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Effect of topical corticosteroids on allergic airway inflammation and disease severity in obstructive sleep apnoea*

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Summary

Background The incidence of sleep-related breathing disorders is correlated with lower and upper airway inflammatory diseases, such as asthma and allergic rhinitis. We hypothesized that corticosteroids treatment would lead to a greater reduction in disease severity in obstructive sleep apnoea syndrome (OSAS) patients with concomitant allergic rhinitis vs. non-allergic OSAS patients by reducing the level of inflammation in upper airway tissues.

Objective This study was performed to determine whether treatment with intranasal corticosteroids could reduce upper airway inflammation and improve sleep parameters in obstructive sleep apnoea syndrome patients with or without concomitant allergic rhinitis.

Methods Obstructive sleep apnoea syndrome patients with ($n = 34$) or without ($n = 21$) documented allergic rhinitis voluntarily enrolled in the study and were assessed at baseline and after corticosteroids treatment for 10–12 weeks. Sleep studies were performed and biopsies were obtained from the inferior turbinate, nasopharynx, and uvula. The apnoea–hypopnoea index, sleep quality, and level of daytime alertness were determined, and immunocytochemistry was used to phenotype tissue inflammation.

Results Standard sleep indices improved following treatment in the entire cohort of obstructive sleep apnoea syndrome patients, with greater improvement seen in the allergic rhinitis group. Allergic rhinitis patients demonstrated significantly improved O_2 saturation and a lower supine apnoea–hypopnoea index score after corticosteroid treatment; similar improvements were not seen in the non-allergic rhinitis group. Eosinophilia was detected at all three sites in the allergic rhinitis group, but not in the non-allergic rhinitis group. Following treatment, fewer eosinophils and CD4 lymphocytes were documented at all three biopsy sites in the allergic group; the reduction in inflammation was less apparent in the non-allergic rhinitis group.

Conclusion This study has provided important molecular and clinical evidence regarding the ability of corticosteroids to reduce upper airway inflammation and improve obstructive sleep apnoea syndrome morbidity patients with concomitant allergic rhinitis.

Keywords allergic rhinitis, corticosteroid, inflammation, sleep apnoea

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Introduction

Epidemiological studies have increasingly correlated the incidence of sleep-related breathing disorders with lower and upper airway inflammatory diseases, such as asthma and allergic rhinitis (AR) [1]. Obstructive

Sleep Apnoea Syndrome (OSAS) is defined as recurrent episodes of upper airway obstruction during sleep, leading to a reduced (hypopnoea) or interrupted (apnoea) airflow [2], in association with symptoms of excessive daytime sleepiness or fatigue. In addition, although fatigue has been associated with waking events during sleep, several studies support the fact that some inflammatory mediators such as cytokines and NO may contribute to the sensation of fatigue [3].

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OSAS has been associated with several risk factors such as upper airway dilator muscle dysfunction [4–6], fat deposition in the neck, posture, abnormal bone morphology in the upper airway, and nasal obstruction [7]. OSAS severity is usually characterized by the apnoea–hypopnoea index (AHI), which is defined as the number of apnoea and hypopnoea events per hour of sleep [8]. The intensity of simple snoring has also been related to fatigue through a mechanism that is not entirely clear.

The nose constitutes the main route of breathing during sleep, and also contributes importantly to respiratory airflow resistance [9, 10]. Indeed, patients with upper airway diseases such as sinusitis, rhinitis, and polyposis display increased nasal resistance when recumbent [11, 12]. As a major outcome of rhinitis, nasal obstruction has been shown to increase the severity of OSAS symptoms. Furthermore, OSAS severity has been shown to be increased in patients with symptomatic AR [13, 14].

The major symptoms of allergic rhinitis are pruritus, sneezing, rhinorrhea, and nasal congestion [15]. Swelling of the upper airway mucosa leading to obstruction is commonly associated with an overabundant Th2 response causing subepithelial oedema. The Th2-type response is characterized by IgE-mediated degranulation and release of inflammatory mediators by mast cells and basophils, which themselves promote Th2-type cytokine production (IL-4, IL-5, and IL-13) which leads to tissue infiltration by lymphocytes and eosinophils. Although lower airway remodelling is widely associated with chronic inflammation in asthma, it remains unclear whether such remodelling of the upper airway (nasal mucosa) occurs in AR [16].

Interestingly, several studies have reported positive effects of intranasal corticosteroids (CS) in reducing the symptoms of allergic and non-allergic rhinitis symptoms. CS treatment has been reported to reduce allergen-induced sneezing, post-nasal drip, and nasal obstruction [17]. Nasal CS treatment is effective in limiting immune responses in the upper airway [18], and recent investigations have revealed that inhaled CS treatment can reduce allergen-induced eosinophilic infiltration in nasal lavage fluids and reverse changes in nasal epithelial integrity [17, 19].

