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MAIN ARTICLE

Effect of modern surgical treatment on the inflammatory/anti-inflammatory balance in patients with obstructive sleep apnoea

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Abstract

Objective: To investigate the inflammatory/anti-inflammatory cytokine balance – T helper 1/T helper 2 ratios – in obstructive sleep apnoea patients, before and after treatment.

Methods: Twenty-eight patients received continuous positive airway pressure treatment and 29 patients who could not tolerate continuous positive airway pressure were scheduled for surgery. Serum levels of interleukins 2, 4 and 10, tumour necrosis factor-alpha, and interferon gamma were analysed by enzyme-linked immunosorbent assays before and three months after treatment.

Results: The success rate of surgical treatment was 65.5 per cent. Mean compliance for the continuous positive airway pressure group was 40.9 per cent. The apnoea/hypopnoea index significantly decreased in both groups after treatment ($p < 0.001$). The interferon gamma/interleukin-4 ratio decreased following surgical treatment ($p = 0.014$), and the interleukin-2/interleukin-4 ratio decreased after treatment in 57 patients in the overall cohort ($p = 0.032$).

Conclusion: After treatment for obstructive sleep apnoea, some ratios reflecting T helper 1/T helper 2 cytokine balance favoured the T helper 2 direction, suggesting a shift to an anti-inflammatory state. Successful surgery and better continuous positive airway pressure compliance can help ameliorate inflammation in obstructive sleep apnoea patients, which may reduce associated morbidities.

Key words: Obstructive Sleep Apnea; Inflammation; Surgical Procedures; Operative; Continuous Positive Airway Pressure; Cytokines

Introduction

Obstructive sleep apnoea (OSA) is a sleep disorder characterised by repetitive episodes of partial or complete upper airway collapse during sleep. These episodes result in intermittent hypoxia and oxyhaemoglobin desaturations.[1,2] The chronic intermittent hypoxia occurring in OSA stimulates transcription factors, such as nuclear factor κB, which trigger the production of proinflammatory mediators.[3,4] Cytokine dysregulation, inflammation and oxidative stress are thought to be the main causes of the cardiovascular morbidity and mortality observed in patients with OSA.[1,5]

Proinflammatory cytokines, such as interleukin (IL)-1β, IL-6 and tumour necrosis factor-α, form a bridge between innate immunity and adaptive immunity, and significant elevations in the serum levels of these markers have been seen in patients with OSA.[6–9] During the activation of adaptive immunity, the anti-inflammatory process is triggered to limit immune activation.

Cytokine profiles divide T helper cells into two basic functional subgroups : T helper 1 (Th1) lymphocytes, which secrete predominantly IL-2 and interferon-γ, and T helper 2 (Th2) lymphocytes, which secrete IL-4 and IL-10.[10] The IL-2 and interferon-γ cytokines produced by Th1 cells have inflammatory properties, whereas IL-4 and IL-10, which control and suppress inflammation, have anti-inflammatory properties.[11] Interferon-γ and IL-10 also regulate activation of these cytokines by inhibiting the proliferation of Th2 clones and Th1 clones, respectively.[10]

Many studies have compared serum levels of inflammatory markers before and after continuous positive airway pressure (CPAP) treatment in patients with OSA.[7,12–22] However, very few studies have examined the effects of surgical treatment on systemic

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inflammation in OSA.[9,23,24] The present study aimed to investigate the inflammatory/anti-inflammatory cytokine balance in patients with OSA before and after surgical treatment, as well as during CPAP treatment. We hypothesised that if surgical therapy is effective, as confirmed by post-operative polysomnography, then the inflammatory process ought to be interrupted as well. By contrast, if the inflammation continues despite significant improvements in OSA severity after surgery, this would suggest that the inflammation process in OSA depends on other underlying pathologies, which need to be taken into consideration.

This study is the first in the English-language literature to investigate the effect of relatively new reconstructive surgical techniques on systemic inflammation, and to assess the effect of treatment on the Th1/Th2 cytokine balance, in adult patients with OSA.

Materials and methods

Subjects

Sixty patients with newly diagnosed OSA were prospectively enrolled in the study at a tertiary referral hospital. All subjects were evaluated with a history review, a detailed physical examination, blood tests (including glucose, liver enzyme, lipid level, renal function and thyroid function tests), chest radiography and electrocardiography.

Patients were excluded if they had chronic pulmonary disease, heart disease, inflammatory disease, renal disease, rheumatological disease, hypertension, diabetes mellitus, dyslipidaemia, a history of OSA surgery, central sleep apnoea predominance in polysomnography, long-term use of medications, were aged less than 18 years or more than 65 years, or had a body mass index (BMI) of more than 35 kg/m^2.

Snoring was subjectively evaluated using a visual analogue scale (VAS), and daytime sleepiness symptoms were measured with the Epworth Sleepiness Scale, before and after treatment.

Patients were divided into two groups according to treatment: a CPAP group and a surgery group. The local ethics committee approved the study protocol, and all participants provided written informed consent.

Surgery group

The surgery group consisted of 30 prospectively enrolled patients with OSA who could not tolerate or were unwilling to use CPAP. The type of surgery was determined based on the clinical findings, anatomical properties and upper airway characteristics of the patients. Twenty-one patients underwent expansion sphincter pharyngoplasty, five underwent anterior palatoplasty, one underwent submucosal minimally invasive lingual excision, two underwent expansion sphincter pharyngoplasty with submucosal minimally invasive lingual excision, and one underwent anterior palatoplasty with submucosal minimally invasive lingual excision.

Post-operative polysomnography was performed three months after surgery. Surgical success was defined as a more than 50 per cent reduction of the apnoea/hypopnoea index, to fewer than 20 events per hour. An OSA cure was defined as a post-operative apnoea/hypopnoea index of fewer than 5 events per hour. The surgery group was divided into two subgroups based on surgical success: successful and non-successful surgery groups.

The anterior palatoplasty procedure was suggested to patients if they had: a redundant soft palate, smaller tonsils (grade 0–2), a vertical or intermediate shaped palate, and primarily antero-posterior velopharyngeal collapse. A 4×1 cm horizontal rectangular strip of mucosa was excised from the soft palate and then the stripped area was sutured with 10–15 vicryl sutures. The muscle layer was completely protected in this procedure. None of these patients underwent tonsillectomy or any other procedures concomitantly.

