



ORIGINAL ARTICLE

Nocturnal temperature controlled laminar airflow for treating atopic asthma: a randomised controlled trial

Robert J Boyle,¹ Christophe Pedroletti,² Magnus Wickman,^{3,4} Leif Bjermer,⁵ Erkka Valovirta,⁶ Ronald Dahl,⁷ Andrea Von Berg,⁸ Olof Zetterström,⁹ John O Warner,¹ for the 4A Study Group*

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¹Department of Paediatrics, Imperial College London, London, UK

²Department of Woman and Child Health, Karolinska Institutet, Stockholm, Sweden

³Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

⁴Sachs' Children's Hospital, Stockholm, Sweden

⁵Department of Respiratory Medicine and Allergology, Lund University Hospital, Lund, Sweden

⁶Terveystalo Allergy Clinic, Turku, Finland

⁷Department of Respiratory Diseases, Aarhus University Hospital, Aarhus, Denmark

⁸Research Institute for Prevention of Children's Allergy and Respiratory Diseases, Marien-Hospital, Wesel, Germany

⁹Allergy Centre, University Hospital of Linköping, Linköping, Sweden

Correspondence to

Professor John O Warner, Professor of Paediatrics, Imperial College London, Wright Fleming Building, Norfolk Place, London W2 1PG, UK; j.o.warner@imperial.ac.uk

For author footnote see end of the article.

Received 22 June 2011

Accepted 11 October 2011



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ABSTRACT

Objective To determine whether environmental control using nocturnal temperature controlled laminar airflow (TLA) treatment could improve the quality of life of patients with persistent atopic asthma.

Design Randomised, double-blind, placebo-controlled, parallel-group trial.

Setting Nineteen European asthma clinics.

Participants 312 patients aged 7–70 with inadequately controlled persistent atopic asthma.

Main outcome measure Proportion of patients with an increase of ≥ 0.5 points in asthma quality of life score after 1 year of treatment.

Results TLA devices were successfully installed in the bedrooms of 282 (90%) patients included in the primary efficacy analysis. There was a difference in treatment response rate between active (143 of 189, 76%) and placebo (56 of 92, 61%) groups, difference 14.8% (95% CI 3.1 to 26.5, $p=0.02$).³ In patients aged ≥ 12 , on whom the study was powered, the difference in response rate was similar—active 106 of 143 (74%), placebo 42 of 70 (60%), difference 14.1% (0.6 to 27.7, $p=0.059$). There was a difference between groups in fractional exhaled nitric oxide change of -7.1 ppb (-13.6 to -0.7 , $p=0.03$). Active treatment was associated with less increase in cat-specific IgE than placebo. There was no difference in adverse event rates between treatment groups.

Conclusion Inhalant exposure reduction with TLA improves quality of life, airway inflammation and systemic allergy in patients with persistent atopic asthma. TLA may be a treatment option for patients with inadequately controlled persistent atopic asthma.

Trial registration number Clinical Trials NCT00986323.

INTRODUCTION

In patients with atopic asthma, the abnormal immune response to inhalant allergens is an important contributor to symptoms.¹ Studies undertaken at high altitude suggest that long-term avoidance of allergens and other exposures can lead to reduced asthma symptoms.^{2–6} Despite positive reports from comprehensive home environmental control programmes,⁷ blinded placebo-controlled studies of air filters and other single-device interventions have failed to demonstrate significant benefit, suggesting that the reduction in allergen exposure is insufficient to impact on airway inflammation.^{8,9} A new device Protexo (Airsonett, Ängelholm, Sweden) has recently been shown to markedly reduce levels of

Key messages

- Can effective environmental control measures improve quality of life for people with asthma?
- Treatment of patients with persistent atopic asthma for 1 year using nocturnal temperature controlled laminar airflow improved asthma-related quality of life and reduced airway inflammation.
- Temperature controlled laminar airflow is a new technology which may be effective for treating patients with allergic asthma.

inhaled allergen and other particles using temperature-controlled laminar airflow (TLA) (personal communication, Dr Robin Gore, 2011). The device distributes a filtered cooled laminar airflow, descending from an overhead position, which displaces aeroallergens from the breathing zone. We undertook a phase III multicentre randomised controlled trial of nocturnal TLA treatment for 1 year to quantify the effect in patients with atopic asthma on quality of life, symptom control, lung function, airway inflammation and markers of systemic allergy (specific IgE and eosinophil count).

METHODS

Participants

We enrolled patients with atopic asthma in a randomised, controlled, parallel-group trial of add-on treatment with TLA or placebo between April 2008 and February 2009. Patients were recruited from 19 European centres. Inclusion criteria were physician's diagnosis of asthma ≥ 1 year prior to study; age 7–70 years; Mini Asthma Quality of Life Questionnaire (mini-AQLQ) or Paediatric Asthma Quality of Life Questionnaire (PAQLQ) (together termed 'AQLQ') score ≤ 5.5 at inclusion; allergic sensitisation to a pet allergen (cat or dog) or house dust mite demonstrated by specific IgE level ≥ 0.70 kU/litre or positive skin prick test (weal diameter \geq histamine control); daily inhaled corticosteroid ≥ 200 μ g/day budesonide/beclomethasone or ≥ 100 μ g/day fluticasone for last 6 months; and features of partly controlled asthma according to Global Initiative for Asthma (GINA) 2006.¹⁰ Exclusion criteria were current active or passive cigarette smoke exposure; inclusion in another allergen avoidance programme or drug trial;

treatment with allergen immunotherapy or omalizumab in previous 2 years (1 year for children); inhaled corticosteroid dose >1200 $\mu\text{g}/\text{day}$ budesonide/beclomethasone or >1000 $\mu\text{g}/\text{day}$ fluticasone. A history of frequent severe asthma exacerbations was not an inclusion criterion for the study. The study was approved by responsible institutional review boards and written informed consent was obtained from all patients/parents. An independent Data and Safety Monitoring Board reviewed efficacy and safety data.

Study intervention

Within 4 weeks from inclusion in the study, an active or placebo TLA device was installed in the bedroom of each study patient. The mode of action of the device is described in the online supplementary material. Patients were asked to turn their device on when they went to bed each night and off in the morning, although the device automatically turned off after 12 h. Placebo devices were identical to active devices, but their filter was bypassed and circulating air not cooled. Treatment compliance was assessed by an electronic counter within the machinery of the devices, which recorded total number of device uses, and total hours of use with the microcontroller MCU PIC18F6622 programmed by Voss Engineering AB, Sweden.

Randomisation and masking

Active or placebo treatment was allocated by the device installation technician according to a randomisation list generated by an independent organisation (APL, Stockholm, Sweden) using Design (Trombult Programming AB, Sweden) in blocks of nine at a ratio of two active to one placebo. The study protocol specified that randomisation would be stratified according to age and gender, but this was not done due to an error in communication with the independent study statistician. The planned stratification of randomisation was however taken into consideration during statistical analyses of outcome data. At the time of device installation and servicing the technician ensured patients and family members were absent from the bedroom and masking of treatment allocation was maintained. Patients, investigators, statisticians and the Trial Steering Committee were masked to treatment allocations through the study.

Trial design

The study was a phase III multicentre, double-blind, placebo-controlled, parallel-group trial. Patients were randomised to receive add-on treatment with Protexo or a placebo device for 1 year. Asthma medications were kept unchanged for the first 3 months, and thereafter adjusted to optimise asthma control by local investigators according to GINA guidelines.¹⁰ Patients were monitored by medical assessments after 1, 3, 6, 9 and 12 months of treatment, and via completion of a diary.

Outcome measures

The primary outcome measure was quality of life assessed by the mini-AQLQ, or in children ≤ 11 years, the PAQLQ.¹¹ A change of 0.5 is considered clinically significant,¹² and the primary outcome analysed was the proportion of patients with a significant increase in mini-AQLQ or PAQLQ score ('responders') after 1 year of treatment. Secondary outcomes were AQLQ score changes, objective measures of airway inflammation (fractional exhaled nitric oxide; FENO), systemic allergy (specific IgE levels to indoor aeroallergens and blood eosinophil count), and airflow obstruction (forced expiratory volume in 1 s, FEV₁; forced expiratory flow at 50% of vital

capacity, FEF₅₀; peak expiratory flow, PEF). Single-breath, online measurement of FENO (NIOX MINO, Aerocrine AB, Stockholm, Sweden) was performed in accordance with the recommendations of the American Thoracic Society.¹³ Spirometry was performed in accordance with international guidelines.¹⁴ Blood eosinophil counts were measured by local hospital laboratories, and specific IgE levels using ImmunoCAP at a single laboratory (Phadia, Denmark; lower limit 0.35 KU/litre). The study was not designed primarily to evaluate effects of TLA on asthma exacerbations, because a history of frequent or severe exacerbations was not an inclusion criterion.

