th, 12th, 24th, 48th hours, as well as, on day 7 and peak expiratory flow (PEF) at baseline and at the 7th day. Daily symptom and medication scores were recorded for all subjects. Immunological studies included phytohemagglutinin induced peripheral blood mononuclear cells culture supernatant for cytokine responses and CD4(+) CD25(+) FOXP3(+) T regulatory cell (T reg) percentage at baseline and day 7.
Results: The changes in PIS and PEF were similar in both treatment groups, with a significant improvement in both values at the 7th day, when compared to baseline. In both groups, significant reductions in symptom and medication scores were observed during the treatment period with no significant difference between the groups. At day 7 of intervention, phytohemagglutinin induced IL-4 level was significantly decreased only in the nebulized group compared to baseline ($p = 0.01$). Evaluation of cytokine responses by means of fold increase (stimulated (S)/unstimulated (US) ratio) revealed a significant reduction in IL-4, IL-5 and IL-17 only in nebulized group ($p = 0.01, 0.01, 0.02$; respectively). The fold increase value of IL-5 was significantly lower at 7th day in nebulized group when compared to systemic one ($p = 0.02$). At 7th day, although in both treatment groups the percentage of T reg cells was suppressed, it remained significantly higher in the nebule one when compared to systemic route ($p = 0.04$).
Conclusion: In the management of moderate acute asthma attack, nebulized CS (2000 mcg daily) was found to be as effective as systemic route with regard to clinical improvement. In addition, immunological parameters were more in favor of nebulized route which may imply a salutary effect of local CS usage.
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1. Introduction

Asthma is a chronic inflammatory disease, characterized by reversible bronchoconstriction and increased mucus production in response to various stimuli. In the majority of patients Th2 cell derived cytokines play a central role in asthma pathogenesis [1]. Due to the enhancement of cell and cytokine accumulation in the lung, regular use of anti-inflammatory drugs like corticosteroid (CS) supply a pivotal role in restricting the airway inflammation. In

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addition to daily symptoms, natural course of asthma is characterized by remissions and attacks which may render in hospital admissions. Whereas daily symptoms are treated with inhaled CS (ICS), relapses frequently require systemic use [1]. The efficacy of systemic use of CS in acute asthma attack is well established [2]. Although ICS had been used in the management of acute asthma [3,4], according to the most recent meta-analysis, there is still insufficient evidence of its use as a replacement therapy for systemic ones [5].

Despite well-established mechanisms of chronic airway inflammation, data on immunopathogenesis of an asthma exacerbation has not been elaborated yet [6]. It has been shown that bronchoprovocation with an allergen leads to increased expression of interleukin (IL)-4, IL-5 and granulocyte-macrophage colony-stimulating factor indicating the critical role of Th2 response during asthma exacerbation [7]. In addition, T regulatory (Treg) cells are known to suppress effector cells and decrease in their numbers has been linked to development and prognosis of the allergic disease [8]. It has been demonstrated that CS exposure leads to enhanced foxp3 mRNA expression in peripheral blood CD4+T cells from adult asthmatics treated with oral and inhaled CS which may contribute to the beneficiary effect of this molecule on asthma control [9]. However, the role of Treg cells during asthma attack still warrants further investigation.

Thereby, we conducted a randomized, double-blind, double-dummy placebo-control study to evaluate the efficacy of nebulized fluticasone propionate treatment in moderate attack in childhood asthma as compared to systemic route. Treatment responses were assessed by monitoring clinical and immunological parameters including; scores of pulmonary index (PIS) and total symptom/medication, peak expiratory flow (PEF) rate as well as cytokine responses and percentages of Treg cells.