The expansion sphincter pharyngoplasty procedure was suggested to patients if they had: lateral pharyngeal wall hypertrophy, grade 0–4 tonsil size, Friedman tongue position 1 to 2, an oblique-shaped palate and narrowing at the distal velopharyngeal sphincter. First, a bilateral tonsillectomy was performed. Then, the palatopharyngeus muscle was identified; its inferior end was transected horizontally, rotated superolaterally, isolated from an inferior to superior direction by monopolar cautery, and left with its superior part attached to the posterior horizontal pharyngeal constrictor muscles. For the rotation of the palatopharyngeus muscle, a tunnel was prepared through an incision made on the anterior surface of the soft palate medial to the hamulus. The first suture for attachment of the muscle was a submucosal, horizontal mattress suture, and the second one was a vertical mattress suture to the fibrous tissues. Finally, the anterior and posterior tonsillar pillars were opposed with vicryl sutures.

In the submucosal minimally invasive lingual excision technique, the intraoral surgical field was visualised with a 30° rigid telescope. For tongue retraction, one retention suture was placed in the midline to pull the tongue anteriorly. After a midline incision was performed by monopolar cautery, two additional traction sutures were placed on the lateral edges of the incision. Submucosal lingual tonsil tissue was removed using a coblator posteriorly and laterally, avoiding mucosal damage to the tongue. After tissue resection was completed, two or three vicryl sutures were placed anteriorly to prevent bulging and to reduce dead space in the midline. The posterior tongue wound was left open to allow any drainage of minimal bleeding or fluids, and to prevent the possibility of any collection that may risk airway safety.

Continuous positive airway pressure group

Thirty newly diagnosed OSA patients, who had no history of prior OSA treatment, were prospectively

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enrolled. Three months of data recorded during the treatment period were obtained from the patients' devices to determine CPAP compliance (percentage of days with at least 4 hours' CPAP use per night). After the determination of mean compliance, the CPAP group was divided into two subgroups: a successful compliance group, comprising patients whose compliance was above the mean compliance value, and a non-successful compliance group, comprising patients whose compliance was below the mean compliance value.

Sleep study

Polysomnography, conducted using a Comet AS40-Plus Amplifier System (Grass Technologies, Astro-Med, West Warwick, Rhode Island, USA), was performed and scored according to the American Academy of Sleep Medicine 2012 guidelines.[25] The apnoea/hypopnoea index, lowest oxygen saturation, mean oxygen saturation, oxygen desaturation index and the percentage of sleep time with oxygen saturation below 90 per cent were recorded.

Inflammatory markers

Peripheral blood samples were obtained from the surgery group before surgery and three months after surgery, and were obtained from the CPAP group before treatment and after three months' recording with the CPAP device. Blood was collected into tubes between 8 and 9 am, and centrifuged at 3000 revolutions per minute for 10 minutes. The supernatant serum was stored at −80 °C in the laboratory until analysis. The levels of all markers were determined by enzyme-linked immunosorbent assays, using the following kits: Human IL-2/IL-4/IL-10/TNF-α (Orgenium, Vantaa, Finland) and Human Interferon-γ (DIA Source, Louvain-la-Neuve, Belgium).

Statistical analysis

Statistical analyses were performed using SPSS for Windows software, version 15.0 (SPSS, Chicago, Illinois, USA). Student's *t*-test was used for the comparison of continuous variables between two groups. Comparisons between more than two groups were assessed by analysis of variance. The comparison of discrete variables between groups was conducted using Fisher's exact test or Pearson's chi-square test. The dependent samples *t*-test for continuous variables and the McNemar test for discrete variables were used for the comparison of dependent groups. Correlations between variables were explored using the Spearman correlation test. Significance was defined as $p < 0.05$.

Results

Two patients in the CPAP group were unable to provide compliance data; hence, a total of 28 patients in that group completed the study. One patient who underwent anterior palatoplasty did not want to undergo post-operative polysomnography; therefore, a total of 29 patients in the surgery group completed the study.

Of the total 57 patients, 53 (93 per cent) were male and 4 (7 per cent) were female, and their ages ranged from 22 to 64 years (mean ± standard deviation (SD) = 42.7 ± 10.2 years). The mean (± SD) ages for the surgery and CPAP groups were 39 ± 9.5 years and 46.4 ± 9.7 years, respectively; this age difference between the groups was statistically significant ($p = 0.005$). No significant differences were noted between the two groups in terms of BMI, apnoea/hypopnoea index, Epworth Sleepiness Scale score, VAS score, serum levels of cytokines or saturation parameters at baseline (all $p > 0.05$) (Table I).

The mean (± SD) apnoea/hypopnoea index values in the surgery group before and after treatment were 30.1 ± 20.5 events per hour and 13 ± 12.4 events per hour, respectively, and this difference was statistically significant ($p < 0.001$) (Table II). Surgical success was achieved in 19 of the 29 patients (65.5 per cent), with a cure of OSA achieved in 8 of those 19 patients (27.6 per cent in 29 patients). The mean (± SD) apnoea/hypopnoea index values in the CPAP group at baseline and during treatment were 35 ± 13.6 events per hour and 4.1 ± 3.2 events per hour, respectively, and the difference was statistically significant ($p < 0.001$). The apnoea/hypopnoea index improved to fewer than 5 events per hour in 24 patients with