Statistical analysis

The study hypothesis was tested by examining the difference in outcome variables between active and placebo groups at the end of the 12-month treatment period. All patients who were randomised and had ≥ 1 day of device treatment were included in the intention-to-treat population and last observation carried forward was used for missing data. Per protocol analyses excluded patients with major protocol violations and/or documented treatment compliance $<80\%$. Results were summarised as mean scores or score changes $\pm 95\%$ CI, or adjusted OR for binary outcomes $\pm 95\%$ CI. The country, gender, years since asthma diagnosis, GINA treatment step at baseline and AQLQ at baseline were variates in the model for adjusted analyses, which were undertaken using analysis of covariance (ANCOVA) for continuous data, and logistic regression for binary data. We calculated the sample size based on a minimum difference of 20% between treatment groups in the proportion of responders (increase in AQLQ ≥ 0.5 points over the 12-month intervention), and a responder rate of 20% in the placebo group. For 80% power and a type I error of 5%, the sample size needed is 186. Allowing for 20% loss to follow-up we planned to recruit 234 patients aged ≥ 12 years, and a proportionate number of patients aged <12 . Planned subgroup analyses were undertaken by age, by asthma treatment intensity at baseline (GINA treatment step), for those with poor symptom control at baseline (Asthma Control Test; ACT <18) and for those with a combination of high-treatment intensity (GINA 4) and poor symptom control at baseline, where guidelines recommend stepping up treatment.^{15 16} An interim analysis was undertaken by the Data and Safety Monitoring Board after all participants reached 3 months, which included the primary outcome measure and safety. No action was taken after interim analysis as no safety issues were reported and early stopping criteria were not met.

Role of the funding source

Aironett AB sponsored the trial. The Trial Steering Committee designed the study and statistical analysis plan. Data were analysed by an independent statistician Fredrik Hansson (Commitum AB). All authors had full access to data and analyses, and vouch for the report's accuracy and completeness.

RESULTS

Three hundred and twelve patients from six countries were randomly allocated to treatment. Treatment groups had similar baseline demographic and clinical characteristics (table 1). Figure 1 shows the flow of patients through the study. A total of 282 of 312 (90%) randomised patients had a study device successfully installed in their bedroom, and were therefore eligible for primary efficacy analysis. Airborne particle counts and mattress dust allergen levels during the study are described in the online supplementary material.

Table 1 Characteristics of study patients at baseline, presented as mean (SD) unless otherwise stated

	Patients randomly allocated to treatment (n=312)		Patients in primary efficacy analysis (n=282)	
	Active (n=207)	Placebo (n=105)	Active (n=189)	Placebo (n=93)
Age (years)	24.2 (16.1)	24.2 (15.6)	24.7 (16.1)	24.9 (16.0)
Age <12, n (%)	51 (25%)	26 (25%)	46 (24%)	22 (24%)
Male sex, n (%)	117 (57%)	50 (48%)	107 (57%)	44 (47%)
Country:				
Sweden, n (%)	99 (48%)	51 (49%)	91 (48%)	47 (51%)
Denmark, n (%)	33 (16%)	22 (21%)	32 (17%)	22 (24%)
UK, n (%)	34 (16%)	19 (18%)	30 (16%)	13 (14%)
Germany, n (%)	23 (11%)	11 (11%)	23 (12%)	10 (11%)
Norway, n (%)	7 (3%)	0 (0%)	7 (4%)	0 (0%)
Finland, n (%)	6 (3%)	1 (1%)	6 (3%)	1 (1%)
BMI (kg/m ²) of adults (>18)	25.8 (4.4)	25.8 (5.2)	25.8 (4.5)	25.7 (5.4)
Duration of asthma (years)	14.6 (12.8)	12.1 (10.5)	14.8 (12.9)	11.7 (10.6)
AQLQ*	4.20 (0.96)	4.27 (0.96)	4.21 (0.92)	4.25 (0.97)
FEV ₁ (% predicted)	89.6 (17.7)	91.8 (16.9)	89.9 (17.6)	91.2 (15.3)
PEF (% predicted)	92.7 (18.8)	92.5 (22.0)	93.3 (18.6)	92.5 (21.3)
FEF ₅₀ (% predicted)	71.3 (27.9)	71.2 (30.4)	71.4 (28.1)	72.4 (29.7)
FENO (ppb)	37.3 (38.0)	34.0 (34.1)	38.5 (38.6)	34.5 (33.3)
Eosinophil count (%)	0.45 (0.62)	0.45 (0.56)	0.45 (0.62)	0.45 (0.56)
ACT score	15.7 (3.9)	16.1 (3.5)	15.8 (3.7)	15.9 (3.5)
Inhaled corticosteroid dose†	586 (445)	610 (372)	576 (368)	616 (376)
House dust mite sensitised, n (%)‡	133 (66%)	70 (69%)	127 (67%)	64 (69%)
IgE Der. pteronyssinus, median (IQR)	3.9 (0.3, 32.7)	4.1 (0.3, 37.3)	3.9 (0.3, 32.7)	4.1 (0.3, 37.3)
IgE Der. farinae, median (IQR)	4.0 (0.3, 31.1)	3.9 (0.3, 29.8)	4.0 (0.3, 31.1)	3.9 (0.3, 29.8)
Cat sensitised, n (%)‡	139 (69%)	73 (72%)	127 (67%)	66 (71%)
IgE cat, median (IQR)	2.0 (0.3, 10.6)	1.0 (0.3, 5.1)	2.0 (0.3, 10.6)	1.0 (0.3, 5.1)
Dog sensitised, n (%)‡	118 (59%)	61 (60%)	109 (58%)	56 (60%)
IgE dog, median (IQR)	1.1 (0.3, 5.4)	0.8 (0.3, 3.8)	1.1 (0.3, 5.4)	0.8 (0.3, 3.8)
Seasonal allergen sensitised, n (%)§	87 (43%)	43 (42%)	85 (45%)	38 (41%)
Total IgE, median (IQR)	281 (109, 662)	257 (120, 506)	281 (109, 662)	257 (120, 506)
Rhinitis, n (%)	191 (95%)	98 (96%)	180 (95%)	88 (95%)
Eczeema, n (%)	49 (24%)	18 (17%)	45 (24%)	17 (18%)
Food allergy, n (%)	28 (14%)	8 (8%)	23 (12%)	7 (8%)

All IgE levels are in kU/litre.

*AQLQ=mini-AQLQ for those aged ≥12 years, PAQLQ for those <12 years.

†Inhaled corticosteroid dose is beclomethasone dipropionate equivalent daily dose.

‡Sensitised=specific IgE level ≥0.70 KU/litre or positive skin prick test (weal diameter to allergen ≥ weal diameter of positive control) to the relevant allergen, taken within 2 years of study enrolment (1 year if ≤12 years).

§Seasonal allergen=grass pollen, birch pollen, mugwort or mould.

ACT, Asthma Control Test; AQLQ, Asthma Quality of Life Questionnaire; BMI, body mass index; Der. farinae, Dermatophagoides farinae; Der. pteronyssinus, Dermatophagoides pteronyssinus; FEF₅₀, forced expiratory flow at 50% of vital capacity; FENO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 s; PAQLQ, Paediatric Asthma Quality of Life Questionnaire; PEF, peak expiratory flow.

Effects of TLA on asthma-related quality of life

Primary efficacy analysis demonstrated a significant difference in AQLQ responder rate between active (143 of 189, 76%) and placebo (56 of 92, 61%) groups after 1 year—absolute difference 14.8% (95% CI 3.1 to 26.5, $p=0.02$; figure 2). Analysis of treatment response rate in participants aged ≥12 years (on whom the study was powered), showed a responder rate of 106 of 143 (74%) in the active group and 42 of 70 (60%) in the placebo group—absolute difference 14.1% (95% CI 0.6 to 27.7, $p=0.059$). In per protocol analysis the difference between treatment groups in responder rate was similar to intention-to-treat analysis, and there were differences of similar magnitude in children <12 when analysed separately, and in those with severe asthma at baseline judged by GINA-defined treatment intensity (table 2).¹⁰ The difference in responder rate was greatest in those with both high-treatment intensity (GINA 4) and poor symptom control (ACT<18) at baseline. Data were also analysed using a ≥1.0 point increase in AQLQ to define treatment response, and these showed similar findings to the analyses using the predefined responder definition of ≥0.5 points (figure 2). In analysis of treatment response rate without any imputation of missing data, a treatment response was seen in 129 of 166 (78%) in the

TLA group at 12 months, and 50 of 79 (63%) in the placebo group—OR 1.87 (95% CI 1.05 to 3.34, $p=0.03$). There was a significant difference between groups in change in the symptom domain of AQLQ, with a mean 0.31 point (95% CI 0.01 to 0.61) greater increase after active versus placebo treatment; 0.70 points (95% CI 0.13 to 1.26) in the subgroup with high treatment intensity and poor symptom control at baseline. Figure 3 shows absolute values for changes in AQLQ in the total study population, and for those with highest treatment intensity (GINA 4; 46% of the study population), poor symptom control (ACT<18; 65%) or both (31%) at baseline. When analysed as a continuous variable we did not find a significant difference between treatment groups for AQLQ change in the total study population, but there was a significant difference in the subgroups with highest treatment intensity, poor symptom control or both.