2. Methods

2.1. Inclusion criteria of patients

The study included 81 consecutive children with acute asthma attack (1–16 years of age) presented to the Pediatric Emergency Department (ED) at Marmara University Hospital. Asthma attack was evaluated by physical examination, including assessment of oxygen saturation, respiratory rate, inspiratory-expiratory ratio, use of accessory muscle, and presence of wheezing. These factors were rated on a 4-point scale (0, no symptoms, to 3, severe symptoms) to calculate the PIS (Supplementary Table 1). Inclusion criteria were PIS between 6 and 12 and/or peak expiratory flow of 60–80% predicted after three doses of nebulized salbutamol at Emergency Room (ER) [10,11]. Those children with concomitant cardiopulmonary disease, systemic CS use during the last 2 weeks prior to the exacerbation, admission to hospital (including patients requiring intense effort to breath, altered state of consciousness, bradycardia, room air oxygen saturation of less than 88%) were excluded. The study protocol was approved by the local ethics committee of Marmara University (IRB number: IRB00009067) and a written informed consent was obtained from all parents. Due to the young age of our patients, a simple oral description of the study was given to participating child in the presence of their parent(s) and a verbal assent was requested. The study was registered to the Australian New Zealand Clinical Trials Registry (ANZCTR). The registry number is ACTRN12615000096550.

2.2. Study design

After the first evaluation at ER, all patients received 0.15 mg of nebulized salbutamol (Ventolin, Glaxo Wellcome, UK) per kilogram through a jet nebulizer (Omron NE-C28 Nebulizer, Japan) three times within 1 h and concomitantly an oxygen flow of 6 L per minute. The design of the study was presented in Fig. 1.

2.3. Randomization

A blocked randomization code (list in blocks of four) was prepared by an independent researcher (EKA, AK) not involved in patient assessment, by using a computer-generated list of random numbers. This researcher prepared sequential sealed packets containing the study drugs in a double-blind, double-dummy fashion for the two study groups;

1) **The fluticasone group (n: 39):** Received active nebulized fluticasone and placebo tablets.
2) **The methylprednisolone group (n: 42):** Received active methylprednisolone tablets orally in addition to placebo nebules (2 ml).

The dose of Fluticasone (Flixotide Nebules, 2 ml, Glaxo Wellcome, UK) was 0.5 mg four times a day for one week and oral methylprednisolone tablet was used at a dose of 1 mg/kg/day for 4 days followed by 0.5 mg/kg/day for three more days (Prednol, Mustafa Nevzat Ilac Sanayii, TR). The placebo of both the nebulizing suspension (distilled water) and methylprednisolone tablets were prepared by Department of Pharmacy at Istanbul University, Division of Pharmaceutical Technology. The placebo of both nebule vials and tablets were indistinguishable from the active ones with respect to appearance, taste and smell. The randomization code was revealed after all the patients had completed the study.

Following the baseline assessment, sequential evaluations of PIS were done at the 1st, 4th, 8th, 12th and 24th at ER. After 24 h, if PIS decreased more than 50% of the baseline value, the patient then was discharged and re-evaluated for PIS and/or PEF measurement at 2nd and 7th day. During the follow up period, all patients were asked to complete daily diaries for their total symptom (including; cough, wheezing, and breathlessness - TSS) which were based on a 3-point scoring system. Zero point stands for no symptoms, whereas 1 for mild, 2 for moderate, and 3 for severe symptoms. The same grading system was used for the short acting β2-agonist requirement (TMS) [12].

Pulmonary function tests were performed at baseline and at 7th day by means of maximal forced expiratory volume curves (Zan Flowhandy II; Zan Messgerate GmbH, Oberthulba, Germany) and expressed as the percentage of predicted for PEF. In patients less than 4 years of age, PEF values were not obtained due to inability to follow instructions. Serum morning cortisol levels were measured at the end of the intervention. In order to evaluate the immunological changes during the management of the attack, phytohemagglutinin (PHA) induced peripheral blood mononuclear cell (PBMC) culture supernatants were analyzed for cytokine responses (IL-4, IL-5, IL-10, IL-13, IL-17) by using Bio-Plex human cytokine assays panels (Bio-Rad Laboratories, Inc., Hercules, CA). TGF-β (Invitrogen®, Camarillo, CA, USA) levels of PBMC culture supernatants were determined using commercial human ELISA kit according to the manufacturer's instructions. CD4, CD25, and Foxp3 expression was estimated by flow cytometry (LSRII instrument, BD Biosciences, San Diego, CA, USA) by using appropriate fluorophore conjugated monoclonal antibodies. The details of the methodology of those analyses were described previously [12].

2.4. Outcome measures

The primary outcome measure was to determine whether nebulized CS could decrease the PIS as efficiently as systemic route.