This study sought to characterize the inflammatory profile in upper airway tissues in OSAS patients, either with or without documented allergic inflammation (i.e. rhinitis). We hypothesized that CS treatment would lead to a greater reduction in disease severity in OSAS patients with AR by reducing the level of inflammation in upper airway tissues. Changes in OSAS severity were assessed by both objective polysomnographic measurements and clinical symptoms.

Methods

Study design and participants

This was a prospective cohort study performed at a tertiary medical centre designed to evaluate the efficacy of intranasal CS on sleep parameters, nasal symptoms, and upper airway inflammation in OSA patients with vs. without AR. The study was approved by Institutional Review Board Services (Aurora, Ontario, approval number: P05515). Patients undergoing evaluation for OSAS were recruited for the study. Two groups of patients were recruited: 21 patients with diagnosed mild to moderate OSAS (AHI = 10–40/h) without AR and 34 patients with OSAS (same AHI criteria) diagnosed with AR as well. Diagnosis of AR was performed by an allergist according to standard ARIA criteria. All patients gave written informed consent. Exclusion criteria included the following: (i) major oxygen desaturation (less than 80%) during sleep, or a level of daytime sleepiness considered potentially dangerous by their treating physician; (ii) patients in whom a significant component of their sleep-disordered breathing (> 55% of AHI) consisted of central sleep apnoea or Cheyne-Stokes respiration; (iii) poorly controlled hypertension or symptomatic cardiac disease; (iv) previous stroke or other major neurological disease; (v) major psychiatric disorders which may affect sleep; (vi) use of sedatives or other medications which have significant effects on daytime alertness; and (vii) use of other anti-inflammatory medications such as oral corticosteroids.

To determine the eligibility for enrolment, the initial evaluation of patient status included the following:

- An assessment of OSAS severity and sleep quality by polysomnography, including evaluations of sleep architecture and composition (arousal index, percentage of different stages of sleep, etc.), oxygen saturation levels, snoring intensity, and the AHI. The AHI was defined according to standard criteria as published by the American Academy of Sleep Medicine [20]; the compound AHI also included respiratory effort-related arousals in addition to apnoeas and hypopnoeas.
- A determination of AR status based on history of allergy over the last 2 years and an assessment by skin prick testing for common aeroallergens.

Procedures

Once enrolled in the study, each patient underwent the following tests:

- Objective measurement of daytime alertness in the sleep laboratory was the primary outcome. This was done using the maintenance of wakefulness test

(MWT) [21], with determination of the mean time to sleep onset according to standard methods;

- Assessment of daytime alertness was assessed as a secondary outcome using well-established sleepiness and sleep apnoea quality-of-life questionnaires, which have been validated for both English- and French-speaking patients (Epworth Sleepiness Scale (ESS) [22] and the Quebec Sleep Questionnaire (QSQ) [23]);
- Evaluation of the intranasal volume was assessed as an additional secondary outcome using rhinometry and transoral nasopharyngoscopy (TON).

All enrolled patients were biopsied from the inferior turbinate (epithelial and subepithelial layers of the nasal mucosa, biopsy size of 2 mm³), from the soft palate (i.e. uvula, biopsy size of 2 mm³), and from the nasopharynx via the transoral route by an operator blinded to the study group of each patient. The first set of biopsies (before treatment) was taken from the left side of the patient. Patients received intranasal CS treatment by nasal spray (mometasone, 200 µg twice daily) for a period of 10–12 weeks. No additional drugs were used, except for acetaminophen use by a few patients. At the end of the medication treatment period, each patient underwent polysomnography, MWT, response to questionnaires, and rhinometry. The patients were biopsied again from the inferior turbinate, nasopharynx, and the uvula on the side contralateral to the first biopsy sites (the right side).

Data collection

Freshly excised tissues, collected before and after treatments, were fixed in paraformaldehyde for a maximum of 12 h, followed by tissue processing and paraffin embedding. Tissue blocks were sectioned at a thickness of 5 µm onto poly-L-lysine coated slides. The degree of inflammatory cell infiltration in tissues was evaluated by histological staining (haematoxylin and eosin; H&E). Inflammatory cell phenotypes were determined by immunocytochemistry (ICC) using monoclonal antibodies: anti-CD68 (macrophages), anti-EG2 (eosinophils), anti-elastase (neutrophils), anti-CD4 (helper T lymphocytes), and anti-CD8 (cytotoxic T lymphocytes).

The indices of OSAS severity evaluated in the study included assessments of sleep architecture and sleep efficiency, the AHI and qualitative patient assessments of fatigue using the ESS and QSQ questionnaires pre- and post-steroid treatment in OSAS patients with or without AR.

Statistical analyses

t-Tests were performed on the changes from baseline for allergic and non-allergic subjects, and to compare changes from baseline between allergic and non-allergic subjects for clinical parameters and inflammation

markers (equivalent to a paired *t*-test). Pearson correlation coefficients were estimated for inflammation markers across the different sites.