Effects of TLA on objective markers of bronchial and systemic allergy and lung function

TLA treatment was associated with a greater decrease in FENO during the study than placebo—mean difference -7.1 ppb (95% CI -13.6 to -0.7 ; $p=0.03$; table 3), which was of greater

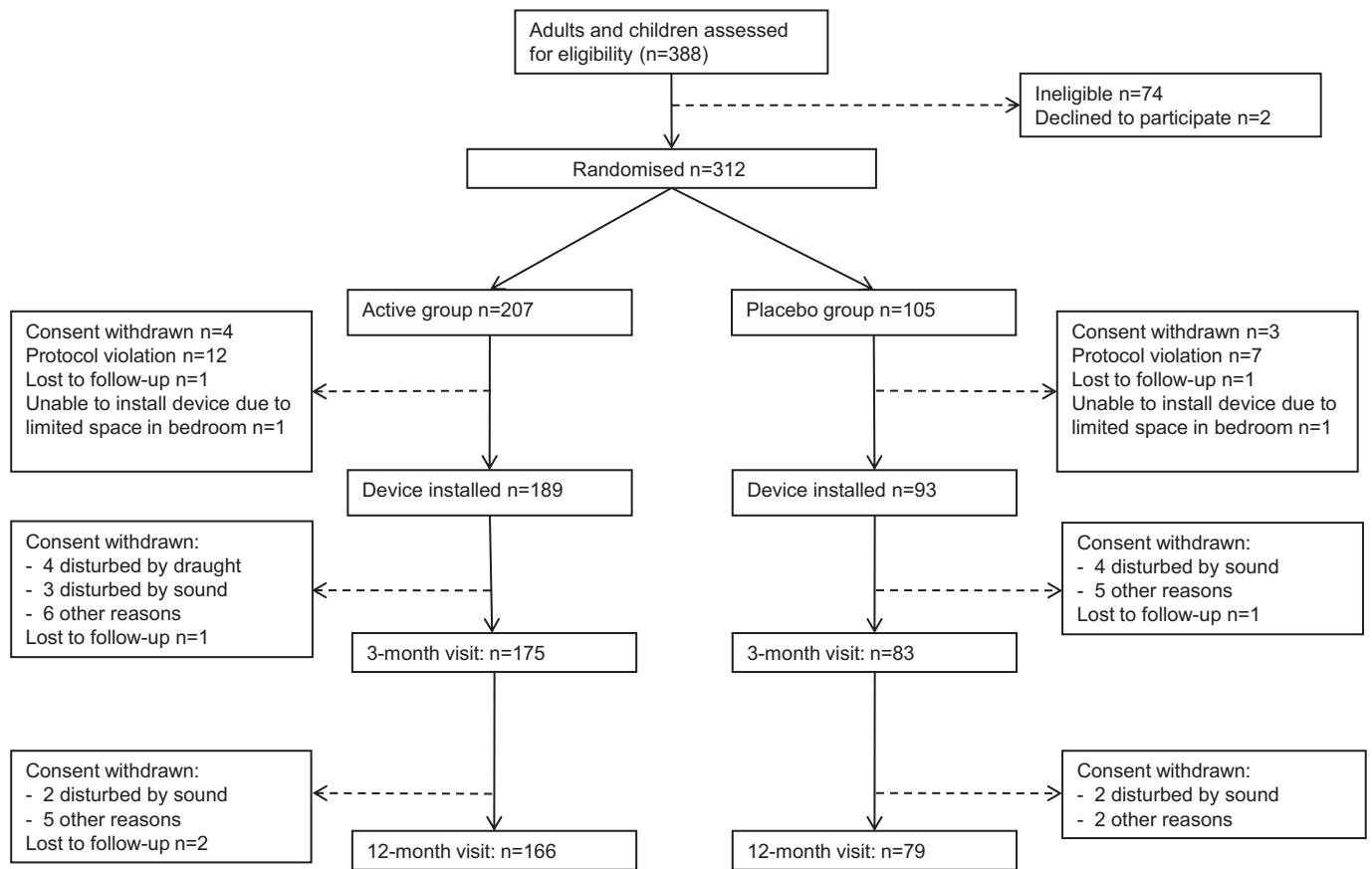


Figure 1 Participant flow through the trial.

magnitude in patients with abnormally raised FENO (>45 ppb) at baseline (mean difference -29.7 ppb; 95% CI -47.2 to -12.2; p=0.001). There was no significant difference in blood eosinophil counts between treatment groups. We found a rise in cat-specific IgE levels relative to baseline level in the placebo group (mean 35%; 95% CI 18% to 53%) and a significantly smaller rise in the active group (mean 8%; 95% CI 0 to 17%; p=0.01; table

3). Lesser increases in levels of specific IgE to house dust mite and dog allergens were also seen in the active versus the placebo group, but the differences between groups were not statistically significant. There was no significant difference between groups in total IgE level change during the study, nor in measures of lung function FEV₁, FEF₅₀ or PEF (table 3).

Effects of TLA on other asthma medication use and asthma exacerbation rates

Although this study was not primarily designed to evaluate TLA effects on asthma medication use or asthma exacerbation rates, medication use and exacerbations by treatment group are presented in online table S4. These data show relatively low rates of severe asthma exacerbations, no significant difference between groups in use of asthma medications and no significant difference between groups in asthma exacerbation rates.¹⁵ When exacerbation data were analysed according to predefined subgroups, there was no significant difference in rate of asthma exacerbations for the whole study population (mean 0.17 TLA; 0.24 placebo; p=0.50), for those with ACT<18 at baseline (mean 0.18 TLA; 0.34 placebo; p=0.28), for those with GINA 4 treatment intensity at baseline (mean 0.24 TLA; 0.40 placebo; p=0.23) or for those with both ACT<18 and GINA 4 at baseline (mean 0.23 TLA; 0.57 placebo; p=0.07).

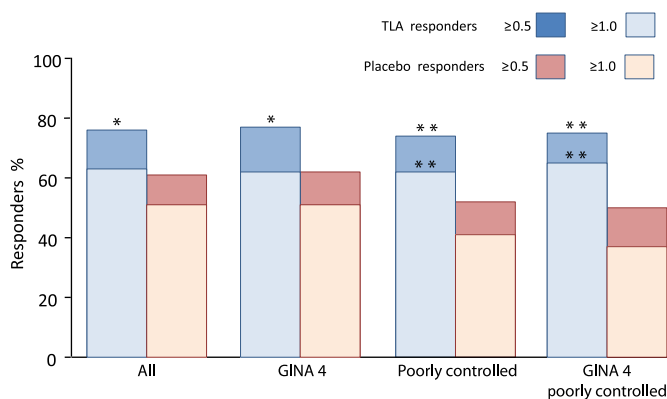


Figure 2 Treatment response rate in patients treated with temperature controlled laminar airflow (TLA) or a placebo device for 1 year, defined as an increase in Asthma Quality of Life Questionnaire score of either ≥0.5 or ≥1.0 point. Data are shown for the total study population (n=282), those with high treatment intensity at baseline (Global Initiative for Asthma, GINA 4; n=129), poor asthma control at baseline (Asthma Control Test, ACT<18; n=184) or both (GINA4/ACT<18; n=87). *p<0.05, **p<0.01 relative to placebo.

Adverse events

In total, 153 (74%) patients in the active and 79 (75%) in the placebo group suffered an adverse event, and 32 (17%) patients in the active and 14 (15%) in the placebo group a serious adverse event. None were treatment related. Further details are given in the online supplementary material.