TABLE I
CLINICAL FEATURES, SATURATION PARAMETERS AND INFLAMMATORY MARKERS AT BASELINE

Parameter	Group	Mean	SD	*p*
AHI	Surgery	30.12	20.51	0.288
	CPAP	34.96	13.58	
ESS score	Surgery	10.86	5.71	0.393
	CPAP	12.20	6.20	
VAS score	Surgery	8.93	1.46	0.865
	CPAP	9.00	1.64	
Mean O_2	Surgery	92.26	2.31	0.323
	CPAP	92.80	1.47	
$SaO_2 < 90\%$	Surgery	10.79	13.80	0.409
	CPAP	15.18	20.79	
Oxygen desaturation index	Surgery	31.87	26.38	0.108
	CPAP	22.63	12.61	
Minimum O_2	Surgery	80.54	6.53	0.749
	CPAP	80.00	5.62	
TNF-α (pg/ml)	Surgery	180.33	430.86	0.053
	CPAP	24.33	27.94	
IL-10 (pg/ml)	Surgery	15.87	35.08	0.557
	CPAP	11.25	24.13	
IL-2 (pg/ml)	Surgery	22.29	32.42	0.933
	CPAP	22.91	23.67	
IL-4 (pg/ml)	Surgery	1.77	2.00	0.657
	CPAP	1.59	0.86	
IFN-γ (IU/ml)	Surgery	0.49	0.24	0.113
	CPAP	0.41	0.11	

SD = standard deviation; AHI = apnoea/hypopnoea index; CPAP = continuous positive airway pressure; ESS = Epworth Sleepiness Scale; VAS = visual analogue scale; mean O_2 = mean oxygen saturation; $SaO_2 < 90\%$ = percentage of sleep time with oxygen saturation below 90 per cent; minimum O_2 = lowest oxygen saturation; TNF = tumour necrosis factor; IL = interleukin; IFN = interferon

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TABLE II
CLINICAL FEATURES AND SATURATION PARAMETERS BEFORE AND AFTER TREATMENT

Parameter	Surgery group ($n = 29$)			CPAP group ($n = 28$)		
	Pre-treatment	Post-treatment	p	Pre-treatment	Post-treatment	p
AHI	30.1 ± 20.5	13 ± 12.4	<0.001*	35 ± 13.6	4.1 ± 3.2	<0.001*
ESS score	10.9 ± 5.7	5 ± 3.8	<0.001*	12.3 ± 6.3	6 ± 4.4	<0.001*
VAS score	8.9 ± 1.5	2.3 ± 1.6	<0.001*	9 ± 1.6	2.9 ± 1.6	<0.001*
Mean O_2	92.3 ± 2.3	93.7 ± 1.7	0.524	92.8 ± 1.5	92.3 ± 4.5	0.34
Minimum O_2	80.5 ± 6.5	85.5 ± 3.7	0.013*	79.7 ± 5.4	83.8 ± 5.4	0.031*
SaO_2 < 90%	10.8 ± 13.8 6.4 (0.4–61.1)†	2.7 ± 3.4 1.8 (0–11.5)†	0.088	15.2 ± 20.8 7.8 (0.1–80.2)†	11.9 ± 19.2 3 (0–65.2)†	<0.001*
Oxygen desaturation index	31.9 ± 26.4	11.4 ± 12.8 6.3 (0.4–45.1)†	0.029*	22.9 ± 12.8	9.7 ± 9.5	0.416

Data represent means ± standard deviations, unless indicated otherwise. *Indicates statistical significance ($p < 0.05$). †Median (range) values. CPAP = continuous positive airway pressure; AHI = apnoea/hypopnoea index; ESS = Epworth Sleepiness Scale; VAS = visual analogue scale; mean O_2 = mean oxygen saturation; minimum O_2 = lowest oxygen saturation; SaO_2 < 90% = percentage of sleep time with oxygen saturation below 90 per cent

CPAP treatment (85.7 per cent). The mean compliance was 40.9 per cent (range, 12.8–91.4 per cent) and the mean daily usage time of CPAP was 4 hours and 59 minutes.

Both surgical and CPAP treatments resulted in significant decreases in Epworth Sleepiness Scale scores, VAS scores and lowest oxygen saturation levels. In addition, the surgical treatment significantly decreased oxygen desaturation index levels ($p = 0.029$), and the CPAP treatment significantly improved the percentage of sleep time with oxygen saturation below 90 per cent ($p < 0.001$) (Table II). No significant BMI changes were noted after treatment in the study population overall.

Surgical treatment resulted in a significant decrease in serum interferon-γ levels ($p = 0.043$). However, the reduction in the serum levels for this marker was not statistically significant in the CPAP group. The serum levels of the other inflammatory markers did not change significantly in either group (Table III). In the CPAP group, the tumour necrosis factor-α decrease was significantly higher in the patients with a BMI above 30 kg/m^2 than in those with a BMI of less than 30 kg/m^2, independent of apnoea/hypopnoea index ($p = 0.014$). In the surgery group, the VAS scores were negatively correlated with serum interleukin (IL)-10 levels ($r = -0.386$, $p = 0.039$).

The successful and non-successful surgery groups consisted of 19 and 10 patients, respectively, and the successful and non-successful CPAP compliance groups consisted of 12 and 16 patients, respectively (Figure 1). The improvements in apnoea/hypopnoea index, VAS scores, Epworth Sleepiness Scale scores, mean oxygen saturation, lowest oxygen saturation and oxygen desaturation index levels were statistically significant in the successful surgery group, but only VAS and Epworth Sleepiness Scale scores were significantly improved in the non-successful surgery group. Improvements in the apnoea/hypopnoea index, VAS scores, Epworth Sleepiness Scale scores, lowest oxygen saturation and oxygen desaturation index levels were observed in the successful and non-successful CPAP compliance groups, and these improvements were statistically significant. Successful CPAP compliance also significantly reduced the percentage of sleep time with oxygen saturation below 90 per cent ($p = 0.014$). The change in serum IL-4 levels between the successful and the non-successful

TABLE III
INFLAMMATORY MARKERS BEFORE AND AFTER TREATMENT

Inflammatory marker	Surgery group ($n = 29$)			CPAP group ($n = 28$)		
	Pre-treatment	Post-treatment	p	Pre-treatment	Post-treatment	p
IL-2 (pg/ml)	22.3 ± 32.4 12.2 (0–153)*	17.6 ± 22.7 9.7 (0.58–110.9)*	0.504	22.9 ± 23.7 15.6 (0–90.2)*	13.9 ± 9.9	0.112
IL-4 (pg/ml)	1.77 ± 2 1.08 (0.37–9.1)*	2.38 ± 2.89 1.71 (0.49–16.08)*	0.178	1.59 ± 0.86	1.55 ± 0.98	0.767
IL-10 (pg/ml)	15.9 ± 35.1 3.8 (2.9–146.28)*	12.8 ± 23.6 3.4 (2.9–117.4)*	0.582	11.2 ± 24.1 2.99 (2.9–115.1)*	13.7 ± 28.7 2.94 (2.9–128.2)*	0.387
TNF-α (pg/ml)	180.3 ± 430.9 14.3 (6.7–1901.9)*	62.7 ± 187.6 14 (5.7–1000.7)*	0.155	24.3 ± 27.9 12.5 (6.9–128.4)*	19.1 ± 14.5	0.366
IFN-γ (IU/ml)	0.49 ± 0.24	0.38 ± 0.09	0.043†	0.41 ± 0.11	0.38 ± 0.1	0.448