A linear mixed model with the nasal symptom score (including nasal congestion, rhinorrhea, itchy nose, ear and palate, sneezing, snoring, and nasal respiration) as the continuous outcome was used to model the treatment effect over time. Random intercepts and random slopes for treatment were included and errors were autoregressively correlated. This model accounts for correlations between observations on the same subject.

Finally, a linear regression model with changes in clinical symptoms as the outcome and changes in inflammation markers as the independent variable was estimated that adjusted for age, BMI, and sex. The results of multiple regression to adjust associations for relevant confounders were nearly identical to simple linear regression, and thus are not presented. An interaction term was included between allergic status and change in markers to assess if the markers responded differently in allergic vs. non-allergic subjects.

Results

Subjects

This study included 21 non-allergic and 34 allergic subjects. As shown in Table 1, the allergic and non-allergic subjects were similar in terms of age, sex, body mass index (BMI), and neck size. At baseline (Table 2), daytime sleepiness was very similar in the non-allergic and allergic groups when measured by the ESS questionnaire. When measured by the QSQ quality-of-life questionnaire, allergic subjects did not have statistically significant different scores than non-allergic subjects ($P = 0.13$). There were no detectable differences in mean sleep latency as determined by the MWT between the two groups (Table 2). Furthermore, there were no differences in nasal symptom parameters at baseline between the allergic and non-allergic groups (data not shown).

Table 1. Patient characteristics

	Allergic patients <i>N</i> = 34 Mean (SEM)	Non-allergic patients <i>N</i> = 21 Mean (SEM)	<i>P</i> -value for difference between the two groups*	<i>N</i> missing
Age	44.8 (9.3)	48 (8.7)	0.14	1
Gender (% male)	64.70	57.10	0.78	2
BMI	29.1 (4.80)	28.7 (5.67)	0.75	4
Neck size	40.4 (4.01)	39.3 (4.01)	0.35	4

**t*-test for continuous data, Chi-square or Fisher exact test for categorical data.

Table 2. Baseline sleep parameters in allergic and non-allergic subjects

	Parameter	Baseline			Adjusted difference [§]	P-value
		Allergic [†]	Non-allergic	P-value [‡]		
Fatigue	ESS	11.4 (4.90)	11.3 (6.19)	0.97	−0.2 (1.0)	0.81
	QSQ	158.5 (37.3)	139.4 (46.26)	0.13	−6.9 (7.6)	0.37
	MWT – mean sleep onset	25.99 (12.4)	23.82 (11.3)	0.52	3.2 (3.4)	0.36
Snoring	TST% snoring	40.75 (21.1)	50.16 (19.1)	0.1	10.1 (5.1)	0.09
	Snoring	6.8 (1.5)	7.4 (1.9)	0.26	0.75 (0.75)	0.32
Apnoea–hypopnoea	AHI TST	28.37 (17.8)	27.7 (12.9)	0.87	−2.6 (3.1)	0.41
	Compound AHI TST	35.5 (18.1)	34.1 (12.5)	0.75	−2.8 (2.8)	0.32
	AHI Supine	55.85 (38.0)	35.8 (18.6)	0.02*	−16.2 (5.3)	< 0.01**
Oxygen Saturation	Min SaO ₂ Nadir	86.42 (6.08)	85.45 (5.19)	0.55	2.9 (1.5)	0.06
	%TST SaO ₂ < 90%	1.12 (1.81)	1.51 (2.64)	0.56	−1.3 (1.3)	0.32
Sleep Architecture	Sleep efficiency	84.65 (11.15)	86.3 (5.98)	0.48	−0.6 (3.0)	0.83
	AI total	39.9 (16.4)	39.4 (12.8)	0.91	0 (3.1)	0.99
	AI resp	33.06 (17.8)	30.7 (11.8)	0.56	−2.4 (2.9)	0.43
	AI spontaneous	6.08 (4.44)	6.50 (4.27)	0.73	0.2 (1.1)	0.82
	%REM	15.12 (4.445)	14.4 (3.86)	0.52	0.0 (1.6)	0.99
	%Stage 1	17.06 (7.76)	16.8 (5.7)	0.9	−1.9 (2.0)	0.36
	%Stage 2	54.12 (6.74)	55.76 (7.14)	0.41	3.0 (2.8)	0.29
	%Stage 3	13.69 (6.58)	13.04 (6.16)	0.72	−1.1 (1.5)	0.46

[†]Indicates that the value is statistically significantly different from 0, i.e. for ESS in allergic subjects, the ESS score was statistically significantly lower than the pre-treatment ESS score.

[‡]Mean and (SD) in allergic patients.

[§]Adjusted difference (SD) and P-value in baseline values between allergic and non-allergic subjects. Adjusted for age, BMI, and sex.

TST, total sleep time.

Bold text and asterisks indicate statistically significant differences. * $P < 0.05$, ** $P < 0.01$.