Table 2 Asthma-related quality of life in active and placebo groups after 1 year of treatment

	Active	Placebo	Difference (95% CI)*	OR (95% CI)*	p Value*
AQLQ responders ≥ 0.5†					
Intention to treat, n/N (%)	143/189 (76%)	56/92 (61%)	14.8% (3% to 27%)	1.92 (1.09 to 3.38)	0.02
Per protocol, n/N (%)‡	106/136 (77%)	40/66 (61%)	16.6% (3% to 30%)	2.22 (1.11 to 4.40)	0.02
<12 years, n/N (%)	37/46 (80%)	14/22 (64%)	16.8% (8% to 38%)	5.57 (1.13 to 27.48)	0.04
≥ 12 years, n/N (%)	106/143 (74%)	42/70 (60%)	14.1% (1% to 28%)	1.89 (0.98 to 3.65)	0.06
GINA 4 group, n/N (%)§	63/82 (77%)	29/47 (62%)	15.1% (2% to 31%)	2.42 (1.05 to 5.60)	0.04
Poorly controlled, n/N (%)¶	93/125 (74%)	30/58 (52%)	22.7% (8% to 38%)	3.45 (1.66 to 7.20)	<0.001
GINA 4 poorly controlled, n/N (%)**	43/57 (75%)	15/30 (50%)	25.4% (4% to 47%)	4.74 (1.48 to 15.19)	0.009
AQLQ responders ≥ 1.0†					
Intention to treat, n/N (%)	119/189 (63%)	47/92 (51%)	14.8% (3% to 26%)	1.58 (0.93 to 2.69)	0.09
Per protocol, n/N (%)‡	89/136 (65%)	33/66 (50%)	15.4% (1% to 30%)	1.85 (0.97 to 3.53)	0.06
<12 years, n/N (%)	33/46 (72%)	11/22 (50%)	21.7% (-3% to 46%)	4.40 (0.99 to 19.57)	0.05
≥ 12 years, n/N (%)	86/143 (60%)	36/70 (51%)	8.7% (-5% to 23%)	1.37 (0.74 to 2.52)	0.31
GINA 4 group, n/N (%)§	51/82 (62%)	24/47 (51%)	15.1% (2% to 31%)	1.96 (0.87 to 4.40)	0.10
Poorly controlled, n/N (%)¶	77/125 (62%)	24/58 (41%)	20.2% (5% to 35%)	2.78 (1.36 to 5.67)	0.005
GINA 4 poorly controlled, n/N (%)**	37/57 (65%)	11/30 (37%)	28.2% (7% to 49%)	8.81 (2.14 to 36.32)	0.003
Change in AQLQ symptom domain					
Intention to treat	1.32 (1.23)	0.99 (1.38)	0.31 (0.01 to 0.61)		0.04
Per protocol‡	1.34 (1.14)	0.96 (1.34)	0.36 (0.01 to 0.71)		0.04
<12 years	1.46 (1.36)	0.93 (1.49)	0.38 (-0.34 to 1.10)		0.29
≥ 12 years	1.27 (1.18)	1.01 (1.36)	0.28 (-0.62 to 0.05)		0.10
GINA 4 group§	1.45 (1.14)	1.00 (1.44)	0.47 (0.03 to 0.91)		0.04
Poorly controlled¶	1.41 (1.24)	0.95 (1.60)	0.58 (0.17 to 0.98)		0.006
GINA 4 poorly controlled**	1.45 (1.15)	0.86 (1.70)	0.70 (0.13 to 1.26)		0.02

*Adjusted analyses controlled for country, gender, years since asthma diagnosis, GINA treatment intensity step at baseline, and AQLQ value at baseline.

†Improvement was classified as ≥ 0.5 or ≥ 1.0 point increase in 'AQLQ' (=mini-AQLQ or PAQLQ) between installation of nocturnal TLA device and assessment 1 year later.

‡Per protocol analyses excluded patients with consent withdrawn and/or with <80% treatment compliance (n=50 active; 27 placebo).

§Asthma treatment intensity at baseline classified according to GINA 2006¹² where GINA step 4 is high treatment intensity.

¶Poorly controlled asthma was defined as ACT score <18 at baseline.

**Poorly controlled asthma in combination with high treatment intensity.

ACT, Asthma Control Test; AQLQ, Asthma Quality of Life Questionnaire; GINA, Global Initiative for Asthma; PAQLQ, Paediatric Asthma Quality of Life Questionnaire.

DISCUSSION

Despite advances in asthma treatment, a significant number of patients have asthma that remains poorly controlled.¹⁷ Previous studies of allergen avoidance measures for treating asthma have been disappointing, leading the authors of a recent Cochrane systematic review of house dust mite control measures for

asthma to comment 'it is doubtful whether further studies... are worthwhile'.⁸ In this trial we investigated the effects of a novel treatment using nocturnal TLA in the homes of patients with atopic asthma. Contrary to previous studies, we found that exposure control using TLA treatment at night has an impact on overall asthma-related quality of life, with a significant

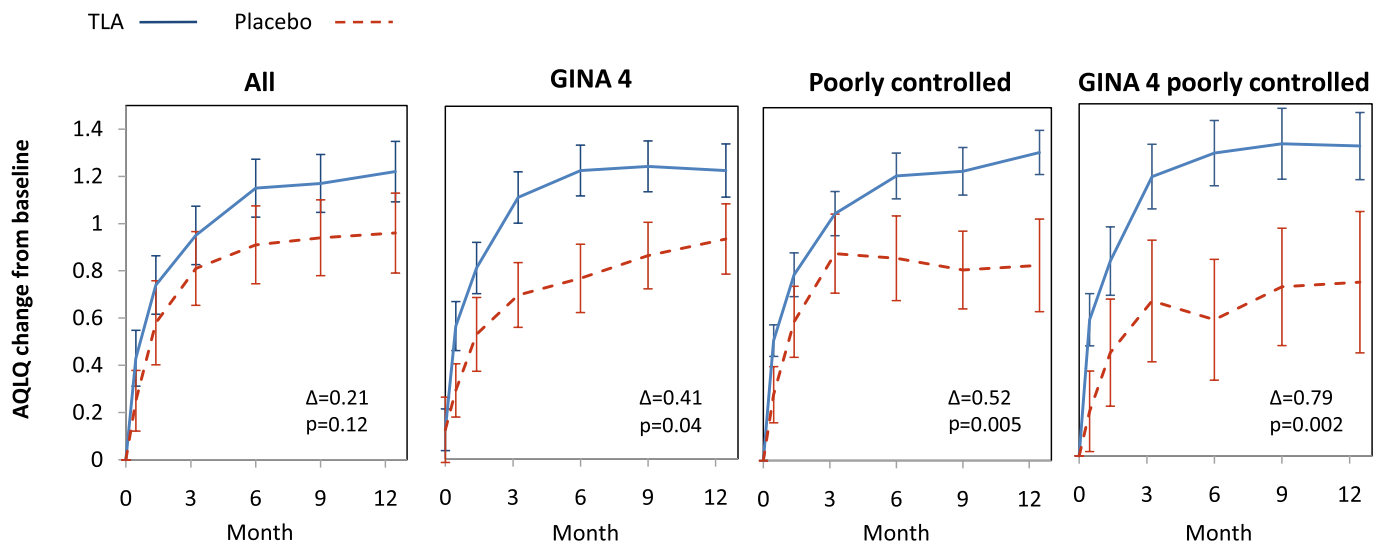


Figure 3 Change in Asthma Quality of Life Questionnaire (AQLQ) score during 1 year of temperature controlled laminar airflow (TLA) (blue line) or placebo (dotted red line) treatment in the whole population (All), those with highest asthma treatment intensity at baseline (Global Initiative for Asthma, GINA 4), those with poor asthma control at baseline (Asthma Control Test, ACT<18), or both (GINA 4, ACT<18). Values are mean \pm 1 SEM. Baseline AQLQ scores were similar in the TLA and placebo groups (total group mean 4.21 TLA, 4.25 placebo; GINA 4 mean 4.14 TLA, 4.14 placebo; ACT<18 mean 3.97 TLA, 3.92 placebo; GINA 4, ACT<18 mean 4.01 TLA, 3.85 placebo).