Data represent means ± standard deviations, unless indicated otherwise. *Median (range) values. †Indicates statistical significance ($p < 0.05$). CPAP = continuous positive airway pressure; IL = interleukin; TNF = tumour necrosis factor; IFN = interferon

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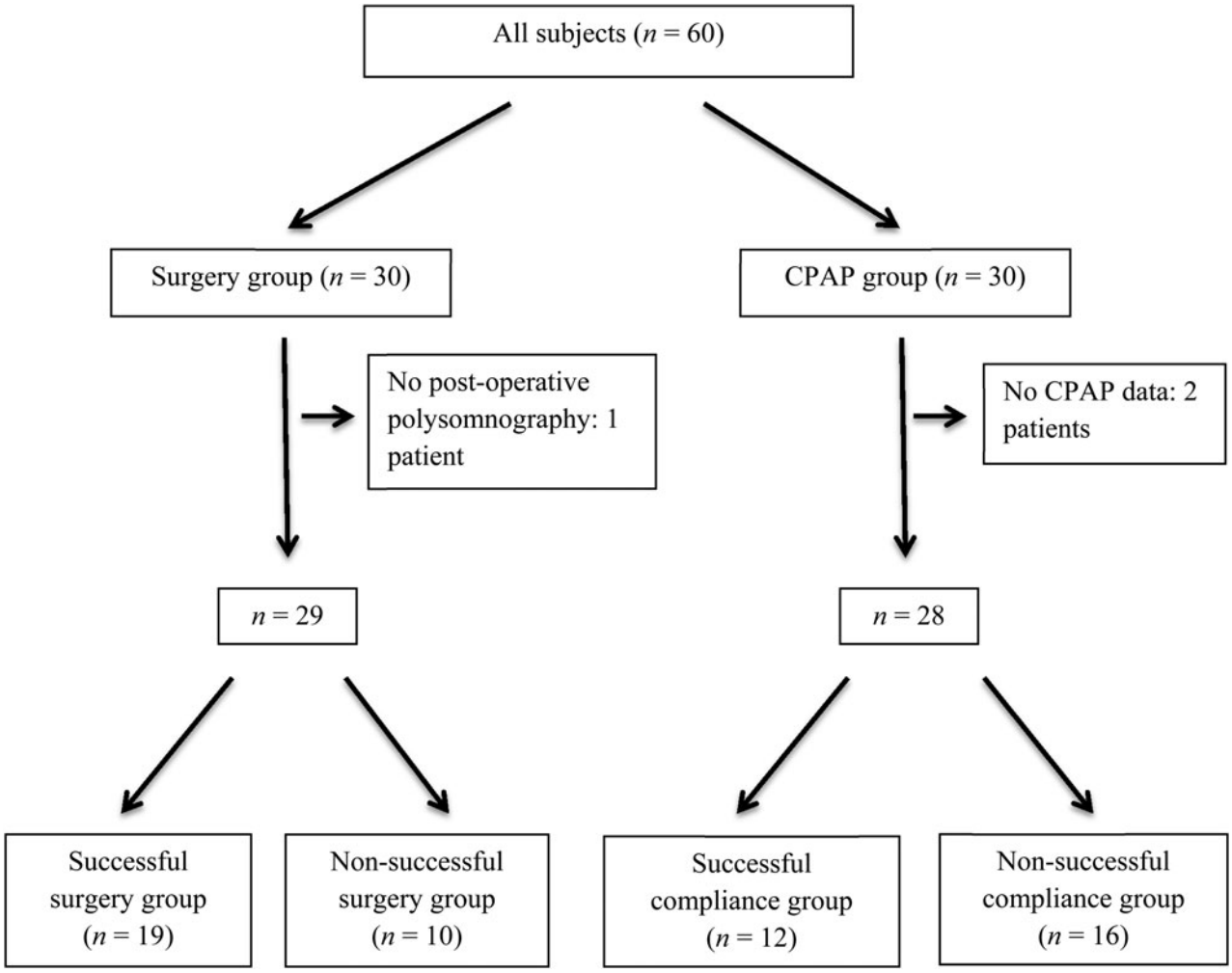

FIG. 1
Flowchart of the patients. CPAP = continuous positive airway pressure

CPAP compliance groups was statistically significant ($p = 0.045$) (Table IV).

After surgery, all six proportional indicators of inflammatory/anti-inflammatory cytokine balance showed decreases. The only significant change was observed in the interferon-γ/IL-4 ratio in the surgery group ($p = 0.014$). After CPAP treatment, four of six proportional indicators of this balance were decreased, while two of the six were slightly increased. No significant differences were observed in the ratio changes in the CPAP group. Analysis of the ratios in all 57 patients, independent of their groups, revealed a reduction in all six ratios, with a statistically significant decrease in the IL-2/IL-4 ratio ($p = 0.032$) (Table V).

When we divided the surgery group into two subgroups according to multilevel or single-level surgical interventions, we found that three patients who underwent multilevel surgery did not show significant improvement in their inflammatory markers. However, single-level surgical treatment resulted in a significant decrease in serum interferon-γ levels ($p = 0.029$) (Table VI).

Discussion

Systemic inflammation has been thought to play a key role in the process that extends co-morbidities in patients with OSA.[5,26] Obtaining an answer to the question 'how does the inflammatory and anti-inflammatory balance change following treatment for OSA?' was the main goal of our work. However, one should take into account that the post-treatment serum levels of inflammatory cytokines evaluated in this study were obtained from a population with a surgical success rate of 65.5 per cent and a mean CPAP compliance of 40.9 per cent. This result should be considered when interpreting the changes in inflammatory and anti-inflammatory markers.