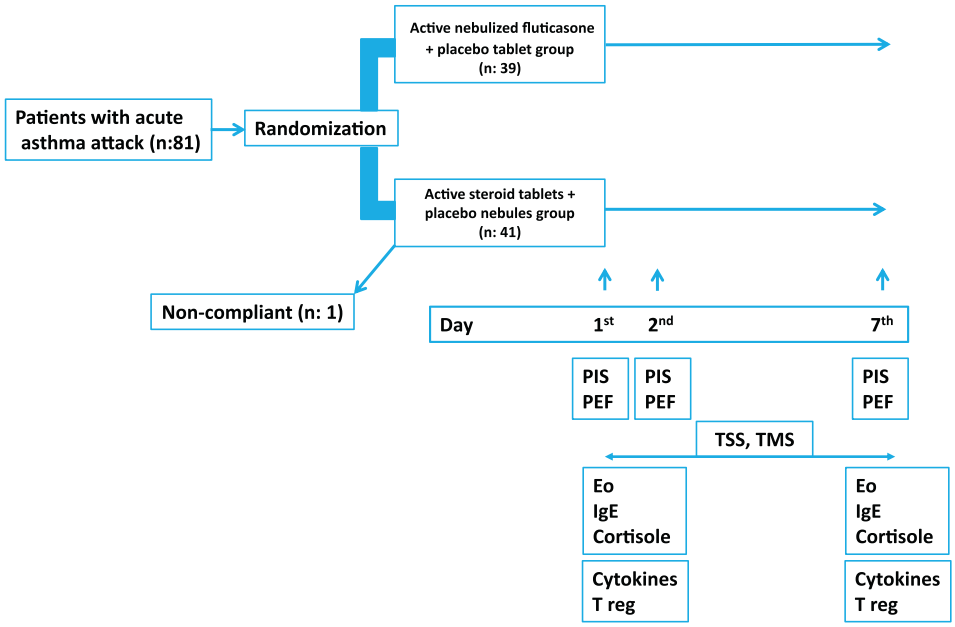

Fig. 1. The study design. PIS: Pulmonary index scoring, PEF: peak expiratory flow, TSS: Total Symptom Score, TMS: Total Medication Score, Eo: Serum eosinophil count.

Secondary outcome measures included changes of total symptom/medication scores, peak expiratory flow, morning cortisol levels, eosinophil and total serum IgE levels. Furthermore, the differences of cytokine responses and percentages of Treg cells were assessed, as well.

2.5. Statistical analysis

Data was described as frequencies and means with standard deviations (SD) unless otherwise indicated and analyzed for continuous variables by using Independent Student's T, Mann Whitney U, paired t and Wilcoxon signed rank test. Differences between the groups were assessed by chi-square analysis for categorical variables. All analyses were performed by using Statistical Package for the Social Sciences (SPSS) program (Version 16.0; SPSS Inc., Chicago, IL, USA) using default settings.

3. Results

3.1. Demographic data

Eighty-two children with asthma exacerbation were included in the study. Following randomization, one patient was excluded due to non-compliance. In total, 81 patients (mean age: 6.1 ± 3.9 years) completed the study protocol. There were 39 patients in the fluticasone group and 41 in methylprednisolone. No significant differences were observed among those groups in terms of gender, age, asthma attack number in the preceding year, value of TSS, PIS, and PEF at baseline (Table 1).

3.2. Primary outcome measure

Pulmonary index scorings decreased significantly during the 7 days treatment period in both groups. Mean baseline PIS decreased from 8 ± 2 to 1 ± 2 in the nebulized CS group ($p = 0.001$) and from 8 ± 1 to 1 ± 1 in the systemic one ($p = 0.001$) (Table 2, Fig. 2A). No differences were detected between the 2 groups in sequential measurement of PIS at the 1st, 4th, 8th, 12th, 24th, 48th hours and lastly, at the 7th day (Fig. 2A). When delta values (Δ post − pretreatment) were analyzed both treatment groups behaved similarly ($p > 0.05$).