Table 3 Objective markers of bronchial inflammation and systemic allergy after 1 year of treatment

	Active	Placebo	Difference (95% CI)	p Value
ΔFENO	-4.88 (-9.3 to -0.4)	2.82 (-3.5 to 9.2)	-7.13 (-13.6 to -0.7)	0.03
ΔFENO (high baseline FENO)*	-27.30 (-37.6 to -17.0)	-2.53 (-24.0 to 18.9)	-29.70 (-47.2 to -12.2)	0.001
Δ Eosinophil count (%)	-0.06 (-0.15 to 0.03)	-0.02 (-0.12 to 0.07)	-0.04 (-0.20 to 0.08)	0.42
Δ FEV ₁ (% predicted)	-0.1 (-1.6 to 1.4)	1.4 (-0.9 to 3.6)	-2.1 (-4.6 to 0.5)	0.11
Δ PEF (% predicted)	2.6 (0.5 to 4.6)	5.1 (2.1 to 8.1)	-2.2 (-5.6 to 1.2)	0.20
Δ FEF ₅₀ (% predicted)	-4.7 (-8.8 to -0.5)	-5.2 (-11.6 to 1.2)	-2.0 (-8.4 to 4.5)	0.55
Total IgE	4.6 (-3.5 to 12.6)	3.7 (-4.1 to 11.5)	2.9 (-9.9 to 15.7)	0.66
Δ Cat-specific IgE	8.2 (-0.4 to 16.7)	35.4 (17.8 to 53.0)	-26.0 (-44.1 to -7.9)	0.005
Δ Dog-specific IgE	-1.9 (-10.4 to 6.5)	13.9 (-10.0 to 38.2)	-14.6 (-36.5 to 7.3)	0.19
Δ Dust mite specific IgE				
Der. farinae	3.5 (-6.2 to 13.2)	13.6 (-0.7 to 27.9)	-8.2 (-25.9 to 9.5)	0.36
Der. pteronyssinus	-2.6 (-9.7 to -4.5)	7.2 (-5.4 to 19.8)	-6.4 (-20.1 to 7.3)	0.36

All data were calculated using last observation carried forward to impute missing values. Data are expressed as mean (95% CI) absolute changes in FENO (ppb), eosinophil count (as % of total white cell count) and lung function variables (as % predicted value), and mean (95% CI) percentage change in total or specific IgE levels (kU/litre) relative to baseline level over the 1-year study period.

*Analysis of change in FENO in the subgroup of patients with abnormally raised FENO (>45 ppb) at baseline. In this group the mean (SD) baseline FENO in ppb was active 81.8 (44.2; n=56) and placebo 80.8 (32.3; n=23). For specific and total IgE and eosinophil counts, n=175 active, 91 placebo; for all other analyses, n=189 active, 93 placebo.

Der. farinae, Dermatophagoides farinae; Der. pteronyssinus, Dermatophagoides pteronyssinus; FEF₅₀, forced expiratory flow at 50% of vital capacity; FENO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 s; PEF, peak expiratory flow.

difference in our primary outcome measure of AQLQ responder rate between active and placebo groups. The reason that nocturnal TLA is successful where so many other approaches have failed may be the profound reduction in inhaled aeroallergen exposure which this treatment achieves. The beneficial effects of TLA treatment on quality of life were not restricted to a specific age group of the study population, although when those aged ≥12 years (on whom the study was powered) were analysed alone, the effect of treatment was of borderline statistical significance. The treatment effect did appear to be greatest in patients with a combination of high asthma treatment intensity and poor asthma control, who represent a significant area of unmet need. Nocturnal TLA treatment also led to reduced airway inflammation measured by FENO, particularly in patients with abnormally raised FENO (>45 ppb) prior to treatment, and interestingly led to modified progression of some allergen-specific IgE compared with placebo. The lesser increase in cat allergen-specific IgE raises the intriguing possibility that nocturnal exposure control may lead to longer-term downregulation of allergic immune responses which has not previously been reported. Given the close relationship between allergen exposure, IgE sensitisation and airway inflammation, these data suggest that nocturnal TLA may work through exclusion of aeroallergens from the breathing zone.^{18 19} We did not find any effect of TLA treatment on measures of lung function such as FEV₁ and PEF, and this is consistent with previous studies which showed that avoidance of aeroallergens can have beneficial effects on asthma symptoms and measures of airway inflammation without affecting lung function.^{4 7} This study was designed to evaluate the effect of TLA on self-reported quality of life, and the small numbers of patients with acute asthma exacerbations during the study limited our power to evaluate whether TLA treatment reduces exacerbation rates. The effects of TLA on quality of life and FENO are consistent with previous pilot work using TLA.²⁰ The clinical effects of nocturnal TLA treatment appear to be applicable to a broad patient group, because our study population included a wide age range of patients sensitised to a variety of perennial allergens, recruited in six countries. Our inclusion criteria for the study were broad, and did not demand formal demonstration of variable airway obstruction—the trial results can therefore be generalised to settings where an asthma diagnosis is made without use of such criteria. It is however possible that

treatment outcomes would differ in a group of patients with asthma included on the basis of meeting objective physiological criteria for airway obstruction or bronchial hyper-responsiveness. We found no evidence of a difference in treatment efficacy between children aged <12 and adolescents/adults. Although the treatment effect in those aged ≥12 was of borderline statistical significance, the effect size was similar to that seen in patients aged <12 and in the whole population. The difference in response rate between treatment groups in those aged ≥12 was not as large as the 20% difference which the study was powered to detect, perhaps due to the very high response rate in the placebo group. Overall our findings support other evidence that nocturnal exposures have a significant impact on inflammation and symptoms in asthma.^{21–23} This may be a consequence of circadian changes in autonomic function, steroid hormones and immune responsiveness. There is also persistent aeroallergen exposure at night, due in part to aeroallergen transfer to the breathing zone via body convection. Together with other studies of TLA, our findings suggest that the clinical effects of TLA can be explained by its ability to break the persistent body convection and thereby reduce aeroallergen exposure.

In conclusion we have demonstrated that nocturnal control of aeroallergen exposure using a novel non-pharmacological treatment TLA can improve quality of life and reduce airway inflammation in adults and children with atopic asthma, without significant adverse effects. Moreover the treatment limited rises in some aeroallergen-specific IgE levels, which have a close relationship with severity and persistence of asthma.^{18 19 24} Nocturnal TLA may be a treatment option for patients with uncontrolled atopic asthma despite high treatment intensity, where guidelines recommend stepping up treatment.¹⁰ Our findings support the importance of focusing exposure control interventions on the breathing zone, and highlight the role of nocturnal exposures in precipitating airway inflammation and symptoms in patients with atopic asthma.

Author footnote

Trial Steering Committee Professor Olof Zetterström (Chair), University Hospital of Linköping, Sweden; Professor Leif Björner, Lund University Hospital, Sweden; Professor Ronald Dahl, Aarhus University Hospital, Denmark; Professor Erka Valovirta, Terveystalo Turku, Finland; Dr Andrea Von Berg, Marien Hospital, Germany; Professor John Warner, Imperial College London, UK; Professor Magnus Wickman, Sachs' Children's Hospital, Stockholm, Sweden.

Data and Safety Monitoring Committee Professor Jan Lötvall (Chair), Göteborg University, Sweden; Professor Stephen Durham, National Heart and Lung Institute, Imperial College London, UK; Fredrik Hansson, Commitum AB.

***4A Study Group Members** Robert Boyle, NIHR Biomedical Research Centre, Imperial College Healthcare NHS Trust and Imperial College London, UK; Christophe Pedroletti, Päivi Söderman and Björn Nordlund, Karolinska University Hospital Solna, Sweden; Per Tunqvist and Magnus Wickman, Sach's Children's Hospital, Sweden; Victoria Strand and Pär Gyllfors, Capio St Görans Hospital, Sweden; Janne Björkander, County Hospital Ryhov, Sweden; Leif Bjermer and Stefan Willers, Lund University Hospital, Sweden; Ann Hammarlund, Hospital of Ängelholm, Sweden; Bill Hesselmar and Nils Åberg, The Queen Silvia Children's Hospital, Sweden; Erka Valovirta and Heikki Valkama, Terveystalo Turku, Finland; Kai-Håkon Carlsen, Karin Lødrup Carlsen and Egil Bakkeheim, Ullevål University Hospital, Norway; Malcolm Sue-Chu, Trondheim University Hospital, Norway; Ronald Dahl, Aarhus University Hospital, Denmark; Vibecke Backer and Lotte Harmsen, Bispebjerg Hospital, Denmark; Claudia Gore, and Heather Hanna, Imperial College London, UK; Christian Virchow and Peter Julius, University of Rostock, Germany; Erika Von Mutius and Caroline von Ehrenstein, University of Munich, Germany; Andrea Von Berg and Christina Beckman, Marien-Hospital Wesel, Germany; Per Olof Wernersson, Allergimottagningen Karlstad, Sweden; Uwe Schauer, St Josef-Hospital Bochum, Germany; Olof Zetterström and Ulla Nyström Kronander, University Hospital of Linköping, Sweden; John Warner, NIHR Biomedical Research Centre, Imperial College Healthcare NHS Trust, and Imperial College London, UK.

Acknowledgements We are grateful to the participants for their assistance in the conduct of this clinical trial.