Baseline values for AHI (apnoeas + hypopnoeas), compound AHI (apnoeas + hypopnoeas + respiratory effort-related arousals), oxygen saturation, and sleep architecture were similar in the allergic and non-allergic groups (Table 2). The parameters presented in Table 2 did not change appreciably after adjustment for age, sex, and BMI (Table 2). However, AHI in the supine body position was higher in the allergic subjects than in the non-allergic group (56 vs. 36, $P < 0.01$).

At baseline, the number of EG2⁺ eosinophils was higher at all three sites in the allergic subjects in comparison to the non-allergic subjects. In addition, the number of CD4⁺ lymphocytes was significantly higher and the number of CD8⁺ lymphocytes was significantly lower in the nasopharynx in allergic subjects compared with non-allergic subjects. No other statistically significant differences in inflammatory cell populations were observed at baseline for the other markers considered, CD68 and elastase (Table 3). These results did not change appreciably when we adjusted for age, sex, and BMI.

Impact of inhaled corticosteroid treatment on sleep parameters

After treatment, the allergic group showed improvement in several indices of sleep-disordered breathing (Table 4).

The lowest level to which oxygen saturation fell during sleep (nadir) was statistically significantly lower ($P = 0.016$) in the non-allergic group compared with the allergic patients (Fig. 1a). In addition, treatment reduced the AHI in the supine body position in allergic subjects significantly more than it did in non-allergic subjects ($P = 0.05$), and this improvement persisted even after adjusting for age, BMI, and sex (Fig. 1b). As a result, the post-treatment supine AHI values (initially worse in the allergic group) were similar in the two groups post-treatment. Other sleep parameters showed no statistically significant differences in terms of the change from baseline in allergic vs. non-allergic subjects (Table 4). Treatment improved subjective fatigue and quality-of-life as measured by the ESS and QSQ questionnaires. There was a trend towards increased sleep latency in the entire cohort ($P = 0.12$). Sleep efficiency increased (borderline statistically significant, $P = 0.06$), and time spent in stage 3 sleep decreased ($P < 0.01$) in allergic patients after treatment (Table 4).

Nasal symptoms

Nasal symptom scores decreased by 0.3 (95% CI: −0.5, −0.1) for each week of the study. Allergic subjects had somewhat higher nasal symptom scores before treatment, but this was not statistically significant, nor was

Table 3. Baseline inflammatory parameters in allergic and non-allergic subjects

		Baseline				
		Allergic (<i>n</i> = 28)		Non-allergic (<i>n</i> = 18)		
Marker	Site	Mean	SD	Mean	SD	<i>P</i> -value [†]
CD4	Inferior turbinate	25.03	11.8	24	7.82	0.706
CD4	Nasopharynx	21.87	8.74	14.38	6.62	0.001***
CD4	Uvula	9.26	3.82	8.57	5.62	0.6283
CD68	Inferior turbinate	13.32	7.13	11.9	6.6	0.4659
CD68	Nasopharynx	8.26	6.31	9.71	4.84	0.3519
CD68	Uvula	3.84	3.68	4.71	3.62	0.4
CD8	Inferior turbinate	12.1	7.17	11.24	6.22	0.6484
CD8	Nasopharynx	4.97	3.77	14.29	5.51	< 0.0001***
CD8	Uvula	4.58	2.78	4.52	3.87	0.9541
EG2	Inferior turbinate	11.52	4.23	3.19	1.57	< 0.0001***
EG2	Nasopharynx	6.16	2.67	1.14	1.11	< 0.0001***
EG2	Uvula	3.1	1.83	0.33	0.66	< 0.0001***
Elastase	Inferior turbinate	8.35	4.25	7.95	4.55	0.7497
Elastase	Nasopharynx	5.74	4.58	6.1	3.86	0.7653
Elastase	Uvula	4.71	2.37	6.14	3.18	0.0873

[†]*P*-value for testing the difference between allergic and non-allergic subjects at baseline.

Bold text and asterisks indicate statistically significant differences ****P* < 0.001.