3.3. Secondary outcome measures

3.3.1. Symptom and medications scores

In the nebulized and systemic CS groups, scorings of total symptom and medication were significantly reduced during the treatment period ($p = 0.001$, Table 2). There were no statistical differences between the group comparisons. Likewise, no significant differences in the delta values (Δ post − pretreatment) were detected, either.

3.3.2. Peak expiratory flow changes

During the intervention, PEF values improved significantly in both groups (Table 2, Fig. 2B). By day 7, values in patients nebulized CS (83.4 ± 20.3%) were equivalent to systemic CS group (85.8 ± 15.9%). Delta values (Δ post − pretreatment) of both groups revealed no differences, either.

3.3.3. Laboratory evaluation

During the management, two modalities were similar with respect to the numbers of leukocyte, eosinophil and the value of total serum IgE level. In addition, serum morning cortisol level displayed no significant difference between group comparisons at 7th day (Data not shown).

3.3.4. Cytokine measurements and T regulatory cell responses

Baseline PHA stimulated and fold increased (stimulated (S)/unstimulated (US) ratio) cytokine measurements were not different between the groups; however, at the 7th day of intervention, PHA induced IL-4 level was significantly decreased only in the nebulized group compared to baseline ($p = 0.01$, Fig. 3A). Moreover, when cytokine fold increases were assessed, within group comparison, significant reduction in fold increase values for IL-4, IL-5 and IL-17 were detected only in the nebulized group, not in systemic one ($p = 0.01, 0.01, 0.02$; respectively, Fig. 3B–D). In addition, the fold increase value of IL-5 was significantly lower at 7th day in nebulized group when compared to systemic one ($p = 0.02$) (Fig. 3C). No

Table 1
The baseline demographic findings of patients.

	Nebulized fluticasone group (n: 39)	Methylprednisolone group (n: 41)	p
Gender (M/F)	13/26	14/27	>0.05[b]
Age (year)	5.9 ± 3.8	6.3 ± 4.3	>0.05[a]
Preceding attack number/year	2.1 ± 0.4	2.25 ± 0.5	>0.05[a]
PIS	8 ± 2	8 ± 1	>0.05[a]
PEF (% predicted)	71.3 ± 15.7	70.8 ± 19.1	>0.05[a]

PIS: Pulmonary index score, PEF: Peak expiratory flow.
[a] Student's T-test.
[b] Chi-square test.

Table 2
The pre and post treatment values of PIS, symptom, medication and PEF.

Parameters	Pre treatment groups			Post treatment groups			Within group comparison – p values[b]	
	Nebulized CS group (n: 39)	Oral CS group (n: 41)	p values[a]	Nebulized CS group (n: 39)	Oral CS group (n: 41)	P values[a]	Nebulized CS group	Oral CS group
PIS	8 ± 2	8 ± 1	0.70	1 ± 2	1 ± 1	0.38	0.001	0.001
TSS	13 ± 4	13 ± 5	0.70	2 ± 3	3 ± 2	0.52	0.001	0.001
TMS	5 ± 1	5 ± 1	0.77	1 ± 1	1 ± 1	0.88	0.001	0.001
PEF	71.3 ± 15.0	70.8 ± 19.0	0.93	83.4 ± 20.3	85.8 ± 15.9	0.65	0.001	0.001

CS: Corticosteroid, PIS: Pulmonary Index Score, TSS: Total Symptom Score, TMS: Total Medication Score, PEF: Peak Expiratory Flow. Due to the incorporation of younger patients, PEF performed only in 30 and 32 of nebulized and oral CS groups, respectively.
[a] Comparison between the groups, Student's T-test.
[b] Comparison between pre and post treatment values, Paired T-test.