Tumour necrosis factor-α (TNF-α) is one of the most studied proinflammatory cytokines in OSA. Studies have shown that serum levels are closely associated with OSA severity, independent of obesity,[27,28] and are linked to nocturnal hypoxemia.[8,24,29] Kataoka *et al*. showed that uvulopalatopharyngoplasty reduced plasma TNF-α levels.[9] Another study reported that treatment with a TNF-α antagonist decreased excessive

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TABLE IV
CHANGES IN SUCCESSFUL SURGERY AND CPAP COMPLIANCE SUBGROUP PARAMETERS BEFORE AND AFTER TREATMENT

Parameter	Surgery group			CPAP group		
	Successful surgery ($n = 19$)	Non-successful surgery ($n = 10$)	Δp^*	Successful compliance ($n = 12$)	Non-successful compliance ($n = 16$)	Δp^*
AHI	<0.001†	0.327	0.058	<0.001†	<0.001†	0.399
ESS score	<0.001†	0.002†	0.499	<0.001†	<0.001†	0.537
VAS score	<0.001†	<0.001†	0.183	<0.001†	<0.001†	0.333
Mean O_2	0.038†	0.356	0.800	0.573	0.162	0.349
$SaO_2 < 90\%$	0.131	0.111	0.837	0.014†	0.426	0.459
Oxygen desaturation index	0.003†	0.215	0.454	0.020†	0.010†	0.518
Minimum O_2	0.012†	0.103	0.213	0.006†	0.045†	0.806
TNF-α	0.137	0.377	0.239	0.321	0.745	0.668
IL-2	0.510	0.934	0.662	0.554	0.063	0.083
IL-4	0.311	0.369	0.911	0.080	0.264	0.045†
IL-10	0.697	0.360	0.218	0.396	0.176	0.387
IFN-γ	0.113	0.241	0.950	0.468	0.868	0.460

Data represent the p-values for the changes in parameter values before and after treatment, unless indicated otherwise. *Δp represents 'the difference of differences'. †Indicates statistical significance ($p < 0.05$). CPAP = continuous positive airway pressure; AHI = apnoea/hypopnoea index; ESS = Epworth Sleepiness Scale; VAS = visual analogue scale; mean O_2 = mean oxygen saturation; $SaO_2 < 90\%$ = percentage of sleep time with oxygen saturation below 90 per cent; minimum O_2 = lowest oxygen saturation; TNF = tumour necrosis factor; IL = interleukin; IFN = interferon

TABLE V
INFLAMMATORY/ANTI-INFLAMMATORY CYTOKINE RATIOS BEFORE AND AFTER TREATMENT

Group	Ratio	Value before treatment	Value after treatment	Direction of change	p
Surgery ($n = 29$)	TNF-α/IL-4	56.16	17.05	Decrease	0.130
	TNF-α/IL-10	33.04	6.11	Decrease	0.129
	IFN-γ/IL-4	0.53	0.27	Decrease	0.014*
	IFN-γ/IL-10	0.11	0.09	Decrease	0.098
	IL-2/IL-4	18.55	7.57	Decrease	0.089
	IL-2/IL-10	3.66	3.65	Decrease	0.996
CPAP ($n = 28$)	TNF-α/IL-4	20.86	14.52	Decrease	0.270
	TNF-α/IL-10	6.85	5.40	Decrease	0.500
	IFN-γ/IL-4	0.29	0.32	Increase	0.312
	IFN-γ/IL-10	0.1042	0.1047	Increase	0.976
	IL-2/IL-4	14.85	10.83	Decrease	0.161
	IL-2/IL-10	5.58	3.87	Decrease	0.215
All patients ($n = 57$)	TNF-α/IL-4	38.51	15.78	Decrease	0.084
	TNF-α/IL-10	19.72	5.75	Decrease	0.110
	IFN-γ/IL-4	0.41	0.30	Decrease	0.051
	IFN-γ/IL-10	0.11	0.10	Decrease	0.238
	IL-2/IL-4	16.70	9.20	Decrease	0.032*
	IL-2/IL-10	4.61	3.76	Decrease	0.387

*Indicates statistical significance ($p < 0.05$). TNF = tumour necrosis factor; IL = interleukin; IFN = interferon; CPAP = continuous positive airway pressure

TABLE VI
INFLAMMATORY MARKERS IN MULTI- VERSUS SINGLE-LEVEL SURGERY SUBGROUPS

Inflammatory marker	Multilevel surgery ($n = 3$)			Single-level surgery ($n = 26$)		
	Pre-treatment	Post-treatment	p	Pre-treatment	Post-treatment	p
IL-2 (pg/ml)	13.34 ± 6.01	10.84 ± 9.16	0.605	23.32 ± 34.11	18.32 ± 23.80	0.528
IL-4 (pg/ml)	1.83 ± 2.14	1.86 ± 0.06	0.981	1.72 ± 2.06	2.44 ± 3.06	0.169
IL-10 (pg/ml)	5.04 ± 1.58	21.64 ± 24.82	0.360	17.11 ± 36.90	11.81 ± 23.75	0.367
TNF-α (pg/ml)	77.43 ± 55.25	93.07 ± 138.6	0.853	192.2 ± 454.15	59.20 ± 194.3	0.148
IFN-γ (IU/ml)	0.32 ± 0.01	0.37 ± 0.12	0.542	0.51 ± 0.25	0.38 ± 0.09	0.029*

Data represent means ± standard deviations, unless indicated otherwise. *Indicates statistical significance ($p < 0.05$). IL = interleukin; TNF = tumour necrosis factor; IFN = interferon

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daytime sleepiness in patients with mild OSA.[30] In our study, the TNF-α levels were reduced in both groups after treatment, but the reduction was not statistically significant. No significant correlation was observed between the changes in TNF-α levels and the changes in apnoea/hypopnoea index. Assessment of the CPAP group independent of apnoea/hypopnoea index revealed that the decrease in TNF-α levels was significantly greater in patients with a BMI above 30 kg/m^2 than in those with a BMI less than 30 kg/m^2 ($p = 0.014$). This result reveals that TNF-α, a cytokine with great reserves in adipose tissue, becomes more sensitive to treatment in patients with a higher BMI. Tumour necrosis factor-α has the potential to be the earliest activated cytokine in OSA in response to hypoxia and desaturation. Alberti *et al*. found a significant increase in the plasma levels of TNF-α in OSA patients immediately after the first apnoea episode.[29] However, we suggest that interleukin (IL)-2 and interferon-γ may be more useful as indicators of a chronic disease such as OSA because these inflammatory cytokines are activated by adaptive immunological responses in the long run.