Funding The study was funded by Airsonett AB. Robert Boyle was supported by a National Institute for Health Research Clinical Lectureship. John Warner is supported by a National Institute for Health Research Senior Fellowship. Robert Boyle and John Warner are supported by a National Institute for Health Research Biomedical Research Centre, and by the MRC and Asthma UK Centre in Allergic Mechanisms of Asthma.

Competing interests None.

Ethics approval Ethics approval was provided by responsible institutional review boards at each site in this multicentre international trial.

Contributors CP, MW, LB, EV, RD, AVB, OZ and JOW designed the study and planned the analyses. All members of the 4A study group participated in patient recruitment for the study. RJB wrote the manuscript with contributions from all authors.

Provenance and peer review Not commissioned; externally peer reviewed.

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Statistical Analysis Plan

‘Clinical analyses for Protexo’

Pr. Mondher TOUMI

MD, PhD, Msc

☎ +33 (0)4 72 43 16 54

mondher.toumi@univ-lyon1.fr

Pr. Michel LAMURE

MD, PhD, Msc

☎ + (33) 4 72 43 16 54

lamure@univ-lyon1.fr

Date: 19/04/2010

Update 1 : 06/08/2010

Update 2 : 06/10/2010

Université Claude Bernard Lyon 1 - Bât. Nautibus

Laboratoire ERIC - Equipe MA2D

43, boulevard du 11 Novembre 1918

69622 Villeurbanne Cedex - France

Preamble

First update contains rational for the choice of LOCF and adjustment on countries. No change was made on statistic analyses planned between this update and the original version.

Second update contains a clarification for the subgroup analysis. No change was made on statistic analyses planned between this update and the original version.

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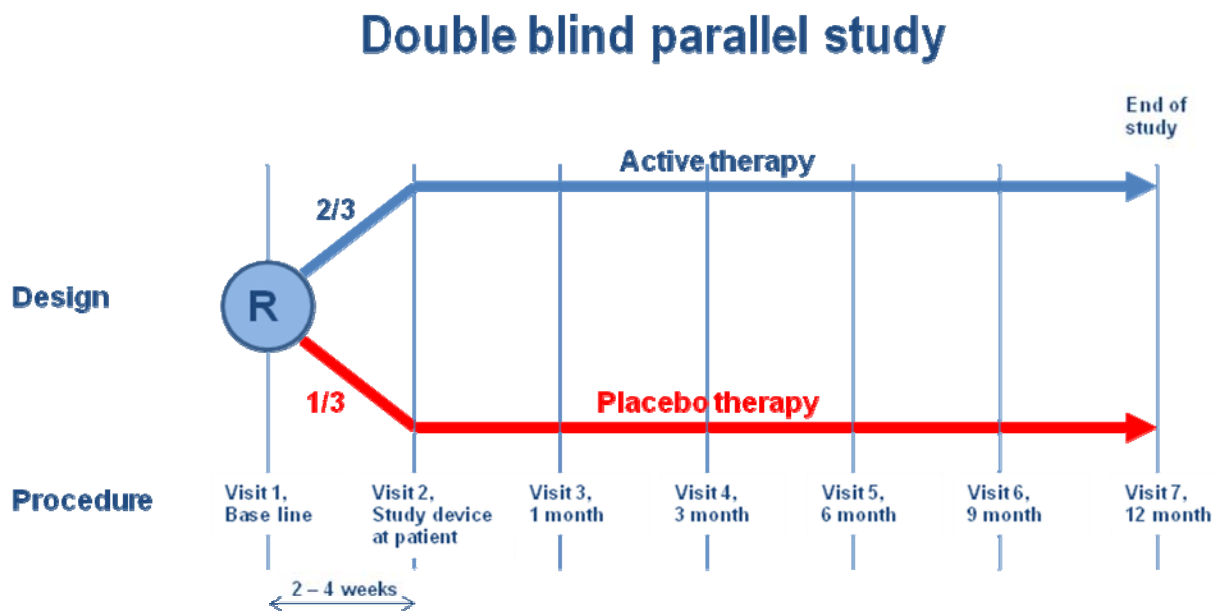
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2. INTRODUCTION

2.1 Trial design

This was a multiple independent, double blind, randomized 52 weeks parallel trial comparing active and placebo treatment with Airsonett Airshower (AA). For ethical reasons the randomization was 2 to 1 for active and placebo treatment, respectively. At visit 1, patients were evaluated against the inclusion and exclusion criteria, randomized and all baseline measures taken. The Airsonett Airshower was installed in the patient’s home within 4 weeks after inclusion, during this time the patient got familiar with the use of the patient asthma diary and adhered to the requirements of the study participation. The first 3 months an unchanged maintenance medication was kept and month 4 - 12 medication was based on control (GINA 2006).

Figure 1. Study design



2.2 Visits

In this statistical analysis plan, we will assume that:

- Visit 1 = baseline
- Visit 2 = 2 weeks visit
- Visit 3 = 6 weeks visit
- Visit 4 = 14 weeks visit
- Visit 5 = 28 weeks visit

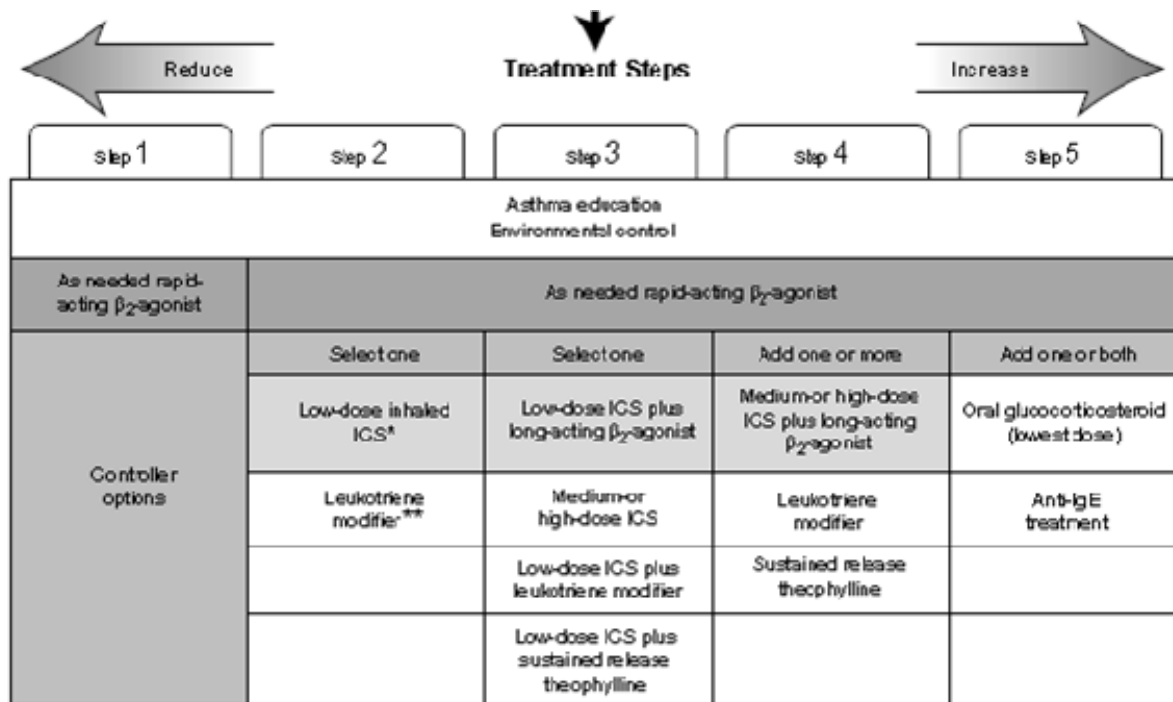
Visit 6 = 41 weeks visit

Visit 7 = 54 weeks visit

3. ANALYSIS POPULATIONS AND DATA DEFINITIONS

3.1 GINA¹ classification

GINA classification is based on treatment intensity, and will be used in adjustment as a proxy of treatment in analysis.



^{*} ICS=inhaled glucocorticosteroids
^{**}-Receptor antagonist or synthesis inhibitor

3.2 Analysis populations

The **Full Analysis Set (FAS)** contains all patients.