Table 4. Post-treatment sleep parameters in allergic and non-allergic subjects

	Parameter	Post-treatment			Change [†]		
		Allergic	Non-allergic	<i>P</i> -value	Allergic	Non-allergic	<i>P</i> -value [‡]
Fatigue	ESS	9.52 (4.83)	9.39 (4.42)	0.93	−1.4 (3.3)*	−1.22 (2.80)	0.85
	QSQ	175.0 (32.17)	169.1 (35.0)	0.58	10.3 (21.7)*	14.1 (22.7)*	0.59
	MWT – mean sleep onset	29.8 (12.7)	26.92 (13.0)	0.46	3.59 (9.75)	0.85 (12.34)	0.43
Snoring	TST% snoring	49.00 (23.1)	48.40 (19.8)	0.92	10.1 (18.8)*	0.32 (17.9)	0.09
	Snoring	6.83 (1.53)	7.44 (1.95)	0.28	−1.5 (2.2)*	−1.89 (2.40)*	0.56
Apnoea–hypopnoea	AHI TST	22.4 (15.2)	26.7 (11.3)	0.27	−2.1 (8.0)	−0.4 (12.2)	0.61
	Compound AHI TST	30.3 (15.3)	34.4 (8.84)	0.25	−1.5 (7.8)	0.2 (10.8)	0.56
	AHI supine	39.7 (23.9)	40.3 (16.6)	0.92	−11.0 (20.6)	3.3 (15.0)	0.01**
Oxygen Saturation	Min SaO ₂ Nadir	88.8 (3.40)	85.5 (4.78)	0.016*	1.8 (4.2)*	−0.3 (5.3)	0.15
	%TST SaO ₂ <90%	1.30 (4.3)	2.78 (3.9)	0.24	0.5 (4.5)	1.8 (3.3)*	0.33
Sleep Architecture	Sleep efficiency	87.0 (9.2)	89.1 (5.5)	0.34	2.6 (11.4)	2.7 (5.0)*	0.97
	AI total	36.5 (12.1)	39.9 (8.46)	0.27	−0.4 (9.6)	−1.5 (11.3)	0.73
	AI resp	28.3 (13.9)	32.07 (7.82)	0.24	−1.3 (8.9)	0.08 (10.6)	0.64
	AI spontaneous	7.03 (4.8)	6.68 (3.5)	0.78	0.5 (2.9)	−0.1 (4.3)	0.58
	%REM	15.5 (3.9)	14.8 (4.0)	0.56	0.2 (5.0)	0.1 (5.3)	0.97
	%Stage 1	18.5 (6.4)	19.3 (8.4)	0.72	1.3 (5.0)	2.7 (8.0)	0.52
	%Stage 2	54.9 (8.21)	54.2 (7.1)	0.75	1.6 (7.5)	−1.8 (10.6)	0.25
	%Stage 3	11.1 (6.6)	11.7 (5.7)	0.75	−3.1 (4.9)*	−1.1 (5.7)	0.22

[†]Indicates that the value is statistically significantly different from 0, i.e. for ESS in allergic subjects, the ESS score was statistically significantly lower than the pre-treatment ESS score.

[‡]*P*-value for testing the difference in the change from baseline in allergic and non-allergic patients.

TST; total sleep time, ESS, Epworth Sleepiness Scale; QSQ, Quebec Sleep Questionnaire; MWT, maintenance of wakefulness test; AHI, apnoea–hypopnoea index.

Bold text and asterisks indicate statistically significant differences. **P* < 0.05, ***P* < 0.01.

there any evidence that the change over time differed by allergy status (not shown). However, there was some evidence that treatment worked better in allergic subjects (Fig. 2a), as the percentage of time spent snoring was initially higher in allergic subjects at baseline, but this was brought down to the same level as non-allergic

patients after treatment. Nasal symptom scores decreased throughout the protocol both as an effect of time (*P* < 0.05) and as a treatment effect (*P* < 0.05) in both allergic and non-allergic groups, with a trend for a better effect of treatment in the allergic group (*P* = 0.056; Fig. 2b).

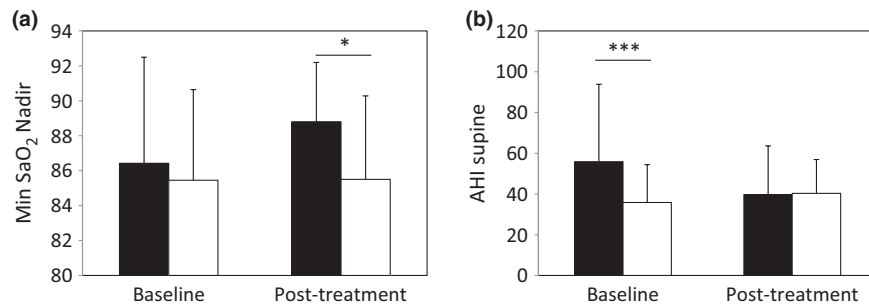


Fig. 1. Effect of treatment on sleep parameters. Change in minimum SaO_2 nadir and apnoea–hypopnoea index supine as a result of intranasal steroid treatment in allergic subjects (black bars), but not in non-allergic subjects (white bars). Statistically significant effects are indicated by * $P < 0.05$ and *** $P < 0.001$.