Interleukin-2 exerts its inflammatory properties by stimulating the synthesis of other cytokines and promoting the proliferation of T cells after the body's interaction with an antigen.[31] A limited number of studies in the literature have reported the IL-2 serum levels in adult patients with OSA, but we have not found any studies demonstrating changes due to treatment. One interesting study revealed that OSA patients who failed to show drops in blood pressure levels during the night had increased serum IL-2 levels, independent of their OSA severity.[32] In our study, serum IL-2 levels were decreased after surgery and after CPAP treatment, but this reduction was not statistically significant in either group. Tumour necrosis factor-α, as the initiator of the inflammatory response, and IL-2, as one of the indicators of the adaptive inflammatory response, showed a significant correlation with each other in both the surgery ($p = 0.038$) and CPAP ($p = 0.006$) groups before treatment.

Interleukin-4 and IL-10 are anti-inflammatory cytokines.[33,34] Studies on OSA have mainly focused on IL-10, which exerts its anti-inflammatory properties by inhibiting proinflammatory cytokines.[34] Increased expression of IL-10 in T-lymphocytes reportedly showed a negative correlation with OSA severity.[35] In addition, IL-10 is known to have beneficial effects in patients with acute coronary syndrome by suppressing over-activation of the proinflammatory signalling system.[36,37] A review investigating the diagnostic capability of inflammatory markers in the assessment of OSA suggested that IL-10 plasma level has the potential to be a good biomarker for identifying or excluding the presence of OSA in adults.[38] The answer to the question 'what causes the increased inflammation in OSA: an increase in proinflammatory cytokines due to chronic intermittent hypoxia or a decrease in the production of anti-inflammatory cytokines due to an unknown mechanism?' remains elusive. A recent study by Jiang *et al*. demonstrated that the TNF-α/IL-10 ratio increased in line with OSA severity.[39] In our study, the TNF-α/IL-10 ratio decreased in both groups after treatment, but this reduction was not statistically significant.

The increase in serum IL-4 levels in the successful CPAP compliance group after treatment was greater than that in the non-successful compliance group. We found that successful CPAP compliance resulted in a greater increase in serum IL-4 levels, which supports the idea that improvement in oxygen saturation with continuous use of CPAP may trigger the anti-inflammatory response by way of IL-4. Serum IL-4 levels were positively correlated with lowest oxygen saturation levels, and were negatively correlated with the percentage of sleep time with oxygen saturation below 90 per cent. The positive correlation between lowest oxygen saturation and IL-4 demonstrates that desaturations may lead to suppression of IL-4 levels in OSA patients. We found no correlation between oxygen saturation values and serum IL-10, but we hypothesise that IL-4 may be more sensitive to inflammation in OSA, and its serum levels cannot be maintained against an inflammatory challenge.

Interferon-γ enhances Th1 cell mediated immunity by affecting macrophages, and leads to the release of proinflammatory cytokines.[40,41] A limited number of studies have shown a relationship between OSA and interferon-γ; these were focused on the elevated interferon-γ serum levels observed in children with OSA.[42,43] A study by Kohler *et al*. demonstrated that four weeks of CPAP treatment did not significantly alter serum interferon-γ levels.[13] To the best of our knowledge, no study available in the literature has evaluated the changes in serum interferon-γ levels after surgical treatment in patients with OSA. When we examined the data for all 57 patients, the only marker that showed significant changes in serum levels after treatment was interferon-γ. The serum interferon-γ levels were decreased after surgical treatment, and this reduction was statistically significant ($p = 0.043$). However, CPAP treatment did not result in any significant difference in serum levels of interferon-γ, in agreement with the study by Kohler *et al*.[13]

No studies in the English-language literature have evaluated the effect of treatment on the Th1 (TNF-α, interferon-γ and IL-2) / Th2 (IL-4 and IL-10) cytokine balance in adult patients with OSA. We detected some decreases in the TNF/IL-4, TNF/IL-10, interferon-γ/IL-4, interferon-γ/IL-10, IL-2/IL-4, and IL-2/IL-10 ratios after treatment. The interferon-γ/IL-4 ratio significantly decreased in the surgery group ($p = 0.014$), which represents a concurrent increase in the serum levels of an anti-inflammatory cytokine or a decrease in the serum levels of an inflammatory cytokine, even with a moderate success rate of 65.5 per cent. The reduction in the IL-2/IL-4 ratio in the study

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group overall was also statistically significant. These results are important as they demonstrate the effect of treatment in adult OSA patients and indicate a shift to an anti-inflammatory state.

The number of patients undergoing multilevel surgery was rather low in this study, but we can comment on the impact of multilevel surgery on inflammatory markers compared to single-level surgery. As seen in Table VI, single-level surgical treatment resulted in a significant decrease in serum interferon-γ levels ($p = 0.029$), while three patients undergoing multilevel surgery did not show significant improvements in inflammatory markers. As shown in Table III, the p value for serum interferon-γ levels in the surgery group overall ($n = 29$) was 0.043. It seems that the data for the three patients undergoing multilevel surgery worsened the p value, reflecting the change of interferon-γ levels in the surgery group. Further prospective studies with large case series are needed to improve power analysis and present exact results of multilevel surgical treatment.

Our study showed that the main indicator for evaluating the inflammation process in OSA was not the apnoea/hypopnoea index. Instead, a change in the serum levels of inflammatory markers had a greater correlation with Epworth Sleepiness Scale and VAS scores, and saturation parameters. Likewise, a change in the saturation parameters was associated with Epworth Sleepiness Scale and VAS scores. In the surgery group, the VAS scores were negatively correlated with serum IL-10 levels. Those patients who ranked their snoring with a higher score also had decreased IL-10 levels and a decreased anti-inflammatory response. Multiple factors that affect the apnoea/hypopnoea index, such as saturation parameters and clinical predictors, can therefore also influence the inflammatory/anti-inflammatory balance.