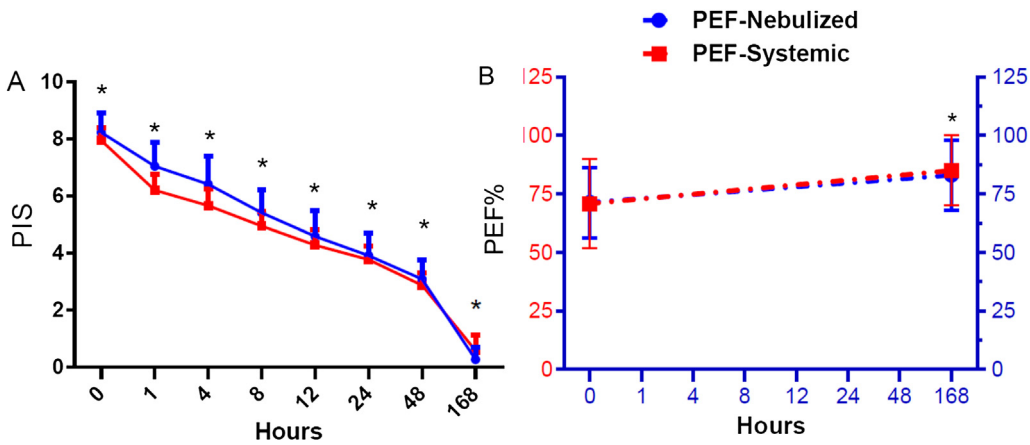

Fig. 2. A, B: Pulmonary index scoring (PIS) and peak expiratory flow (PEF) values of the study groups during the treatment period. A. Mean baseline PIS decreased significantly in both treatment routes (Paired t test, p = 0.001). B. PEF values improved significantly in both groups (Paired t test, p = 0.001). No differences were detected between the 2 groups in sequential evaluation of PIS and PEF (Independent Student's T). (*) indicate significancy.

significant difference for IL-10, IL-13 and TGF-β levels was detected between the 2 groups (Supplementary Fig. 1).

While baseline PHA induced CD4+CD25+FOXP3+ cells percentage was similar between the systemic (4.1% ± 2.9) and nebule (7.2% ± 5.6) groups, at 7th day of therapy, it was suppressed in both and found be significantly higher in nebulized CS receiving group (4.5% ± 3.3) compared to the systemic ones (1.8% ± 1.9) (p = 0.04, Fig. 4).

4. Discussion

This prospective, randomized, double-blind, double-dummy, placebo-controlled study demonstrated that nebulized fluticasone therapy is as effective as systemic CS in reducing pulmonary index scoring, scores of symptom and medication in children with moderate asthma attack. Furthermore, changes in the cytokine responses were in favor of nebulized therapy.

For management of acute asthma the current guidelines recommend a short course of oral or parenteral CS [1]. Despite their effectiveness, there are serious side effects associated with CS usage [1,13]. It is well established that growth velocity suppression as well as adrenal gland insufficiency can occur in children after a prolonged use of systemic steroid. Meanwhile, even short, intermittent courses may have a negative effect on growth and adrenal system [14,15]. In this study, by use of nebulized route, we aim to minimize those side effects in children with asthma. Furthermore, nebulized form has an advantage over oral route regarding the ease of administration for young children.

Previously, the efficacy of nebulized steroids has been addressed during exacerbation of asthma by some studies [4,10,16–18]. In one, Devidayal et al. compared nebulized budesonide to oral prednisolone in the early period during the emergency room management of acute asthma [18]. Following three doses of nebulized budesonide or placebo, more patients in the former group recovered