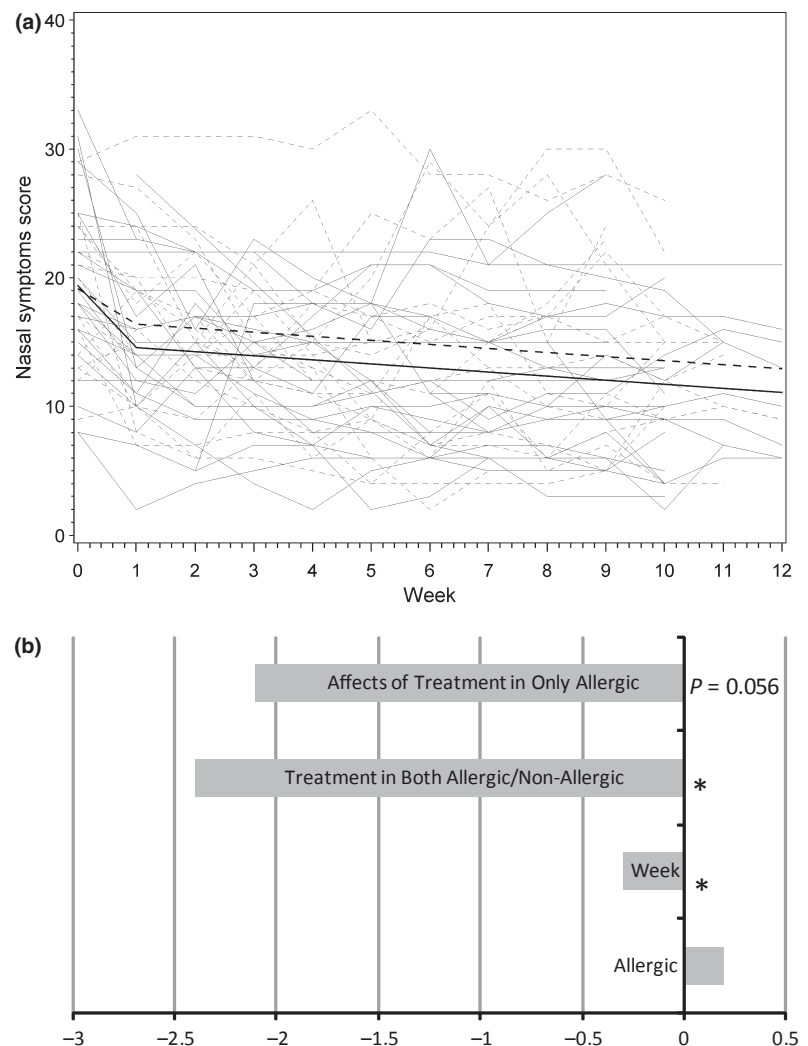


Fig. 2. Effect of treatment on nasal symptoms. (a) Individual (thin lines) and predicted (thick lines) trajectories of nasal symptom scores over time for allergic (solid lines) and non-allergic (dashed lines) subjects. (b) Nasal symptom scores by regression covariates. *Indicates a statistically significant effect ($P < 0.05$).

Impact of inhaled corticosteroid treatment on inflammatory markers

Strong evidence was found for an effect of inhaled corticosteroid treatment on EG2^+ eosinophils (Table 5). The numbers of these cells were higher in allergic patients at all three sites compared with what was observed in the

non-allergic patients before ($P < 0.0001$) and after treatment ($P < 0.05$), inhaled corticosteroids significantly reduced eosinophilia in the allergic patients ($P < 0.0001$) at all three sites, but only in the nasopharynx for non-allergic patients ($P < 0.001$). The effect of treatment on EG2^+ cells was statistically different ($P < 0.0001$) in the allergic and non-allergic groups (Fig. 3a; Table 5).

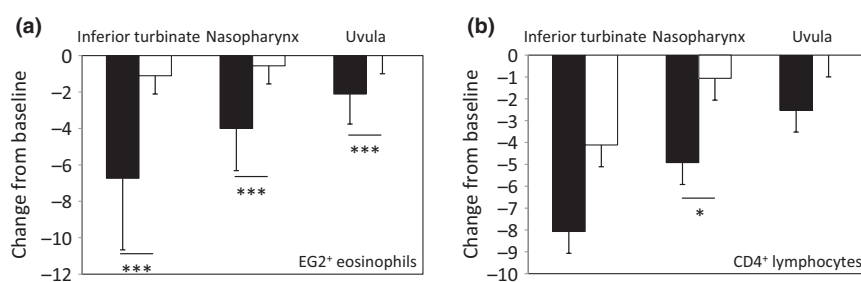


Fig 3. Effect of treatment on upper airway inflammation. Changes in the numbers of (a) CD4⁺ lymphocytes and (b) EG2⁺ eosinophils in the inferior turbinate, nasopharynx, and uvula in allergic subjects (black bars) and non-allergic subjects (white bars). Statistically significant effects are indicated by * $P < 0.05$ and *** $P < 0.001$.