- **The effect of surgical treatment or continuous positive airway pressure on T helper 1 (Th1)/T helper 2 (Th2) cytokine balance in adult obstructive sleep apnoea (OSA) patients is not known**
- **This study demonstrates the effect of treatment on the Th1/Th2 cytokine balance**
- **It also shows the effect of relatively new reconstructive surgical techniques on inflammation**
- **Successful surgical treatments for OSA may interrupt the inflammatory process by changing the Th1/Th2 cytokine balance towards the Th2 direction**

This study has some limitations. When we assessed the groups according to surgical success and success in CPAP compliance, the subgroups consisted of a relatively lower number of patients. Additionally, more successful surgical procedures or better CPAP compliance could provide greater amelioration of inflammation, and could demonstrate the exact anti-inflammatory response. Thus, the study design should be adapted to select only those patients who undergo successful surgery or have excellent CPAP compliance.

Conclusion

This study shows that surgical treatments for OSA may affect and interrupt the inflammatory process by changing the Th1/Th2 cytokine balance towards the Th2 direction, to an anti-inflammatory state. The most effective factor in this process – whether it is intermittent hypoxemia or another factor – will be the subject of further studies. A larger study population is needed to uncover the exact effect of successful surgery and CPAP compliance. If the present study, with a surgical success rate of 65.5 per cent and a CPAP compliance of 40.9 per cent, can reveal these promising results, a more successful treatment for OSA may further reduce the inflammation and systemic co-morbidities associated with this condition.

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References

1 Garvey JF, Taylor CT, McNicholas WT. Cardiovascular disease in obstructive sleep apnoea syndrome: the role of intermittent hypoxia and inflammation. *Eur Respir J* 2009;**33**:1195–205
2 Dempsey JA, Veasey SC, Morgan BJ, O'Donnell CP. Pathophysiology of sleep apnea. *Physiol Rev* 2010;**90**:47–112
3 Semenza GL. Hypoxia-inducible factor 1: control of oxygen homeostasis in health and disease. *Pediatr Res* 2001;**49**:614–17
4 Htoo AK, Greenberg H, Tongia S, Chen G, Henderson T, Wilson D *et al*. Activation of nuclear factor kappaB in obstructive sleep apnea: a pathway leading to systemic inflammation. *Sleep Breath* 2006;**10**:43–50
5 Ryan S, Taylor CT, McNicholas WT. Systemic inflammation: a key factor in the pathogenesis of cardiovascular complications in obstructive sleep apnoea syndrome? *Thorax* 2009;**64**:631–6
6 Nadeem R, Molnar J, Madbouly EM, Nida M, Aggarwal S, Sajid H *et al*. Serum inflammatory markers in obstructive sleep apnea: a meta-analysis. *J Clin Sleep Med* 2013;**9**:1003–12
7 Yokoe T, Minoguchi K, Matsuo H, Oda N, Minoguchi H, Yoshino G *et al*. Elevated levels of C-reactive protein and interleukin-6 in patients with obstructive sleep apnea syndrome are decreased by nasal continuous positive airway pressure. *Circulation* 2003;**107**:1129–34
8 Vgontzas AN, Papanicolaou DA, Bixler EO, Kales A, Tyson K, Chrousos GP. Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. *J Clin Endocrinol Metab* 1997;**82**:1313–16
9 Kataoka T, Enomoto F, Kim R, Yokoi H, Fujimori M, Sakai Y *et al*. The effect of surgical treatment of obstructive sleep apnea syndrome on the plasma TNF-alpha levels. *Tohoku J Exp Med* 2004;**204**:267–72
10 Swain SL, Bradley LM, Croft M, Tonkonogy S, Atkins G, Weinberg AD *et al*. Helper T-cell subsets: phenotype, function and the role of lymphokines in regulating their development. *Immunol Rev* 1991;**123**:115–44
11 Girón-González JA, Moral FJ, Elvira J, García-Gil D, Guerrero F, Gavilán I *et al*. Consistent production of a higher TH1:TH2 cytokine ratio by stimulated T cells in men compared with women. *Eur J Endocrinol* 2000;**143**:31–6