The **Intention-To-Treat (ITT) analysis set** consists of all randomized patients who have had at least one treatment day with the Airshower. 9 subsets are derived from ITT set:

¹ Pocket guide for asthma management and prevention, A Pocket Guide for Physicians and Nurses Revised 2006

- × **ITT**: all ITT population
- × **ITT-P12**: Pediatric ITT population (<12 years)
- × **ITT-A12**: Adults ITT population (≥ 12 years)
- × **ITT-P18**: Pediatric ITT population (<18 years)
- × **ITT-A18**: Adults ITT population (≥ 18 years)
- × **ITT-R**: ITT patients with rhinitis at baseline
- × **ITT-STB**: ITT stable² patients
- × **ITT-M-GINA**: ITT patients in step 3 or step 4 according to GINA classification
- × **ITT-S-GINA**: ITT patients in step 4 according to GINA classification

Patients, who drop out after randomization and before the first use of the Airshower, will be excluded from the intention-to-treat analysis.

The **Per Protocol-52 (PP52) analysis set** consists of all randomized patients who have used the Airshower minimum 80% of the 52 week trial period, as recorded on a data chip in the machine and 80% of the last 3 weeks prior to visit month 3 and 12. In the same way that for ITT, 9 subsets are derived from PP set:

- × **PP52**: all PP52 population
- × **PP52-P12**: Pediatric PP52 population (<12 years)
- × **PP52-A12**: Adults PP52 population (≥ 12 years)
- × **PP52-P18**: Pediatric PP52 population (<18 years)
- × **PP52-A18**: Adults PP52 population (≥ 18 years)
- × **PP52-R**: PP52 patients with rhinitis at baseline
- × **PP52-STB**: PP52 stable patients
- × **PP52-M-GINA**: PP52 patients in step 3 or step 4 according to GINA classification
- × **PP52-S-GINA**: PP52 patients in step 4 according to GINA classification

The **Per Protocol-12 (PP12) analysis set** consists of all randomized patients who have used the Airshower minimum 80% of the 12 week trial period, as recorded on a data chip in the machine

² A patient is considered stable if absolute difference between AQLQ score at V2 and AQLQ score at V1 is <0.5.

and 80% of the last 3 weeks prior to visit month 3 and 12. In the same way that for ITT, 9 subsets are derived from PP set:

- × **PP12:** all PP12 population
- × **PP12-P12:** Pediatric PP12 population (<12 years)
- × **PP12-A12:** Adults PP12 population (≥ 12 years)
- × **PP12-P18:** Pediatric PP12 population (<18 years)
- × **PP12-A18:** Adults PP12 population (≥ 18 years)
- × **PP12-R:** PP12 patients with rhinitis at baseline
- × **PP12-STB:** PP12 stable patients
- × **PP12-M-GINA:** PP12 patients in step 3 or step 4 according to GINA classification
- × **PP12-S-GINA:** PP12 patients in step 4 according to GINA classification

All analyses will be run on each of the 27 populations.

Further, analyses will be run on asthma severity defined as the intensity of treatment required to control the patient's asthma. Sub-group of treatment intensity (GINA) in combination with the activity of the underlying disease (ACT) according to the definition of asthma severity in ATS/ERS statement^{3,4} will be created.

The FAS contains 312 patients, the ITT set contains 282 patients, the PP52 set contains 205 patients and the PP12 set contains 230 patients.

	Nb of patients (TLA + placebo)		Nb of patients (TLA + placebo)		Nb of patients (TLA + placebo)
ITT	282 (189 + 93)	PP12	230 (157 + 73)	PP52	205 (139 + 66)
ITT-P12	68 (46 + 22)	PP12-P12	61 (41 + 20)	PP52-P12	58 (39 + 19)
ITT-A12	214 (143 + 71)	PP12-A12	169 (116 + 53)	PP52-A12	147 (100 + 47)
ITT-P18	152 (104 + 48)	PP12-P18	129 (90 + 39)	PP52-P18	119 (80 + 39)
ITT-A18	130 (85 + 45)	PP12-A18	101 (67 + 34)	PP52-A18	86 (59 + 27)
ITT-R	268 (180 + 88)	PP12-R	220 (151 + 69)	PP52-R	197 (134 + 63)
ITT-STB	129 (87 + 42)	PP12-STB	105 (74 + 31)	PP52-STB	96 (65 + 31)
ITT-M-GINA	228 (153 + 75)	PP12-M-GINA	187 (127 + 60)	PP52-M-GINA	166 (113 + 53)
ITT-S-GINA	129 (82 + 47)	PP12-S-GINA	111 (70 + 41)	PP52-S-GINA	96 (58 + 38)

ITT- UnC*	183 (125 + 58)	PP12- ACT<18	149 (103 + 46)	PP52- ACT<18	129 (89 + 40)
ITT-M- GINA- UnC*	153 (103 + 50)	PP12-M- GINA- UnC*	126 (85 + 41)	PP52-M- GINA- UnC*	109 (74 + 35)
ITT-S- GINA- UnC*	87 (57 + 30)	PP12-S- GINA- UnC*	74 (47 + 27)	PP52-S- GINA- UnC*	62 (37 + 25)

*UnC: Uncontrolled asthma according to section 5.4 clinical assessments

³ Reddel HK et al, An Official American Thoracic Society/European Respiratory Society Statement: Asthma Control and Exacerbations. American Journal of Respiratory and Critical Care Medicine Vol 180. pp. 59-99, (2009)

⁴ Taylor DR et al. A new perspective on concepts of asthma severity and control. Eur Respir J 2008;32: 545-554.

4. STATISTICAL CONVENTIONS

ITT populations will be analyzed using the LOCF (Last Observation Carried Forward) technique. PP12 and PP52 populations will be described using OC (Observed Case) technique.

All continuous variables will be described using number of valid values, number of missing values, mean, standard deviation, 95%CI, min-max, median, and Q1-Q3.

All categorical variables will be described using number of valid values, number of missing values and percentages.

When provided, graphs will show the evolution of the considered outcome, from V1 to V7. SD of means will be provided on the graph.

Rationale for using LOCF method

How to integrate missing data, and in particular drop outs in an analysis, has always been a significant issue.

The Last Observation Carried Forward (LOCF) imputation method can be used when data are longitudinal (i.e. repeated measures have been taken per subject by time point). The last observed non-missing value is used to fill in missing values at a later point in the study. Therefore one makes the assumption that the response remains constant at the last observed value. [LOCF Method and Application in Clinical Data Analysis Huijuan Xu, Biogenidec, Inc., 2009]

The FDA has traditionally viewed LOCF as the preferred method of analysis, considering it likely (but not certain) to be conservative and clearly better than using observed cases (OC).

There is no perfect method to handle missing data. Nevertheless it is important to define it in the SAP, a priori. It is also important to choose one accepted by authorities (FDA and EMEA). Here are other considerations:

One must be sure that the method used does not penalize the product which shows less drop outs. If ever the product is not so efficient, or not well tolerated, there might be lots of drops outs in the active group.

There are 2 situations in clinical trials:

- If the disease is progressive, and if the treatment of interest aims at slowing the progression of the disease, OC (Observed Case) should be chosen. With LOCF, the treatment with the more drops out would be favored.
- If the treatment aims at improving any outcome, it is the contrary. LOCF should be chosen.

The alternative to LOCF is MMRM (which integrates use of all points, and then gives more power). But LOCF is the method the most frequently used in all clinical trials that are done for registering, and completely accepted by authorities.

Due to the relatively high patient dropout rate of this study, analysis will be conducted on two different datasets: one on ITT with an imputation of missing values according to the LOCF methodology and the other on PP in the absence of data imputation (that is, using OC method). It is common in clinical trials to analyze ITT population using the LOCF method, and to make a sensibility analysis working on PP population, using OC method.

5. BASELINE DESCRIPTION

5.1 Demographical characteristics

Sex, age, ethnic origin, weight, height, BMI, country and site at baseline will be described.

5.2 Medical History

Types of allergen at baseline will be described using the following variables:

- Dust mites
- Cat
- Other allergens
- No. of Perenn
- Pollen
- Perenn and Pollen

- Total number of allergens (as continuous and categorical variable)
- 1, 2 or more than 3 allergens

Presence of any significant medical history at baseline will be described.

5.3 Physical Examination

Presence of nasal polyps, septum deviation and rhinitis at baseline will be described.

5.4 Clinical assessment

ACT groups at baseline will be described. It was defined as follows:

- 0-17: Uncontrolled
- 18-19: Partially controlled
- ≥ 20 : Controlled

FENO groups at baseline will be described. It was defined as follows:

- 5-25: Normal
- 26-45: Increased risk
- > 45 : High risk

GINA groups at baseline will be described. Please refer to 3.1 for definition.

6. EFFICACY ANALYSIS

6.1 Responder rate

6.1.1 Primary endpoint

A short version of the AQLQ (Asthma Quality of Life Questionnaire), the mini-AQLQ, has been developed and fully validated. This instrument has 15 items and each item has the same 7 severity levels as the original (1 = severe impairment to 7 = no impairment). It consists of 5 items on symptoms, 4 items on activity limitations, 3 items on emotional function, and 3 items concerning environmental stimuli. For children under the age of 12 the P-AQLQ was used.