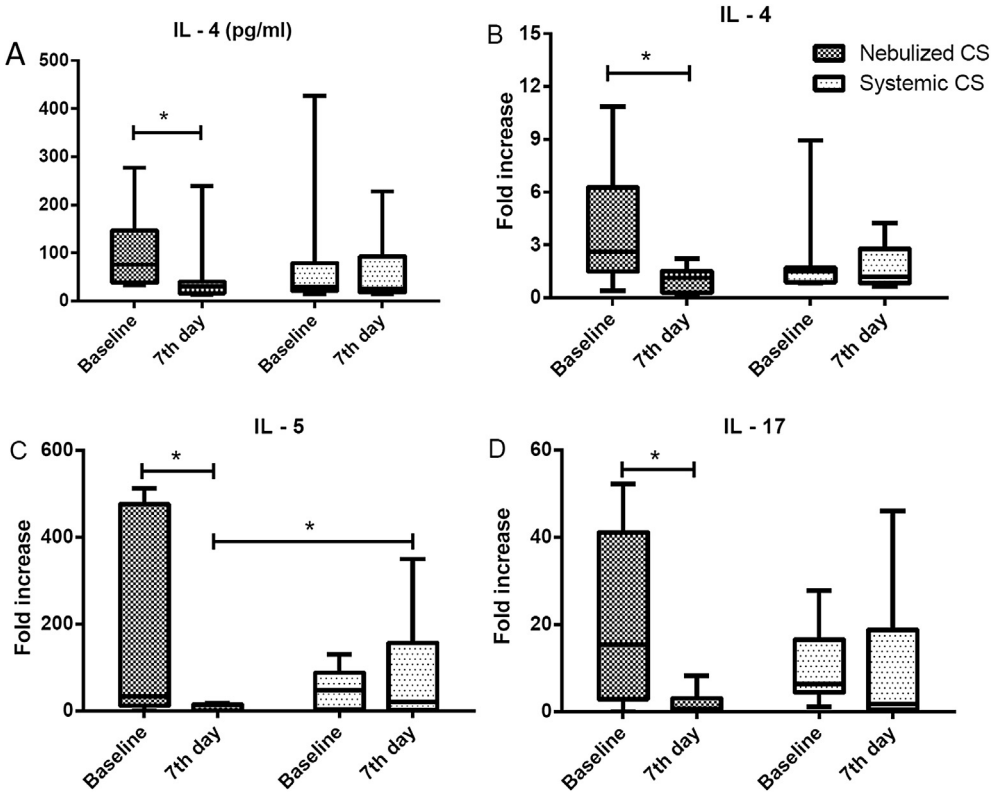

Fig. 3. A–D: Levels of secreted and fold increased cytokines after PBMCs stimulation with phytohemagglutinin (PHA) at baseline and 7th day of treatment in both study groups. A. IL-4 decreased significantly only in nebulized CS group (Wilcoxon signed-rank test, $p = 0.01$). B–D. Within group comparison, significant reduction in fold increase values for IL-4, IL-5 and IL-17 were detected only in the nebulized group (Wilcoxon signed-rank test, $p = 0.01, 0.01, 0.02$; respectively). At 7th day, significant difference was observed between 2 groups in fold increase of IL-5 favored nebule form (Mann Whitney U test, $p = 0.02$). (*) indicate significancy.

significantly in terms of oxygen saturation, respiratory rate, scoring of pulmonary index and respiratory distress, with a higher discharge rate at the end of 2nd hour. Another study conducted by Volovitz B et al. revealed that children receiving high dose (1600 mcg) budesonide turbohaler compared to oral prednisolone (2 mg/kg) showed equivalent improvement in PIS and PEF. Meanwhile, children treated with budesonide demonstrated an earlier clinical response than those receiving systemic CS [10].

While several studies investigated the role of inhaled budesonide in the treatment of acute asthma attacks [16], fluticasone propionate in this setting was not studied adequately. In one study conducted by Manjra AI et al. showed that high dose nebulized fluticasone propionate (1 mg b.d.) resulted in significantly higher increases in PEF values over 7 days compared to oral group [4]. The authors concluded that nebulized fluticasone therapy was at least as effective as oral prednisolone in the treatment of asthma attacks. Of note, this study was hampered with the fact that it was not carried out at ER setting. While inhaled corticosteroids used alone or in combination with systemic corticosteroids helped to relieve asthma attack symptoms, there are still unresolved issues related to their use in terms of dosage, frequency and the duration, partly due to heterogeneity among those studies.

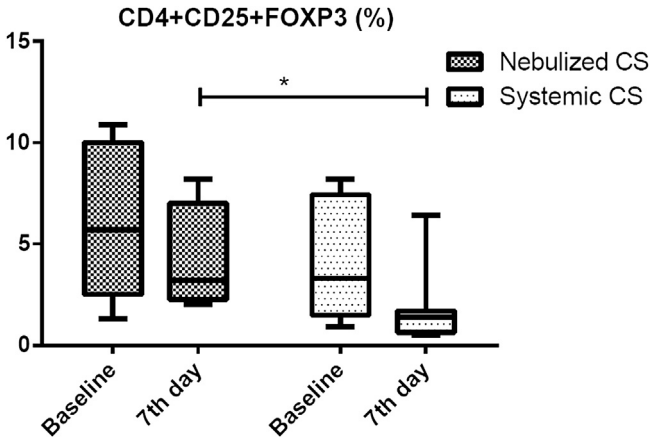

Fig. 4. Percentage of $CD4^{+}CD25^{+}FOXP3^{+}$ cells in the peripheral blood (Mann–Whitney U-test). The reduction of cells percentage was more in the systemic treatment group when compared to nebule ones ($p = 0.04$). (*) indicate significancy.