Table 5. Post-treatment inflammatory parameters in allergic and non-allergic subjects

Marker	Site	Post-treatment						Change					
		Allergic	SD	Non-allergic	SD	P -value [†]	Allergic [‡]	SD	P -value [¶]	Non-allergic [§]	SD	P -value ^{††}	P -value ^{‡‡}
CD4	Inferior turbinate	17.89	6.7	18.72	6.75	0.6857	-8.07	9.57	< 0.01**	-4.11	6.45	0.02*	0.1
CD4	Nasopharynx	16.52	5.83	14.22	5.26	0.1778	-4.92	5.75	< 0.01**	-1.06	5.31	0.41	0.03*
CD4	Uvula	7.14	3.58	8.39	4.41	0.3225	-2.52	3.69	< 0.01**	0	4.64	1	0.06
CD68	Inferior turbinate	13.46	5.26	7.89	4.06	0.0002***	-0.19	6.28	0.88	-4.11	5.91	0.01*	0.04*
CD68	Nasopharynx	7.96	3.57	10	5.34	0.1666	0.42	4.34	0.62	0	4.49	1	0.76
CD68	Uvula	2.57	2.71	2.89	2.91	0.713	-1.78	3.88	0.02*	-1.61	3.87	0.1	0.89
CD8	Inferior turbinate	10.14	5.22	10.17	6.4	0.9895	-1.41	6.7	0.28	-0.78	9.18	0.72	0.8
CD8	Nasopharynx	6.26	3.69	10.44	2.36	< 0.0001***	1.58	3.55	0.03*	-4.39	4.83	< 0.01**	< 0.01**
CD8	Uvula	4.39	2.57	4.17	3.09	0.7979	-0.15	3.38	0.82	-0.83	5.66	0.54	0.65
EG2	Inferior turbinate	5.14	3.24	2.28	2.85	0.0031**	-6.74	3.93	< 0.0001***	-1.11	3.08	0.14	< 0.00001***
EG2	Nasopharynx	2.48	2.08	0.67	1.08	0.0004***	-4	2.32	< 0.0001***	-0.56	0.86	0.01**	< 0.00001***
EG2	Uvula	1.21	1.37	0.39	0.85	0.0154*	-2.11	1.65	< 0.0001***	0	0.91	1	< 0.00001***
Elastase	Inferior turbinate	8.14	4.66	9.39	4.24	0.3558	0.3	4.32	0.72	2.39	3.79	0.02*	0.09
Elastase	Nasopharynx	8.48	4.43	7.94	4.25	0.6854	2.96	3.26	< 0.0001***	1.67	4.61	0.14	0.31
Elastase	Uvula	5.14	2.1	5.11	1.78	0.9564	0.26	2.71	0.62	-1.17	2.83	0.1	0.1

[†] P -value for testing the difference between allergic and non-allergic subjects post-treatment.

[‡]Based on 26 or 27 subjects.

[§]Based on 18 subjects.

[¶] P -value for change in marker from baseline in the allergic subjects.

^{††} P -value for change in marker from baseline in the non-allergic subjects.

^{‡‡} P -value for difference in change from baseline in allergic vs. non-allergic subjects.

Bold text and asterisks indicate statistically significant differences. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

The next strongest evidence was for CD4⁺ lymphocytes. At baseline, there were more CD4⁺ lymphocytes in the nasopharynx in allergic subjects vs. non-allergic subjects ($P < 0.001$). After treatment, there were no longer any significant differences in CD4⁺ cell counts at any site between the allergic and non-allergic subjects ($P > 0.05$); this translated into a better CS treatment effect in the allergic patients ($P = 0.03$; Fig. 3b). Treatment significantly reduced CD4⁺ lymphocyte numbers at all sites in the allergic patients ($P < 0.01$; Table 5).

Overall, CD4⁺ and EG2⁺ cell numbers decreased at all sites post-treatment compared with baseline. This pattern was also observed in allergic subjects, although the reduction in these inflammatory cell types was more

profound. There was evidence that the decrease in inflammation in allergic subjects differed from the decrease in non-allergic subjects (Table 5). There was less evidence for an effect of inhaled corticosteroids on the other markers assessed in this study (CD8, CD68, and elastase) in both the allergic and non-allergic groups of patients (Table 5).

Adverse events

No patients had to stop due to bleeding or dryness or other intolerance to intranasal CS. One case of nosebleed occurred after biopsy that was stopped with AG nitrate cauterization.

Discussion

In this study, the differences in the impact of corticosteroid treatment on OSAS symptoms and nasal inflammation were investigated in allergic and non-allergic OSAS patients. Following treatment, our results showed a significant improvement in OSAS symptoms and reduced upper airway inflammation in the entire cohort, but these changes were more marked in allergic subjects compared with non-allergic patients. Various symptoms such as fatigue, hypersomnolence, decreased alertness, or diminished productivity have been associated with AR, and have long been interpreted as side-effects of allergy medication. However, some studies now report that this diminished quality-of-life may be linked with AR-induced nasal congestion causing sleep fragmentation [24]. The use of nasal decongestants and CS, through their effect on nasal airflow resistance, has been controversially reported to diminish OSAS symptom severity [25–27]. It has also been suggested that many inflammatory mediators associated with allergic rhinitis may directly influence nocturnal sleep [24], and a number of studies have also found an association between inflammation and sleep disturbances [28, 29]. However, few studies have characterized the inflammatory profile in upper airway tissues of patients with OSAS [30].