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12 Arias MA, García-Río F, Alonso-Fernández A, Hernanz A, Hidalgo R, Martínez-Mateo V *et al*. CPAP decreases plasma levels of soluble tumour necrosis factor-alpha receptor 1 in obstructive sleep apnoea. *Eur Respir J* 2008;**32**:1009–15
13 Kohler M, Ayers L, Pepperell JC, Packwood KL, Ferry B, Crosthwaite N *et al*. Effects of continuous positive airway pressure on systemic inflammation in patients with moderate to severe obstructive sleep apnoea: a randomised controlled trial. *Thorax* 2009;**64**:67–73
14 Wu KM, Lin CC, Chiu CH, Liaw SF. Effect of treatment by nasal continuous positive airway pressure on serum high mobility group box-1 protein in obstructive sleep apnea. *Chest* 2010; **137**:303–9
15 Jullian-Desayes I, Joyeux-Faure M, Tamisier R, Launois S, Borel AL, Levy P *et al*. Impact of obstructive sleep apnea treatment by continuous positive airway pressure on cardiometabolic biomarkers: a systematic review from sham CPAP randomized controlled trials. *Sleep Med Rev* 2015;**21**:23–38
16 Xie X, Pan L, Ren D, Du C, Guo Y. Effects of continuous positive airway pressure therapy on systemic inflammation in obstructive sleep apnea: a meta-analysis. *Sleep Med* 2013;**14**: 1139–50
17 Ishida K, Kato M, Kato Y, Yanagihara K, Kinugasa Y, Kotani K *et al*. Appropriate use of nasal continuous positive airway pressure decreases elevated C-reactive protein in patients with obstructive sleep apnea. *Chest* 2009;**136**:125–9
18 Ip MS, Lam B, Chan LY, Zheng L, Tsang KW, Fung PC *et al*. Circulating nitric oxide is suppressed in obstructive sleep apnea and is reversed by nasal continuous positive airway pressure. *Am J Respir Crit Care Med* 2000;**162**:2166–71
19 Harsch IA, Konturek PC, Koebnick C, Kuehnlein PP, Fuchs FS, Pour Schahin S *et al*. Leptin and ghrelin levels in patients with obstructive sleep apnoea: effect of CPAP treatment. *Eur Respir J* 2003;**22**:251–7
20 Guo Y, Pan L, Ren D, Xie X. Impact of continuous positive airway pressure on C-reactive protein in patients with obstructive sleep apnea: a meta-analysis. *Sleep Breath* 2013;**17**:495–503
21 Baessler A, Nadeem R, Harvey M, Madbouly E, Younus A, Sajid H *et al*. Treatment for sleep apnea by continuous positive airway pressure improves levels of inflammatory markers – a meta-analysis. *J Inflamm* (*Lond*) 2013;**10**:13
22 Karamanlı H, Özol D, Ugur KS, Yıldırım Z, Armutçu F, Bozkurt B *et al*. Influence of CPAP treatment on airway and systemic inflammation in OSAS patients. *Sleep Breath* 2014;**18**: 251–6
23 Chu L, Li Q. The evaluation of adenotonsillectomy on TNF-alpha and IL-6 levels in obese children with obstructive sleep apnea. *Int J Pediatr Otorhinolaryngol* 2013;**77**:690–4
24 Constantinidis J, Ereliadis S, Angouridakis N, Konstantinidis I, Vital V, Angouridaki C. Cytokine changes after surgical treatment of obstructive sleep apnoea syndrome. *Eur Arch Otorhinolaryngol* 2008;**265**:1275–9
25 Berry RB, Budhiraja R, Gottlieb DJ, Gozal D, Iber C, Kapur VK *et al*. Rules for scoring respiratory events in sleep: update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine. *J Clin Sleep Med* 2012;**8**:597–619
26 Calvin AD, Albuquerque FN, Lopez-Jimenez F, Somers VK. Obstructive sleep apnea, inflammation, and the metabolic syndrome. *Metab Syndr Relat Disord* 2009;**7**:271–8
27 Minoguchi K, Tazaki T, Yokoe T, Minoguchi H, Watanabe Y, Yamamoto M *et al*. Elevated production of tumor necrosis factor-alpha by monocytes in patients with obstructive sleep apnea syndrome. *Chest* 2004;**126**:1473–9
28 Ciftci TU, Kokturk O, Bukan N, Bilgihan A. The relationship between serum cytokine levels with obesity and obstructive sleep apnea syndrome. *Cytokine* 2004;**28**:87–91
29 Alberti A, Sarchielli P, Gallinella E, Floridi A, Mazzotta G, Gallai V. Plasma cytokine levels in patients with obstructive sleep apnea syndrome: a preliminary study. *J Sleep Res* 2003; **12**:305–11
30 Vgontzas AN, Zoumakis E, Lin HM, Bixler EO, Trakada G, Chrousos GP. Marked decrease in sleepiness in patients with sleep apnea by etanercept, a tumor necrosis factor-alpha antagonist. *J Clin Endocrinol Metab* 2004;**89**:4409–13
31 Wagner H, Hardt C, Heeg K, Pfizenmaier K, Stotter H, Rollinghoff M. The in vivo effects of interleukin 2 (TCGF). *Immunobiology* 1982;**161**:139–56
32 Sarinc Ulasli S, Sarıaydın M, Gunay E, Halici B, Celik S, Koyuncu T *et al*. Effects of nondipping pattern on systemic inflammation in obstructive sleep apnea. *Sleep Breath* 2015; **19**:1185–90
33 Ransom J, Fischer M, Mosmann T, Yokota T, DeLuca D, Schumacher J *et al*. Interferon-gamma is produced by activated immature mouse thymocytes and inhibits the interleukin 4-induced proliferation of immature thymocytes. *J Immunol* 1987;**139**:4102–8
34 de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med* 1991;**174**:1209–20
35 Dyugovskaya L, Lavie P, Lavie L. Lymphocyte activation as a possible measure of atherosclerotic risk in patients with sleep apnea. *Ann N Y Acad Sci* 2005;**1051**:340–50
36 Heeschen C, Dimmeler S, Hamm CW, Fichtlscherer S, Boersma E, Simoons ML *et al*. Serum level of the antiinflammatory cytokine interleukin-10 is an important prognostic determinant in patients with acute coronary syndromes. *Circulation* 2003;**107**: 2109–14
37 Potteaux S, Deleuze V, Merval R, Bureau MF, Esposito B, Scherman D *et al*. In vivo electrotransfer of interleukin-10 cDNA prevents endothelial upregulation of activated NF-kappaB and adhesion molecules following an atherogenic diet. *Eur Cytokine Netw* 2006;**17**:13–18
38 De Luca Canto G, Pacheco-Pereira C, Aydinoz S, Major PW, Flores-Mir C, Gozal D. Biomarkers associated with obstructive sleep apnea: a scoping review. *Sleep Med Rev* 2014;**23**:28–45
39 Jiang H, Cao H, Wang P, Liu W, Cao F, Chen J. Tumour necrosis factor-alpha/interleukin-10 ratio in patients with obstructive sleep apnoea hypopnoea syndrome. *J Laryngol Otol* 2015;**129**: 73–8
40 Billiau A. Interferons and inflammation. *J Interferon Res* 1987; **7**:559–67
41 Gately MK, Warrier RR, Honasoge S, Carvajal DM, Faherty DA, Connaughton SE *et al*. Administration of recombinant IL-12 to normal mice enhances cytolytic lymphocyte activity and induces production of IFN-gamma in vivo. *Int Immunol* 1994;**6**:157–67
42 Tam CS, Wong M, McBain R, Bailey S, Waters KA. Inflammatory measures in children with obstructive sleep apnoea. *J Paediatr Child Health* 2006;**42**:277–82
43 Waters KA, Mast BT, Vella S, de la Eva R, O'Brien LM, Bailey S *et al*. Structural equation modeling of sleep apnea, inflammation, and metabolic dysfunction in children. *J Sleep Res* 2007;**16**:388–95

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