Cochrane Review from 2012 concluded that, there was insufficient evidence to support the use ICS as an alternative to systemic corticosteroid in acute asthma attacks [5]. Inspired by this conclusion, we set out to compare the two routes; oral steroids/nebulized fluticasone propionate in terms of clinical efficacy and immunological response in management of acute asthma in children. Findings of our study revealed an equal efficacy of nebulized steroids and oral form, from the beginning of the treatment period till 7th day regarding all measurements of PIS, as well as, PEF evaluations. During the management of acute asthma in children, nebulized CS use can be suggested as a viable alternative to systemic route due to less cumulative corticosteroid dose administered along with comparable clinical efficacy.

The immunological changes that take place during asthma attack still remains elusive. The predominance of Th2 cytokines have been linked with clinical deterioration [19]. The study conducted by Robinson D et al. showed that in patients with atopic asthma after stimulation with allergen, the expressing of mRNA in

bronchoalveolar lavage (BAL) for IL-4, IL-5 and granulocyte-macrophage colony-stimulating factor were significantly increased implying the role of Th2 response in attacks [7]. Recently, Calderon C et al. demonstrated that increasing IL-13, rather than IL-4 plays a proinflammatory role during acute severe asthma, whereas IFN-gamma responses were associated with recovery [20]. In addition, IL-17 was found to be increased in serum and BAL correlating with airway hypersensitivity in asthmatic patients [21]. In the management of asthma attacks, it was shown that oral CS caused control of inflammation by reducing IL-5 and soluble CD25 [22]. In the present study, a significant fold increase reduction of IL-4, IL-5 and IL-17 levels which were appeared only in nebulized group may be suggestive for salutary effect of local CS usage. On the other hand, our study was set in ER, therefore allergic sensitization and viral etiology could not be assessed in our patients. Determination of those parameters in patients receiving CS via different routes can precisely figure out the impact of CS on cytokines responses.

Originally, advocation of CSs via inhalation route was based on their rapid onset of action with fewer side effects on the target organ [16,17]. Furthermore, it was shown that in addition to genomic anti-inflammatory responses, corticosteroids exert non-genomic effects, as well, including local airway vasoconstriction which occurs by inhalation route, only [23,24]. This unique effect leads to decrease in mucosal edema and bronchoconstriction. Better downregulation of Th2 cytokines by nebulized steroids demonstrated in our study may partially be explained by the combined genomic and non-genomic effects of the medication when compared to systemic use.

Previously, Karagiannidis C et al. demonstrated significant increase of foxp3 mRNA expression in asthmatic patients treated with high dose inhaled (up to 2000 mg of fluticasone or equivalent per day) and/or systemic steroid when compared to healthy controls [9]. This study suggests the beneficial effect of CS on asthma disease control via inducing Treg cells. However, during an asthma attack, role of CS on Treg response has not been elucidated, yet. According to our results, while baseline T reg percentages were similar between two groups, at the end of therapy, it was found be significantly higher in nebulized CS receiving group compared to the systemic ones. Although clinical efficacy was found to be equal between the two treatment groups, significantly higher T reg counts at 7th day may support the preference of nebulized CS therapy during moderate asthma attacks.

In conclusion, our study confirmed the previous experience indicating an equal clinical efficacy of high dose nebulized fluticasone propionate to oral steroids in moderate asthma attacks in children. From a clinical point of view, fewer side effects, rapid onset of action along with an equal clinical efficacy makes nebulized CS preferable alternative to systemic use in acute moderate asthma attack.

Conflict of interest

No.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.rmed.2015.07.007.