Intranasal CS have been reported to reduce nasal congestion and daytime sleepiness, to improve sleep and quality-of-life compared with placebo in patients with AR [31]. However, these improvements were mostly obtained through subjective assessments (i.e. questionnaires) and correlations with objective measurements are lacking. The results of this study confirm these previous findings, and demonstrate that treatment with intranasal mometasone led to objective improvements in nasal symptom scores and subjective improvements in daytime sleepiness in all subjects, regardless of AR comorbidity. However, the extent to which reductions in the number of apnoea and hypopnoea events (AHI) can be achieved by medication remains controversial [31]. As seen in this study, treatment with intranasal corticosteroids resulted in a number of improvements in sleep parameters in all subjects, especially in terms of subjective sleep assessments, snoring, and oxygen saturation. However, these improvements were more profound in the allergic group. In addition, the allergic group showed a statistically significant improvement in the supine AHI score compared with non-allergic subjects after a course of intranasal corticosteroids. A recent study investigating the impact of inhaled mometasone therapy in patients with allergic rhinitis did not show the same improvement in AHI observed in this study; however, improvements in other sleep parameters, including nasal symptoms and

subjective sleep assessments were seen [32]. One possible explanation of the variable impact of nasal steroid treatment on AHI may be related to the duration of treatment.

In this study, we observed, in our baseline assessment, that OSAS patients with AR demonstrated higher numbers of CD4⁺ lymphocytes, but fewer CD8⁺ lymphocytes in the nasopharynx and significantly increased eosinophils numbers were found at all three investigated biopsy sites compared with non-AR OSAS patients, confirming that allergic-type inflammation of the upper respiratory tissues is a clinical feature of allergic rhinitis. We have previously reported increased inflammation in the upper airways in OSAS patients. Specifically, Boyd et al. [33] demonstrated that OSAS patients had significantly greater inflammatory cell infiltration within the mucosal and muscular compartments of the upper airways, both of which were characterized by substantial increases in activated (CD25⁺) T lymphocytes.

Corticosteroid treatment also had significant effects on the degree of inflammation in all OSAS patients, although these changes were more profound and widespread in OSAS patients with allergic rhinitis. In the group of patients with AR, intranasal CS treatment reduced the numbers of eosinophils and CD4⁺ T-helper cells at all three investigated sites, an effect which was not observed to the same extent in the non-allergic group of patients. This study supports previous findings showing reduced nasal inflammation in response to CS treatment [17, 19], with the added novelty of demonstrating the broad effect of CS treatment on multiple cell types (eosinophils and T-helper lymphocytes) at multiple sites in the upper airways in patients with AR. It should also be mentioned that CD4 and CD8 cells may be influenced by neurogenic inflammation in OSA, and this component is less influenced by INCS [34, 35].

Although the precise relationship between upper airways inflammation and sleep disturbances is currently unclear, it has been shown that increased nasal resistance due to inflammation plays a role in sleep-disordered breathing [14]. A previous study investigating the association between inflammatory cell subsets in the peripheral blood and the severity of sleep apnoea showed no correlation between lymphocyte numbers in the blood and AHI [36]. Taken together, these results support the notion that peripheral blood inflammation may have little predictive power for determining the severity of OSAS symptoms; instead, a more invasive evaluation of inflammation at the site of the disease (upper airway tissues) may be required to effectively investigate the relationship between inflammation and the severity of sleep apnoea.

This study has limitations, specifically the lack of a healthy control group, a group with snoring only, as

well as placebo treatment control groups of OSAS patients with and without AR. Regarding the first point, an attempt was made to recruit healthy control subjects and subjects with snoring for this study, but this proved to be quite difficult given the invasive nature of the assessments used. The absence of a placebo group was necessary, given the number of available subjects, to meet the statistical criteria to ensure the detection of significant differences. These shortcomings will be addressed in subsequent studies.

In conclusion, this study has demonstrated that OSAS is associated with a variable inflammatory cell profile in patients with allergic compared with non-allergic patients at multiple sites in the upper airways; treatment with intranasal corticosteroids markedly improves the inflammatory profile in OSAS patients with AR; and clinical improvements were more profound in OSAS patients with AR comorbidity following treatment with CS. This study has provided important molecular and clinical evidence regarding the ability of corticosteroids to reduce upper airway inflammation and improve obstructive sleep apnoea syndrome morbidity patients with concomitant allergic rhinitis.

Author contributions

F.L. conducted the biopsies and nasal assessments and initiated the draft of the manuscript. B.J.P. and N.D. performed all the sleep assessments. Q.H. supervised the analysis of inflammatory markers. J.R.J. performed data analysis, prepared the figures and tables, and was involved in preparation of the manuscript. P.L. developed the scoring sheets for the clinical data, provided clinical support, and performed data collection. N.B., G.-O.K., M.A., and C.G. performed ICC and histological analysis. A.B. and D.H. performed the statistical analysis. Q.H., F.L., and B.J.P. designed and supervised the study. All authors provided intellectual contributions and approved the final draft of the manuscript.

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Conflicts of interest

None of the authors have a conflict of interest to report.

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