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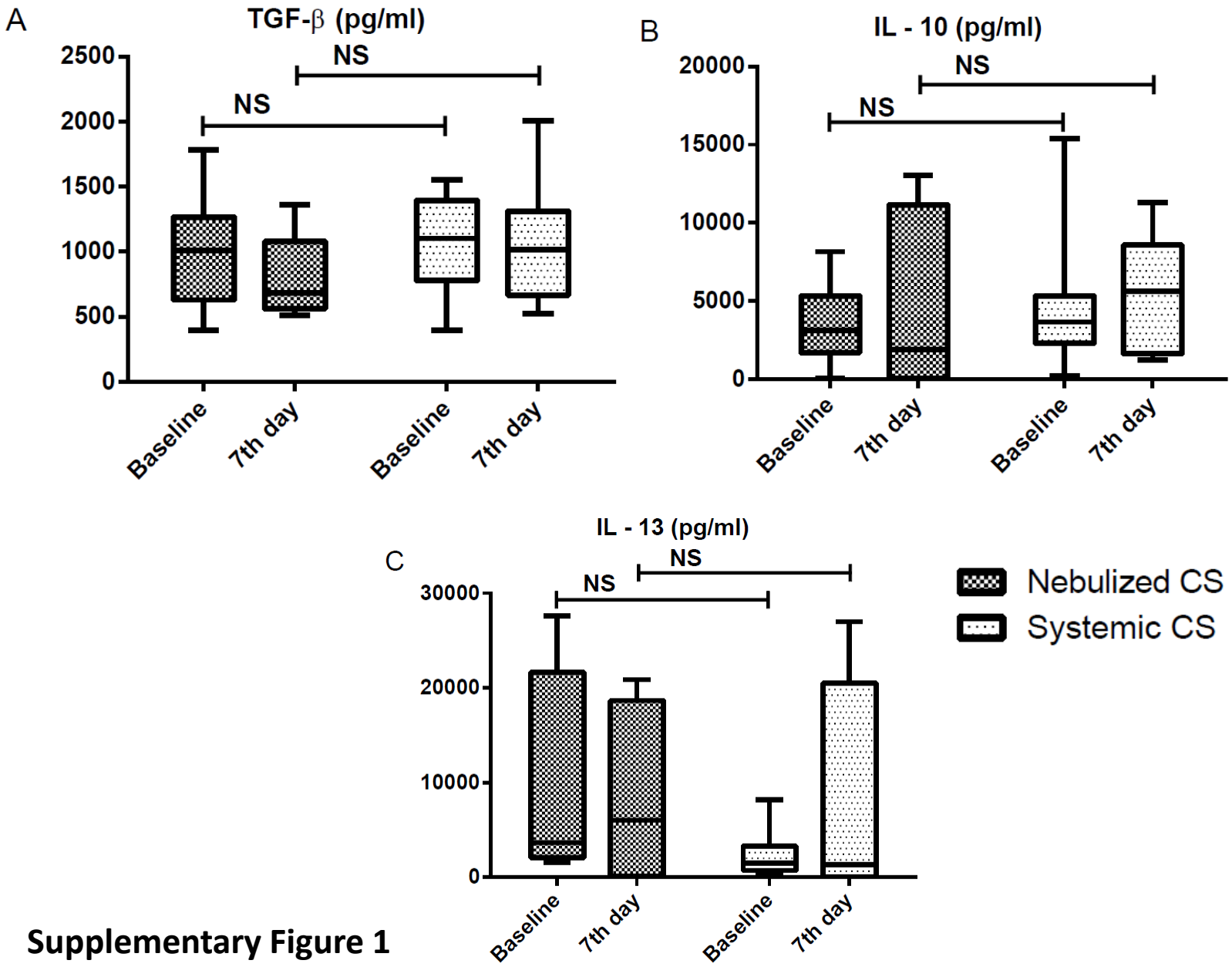
Supplementary Table 1. Pulmonary Index Score

Score	Respiratory rate	Wheezing	Inspiratory/ expiratory ratio	Accessory muscle use	Oxygen saturation
0	< 30	None	2:1	None	99-100
1	31-45	End expiration	1:1	+	96-98
2	46-60	Entire expiration	1:2	++	93-95
3	> 61	Inspiration and expiration	1:3	+++	< 93



Supplementary Figure 1