The primary endpoint, **responder rate**, is defined as follows: a patient will be considered as a responder if the increase at V7 compared to baseline in mean total AQLQ (mini-AQLQ or P-AQLQ) score is at least 0.5 units. Otherwise the patients are categorized as non responder.

The population considered for the primary endpoint will be the ITT population.

Responder rate at V7 will be compared between the treatment groups, using 3 tests:

- Chi2 test
- Adjusted test: logistic regression, adjusting on treatment, baseline AQLQ score, time since disease onset, GINA classification at baseline, and stratified on country
- Repeated observations model will be also used to account for heterogeneity between individuals and correct for potential bias associated with drop-outs (same adjustment will be used)

Note: some patient has been using both mini-AQLQ and P-AQLQ, the list describing if it is AQLQ or PAQLQ that should be used is in appendix 9.1.

Responder rates will also be described at each visit.

6.1.2 Sensitivity analysis on primary endpoint

Responder rate at V4 and sustained responders rate (responders at V4, who remain responders at V5, V6 and V7) will also be described and compared in the same way as primary endpoint as a sensitivity analysis.

6.2 AQLQ score

6.2.1 AQLQ score change

AQLQ (mini-AQLQ and P-AQLQ) scores will be described at each visit. Evolution graphs will be provided.

Change from baseline in AQLQ (mini-AQLQ and P-AQLQ) score between will be described in the same way. Comparison between treatment groups will be provided using 3 tests:

- Ttest
- Adjusted test: analysis of covariance regression (ANCOVA), adjusting on treatment, baseline AQLQ score, time since disease onset, GINA classification at baseline, and country
- Repeated observations model will be also used to account for heterogeneity between individuals and correct for potential bias associated with drop-outs (same adjustment will be used)

AQLQ change V4-V1 will also be analyzed.

6.3 AQLQ subscores

Mini-AQLQ can be assessed using 4 subscales scores:

- Symptoms subscore: mean of items 1, 4, 6, 8 and 10
- Emotions subscore: mean of items 3, 5 and 9

- Activities subscore: mean of items 12, 13, 14 and 15
- Environment subscore: mean of items 2, 7 and 11

In the same way, P-AQLQ can be assessed using 3 subscales scores:

- Symptoms subscore: mean of items 4, 6, 8, 10, 12, 14, 16, 18, 20 and 23
- Emotions subscore: mean of items 5, 7, 9, 11, 13, 15, 17 and 21
- Activities subscore: mean of items 1, 2, 3, 19 and 22

These subscores will be described at each visit. Evolution graphs will be provided.

Change from baseline in subscores will be described in the same way. Comparison between treatment groups will be provided using 3 tests:

- Ttest
- Adjusted test: analysis of covariance regression (ANCOVA), adjusting on treatment, baseline subscore, time since disease onset, GINA classification at baseline, and country
- Repeated observations model will be also used to account for heterogeneity between individuals and correct for potential bias associated with drop-outs (same adjustment will be used)

6.4 AQLQ sleep items

6.4.1 AQLQ sleep items scores

Items concerning quality of sleep of the patient will be analyzed.

One item is referring to the quality of sleep in the mini-AQLQ: item 8 “Have difficulty getting a good night’s sleep as a result of asthma? ».

Two items are referring to the quality of sleep in the P-AQLQ: item 16 “Wake-up during the night because of asthma? », and item 20 “Have trouble sleeping at night because of your asthma? »

These items will be described at each visit. Evolution graphs will be provided.

Change from baseline in scores will be described in the same way. Comparison between treatment groups will be provided using 3 tests:

- Ttest
- Adjusted test: analysis of covariance regression (ANCOVA), adjusting on treatment, baseline score, time since disease onset, GINA classification at baseline, and country
- Repeated observations model will be also used to account for heterogeneity between individuals and correct for potential bias associated with drop-outs. Same adjustment will be used.

6.4.2 AQLQ sleep items responders

A responder patient is defined as follows: a patient will be considered as a responder if the increase at V7 compared to baseline in AQLQ sleep item score is at least 0.5 units. Otherwise the patients are categorized as non responder.

Responder rate at V7 will be compared between the treatment groups, using 3 tests:

- Chi2 test
- Adjusted test: logistic regression, adjusting on treatment, baseline ACT score, time since disease onset, GINA classification at baseline and stratified on country
- Repeated observations model will be also used to account for heterogeneity between individuals and correct for potential bias associated with drop-outs. Same adjustment will be used.

Responder rates will also be described at each visit.

6.5 ACT scale

6.5.1 ACT scale scores

ACT scores will be described at each visit. Evolution graphs will be provided.

Change from baseline in ACT score will be described in the same way. Comparison between treatment groups will be provided using 3 tests:

- Ttest
- Adjusted test: analysis of covariance regression (ANCOVA), adjusting on treatment, baseline ACT score, time since disease onset, GINA classification at baseline, and country
- Repeated observations model will be also used to account for heterogeneity between individuals and correct for potential bias associated with drop-outs. Same adjustment will be used.

ACT change V4-V1 will also be analyzed.

6.5.2 ACT scale responders

A responder patient is defined as follows: a patient will be considered as a responder if the increase at V7 compared to baseline in mean total ACT score is at least 3 units. Otherwise the patients are categorized as non responder.

Responder rate at V7 will be compared between the treatment groups, using 3 tests:

- Chi2 test
- Adjusted test: logistic regression, adjusting on treatment, baseline ACT score, time since disease onset, GINA classification at baseline and stratified on country

- Repeated observations model will be also used to account for heterogeneity between individuals and correct for potential bias associated with drop-outs. Same adjustment will be used.

Responder rates will also be described at each visit.

Responder rate at V4 will also be compared in the same way.

6.5.3 ACT classification

ACT scores will be categorized into 3 classes for each visit, defined as follows:

- 0-17: Uncontrolled
- 18-19: Partially controlled
- ≥ 20 : Controlled

ACT classes will be provided at each visit (except V3 as assessment was not done).

Change in classes will be described at each visit, using 3 classes:

- worse
- no change
- improvement

Change in classes at V7 will be compared between the treatment groups using 2 tests:

- Chi2 test
- Adjusted test: logistic regression, adjusting on treatment, baseline ACT score, time since disease onset, GINA classification at baseline and country

ACT change group V4-V1 will also be analyzed.

6.6 Specific allergens

Specific groups will be analyzed for this outcome: patients cat allergic at baseline, and patients dust mites allergic at baseline.

6.6.1 Total IgE

Total IgE will be described at V1 and V7. Evolution graphs will be provided.

Relative change³ from baseline in IgE will be described in the same way. Comparison between treatment groups will be provided using 2 tests:

- Ttest

³ Relative change = $(V7-V1)/V1$

- Adjusted test: analysis of covariance regression (ANCOVA), adjusting on treatment, baseline value, time since disease onset, GINA classification at baseline and country

6.6.2 Specific IgE

Specific IgE (d1, d2, e1, e5, max(d1,d2), and max(d1,d2)+e1+e5) will be described as specific IgE at V1 and V7. Evolution graphs will be provided.

Relative change from baseline in specific IgE will be described in the same way. Comparison between treatment groups will be provided using 2 tests:

- Ttest
- Adjusted test: analysis of covariance regression (ANCOVA), adjusting on treatment, baseline value, time since disease onset, GINA classification at baseline and country

Specific IgE will also be analyzed as percentage of total IgE in the same way.

6.7 FENO and Spirometry

6.7.1 FENO and Spirometry

FENO and all spirometry variables (FEV1, PEF and FEF50, as percentages of reference) will be described at each visit. Evolution graphs will be provided.

Change from baseline in FENO and all spirometry variables (FEV1, PEF and FEF50) score will be described in the same way. Comparison between treatment groups will be provided using 3 tests:

- Ttest
- Adjusted test: analysis of covariance regression (ANCOVA), adjusting on treatment, baseline score, time since disease onset, sever GINA classification at baseline, and country
- Repeated observations model will be also used to account for heterogeneity between individuals and correct for potential bias associated with drop-outs. Same adjustment will be used.

FENO and all spirometry variables change V4-V1 will also be analyzed.

6.7.2 FENO classification

FENO quantitative variable will be categorized into 3 classes for each visit, defined as follows:

- 5-25: Normal
- 26-45: Increased risk
- > 45: High risk

FENO classes will be provided at each visit.

Change in classes will be described at each visit, using 3 classes:

- worse
- no change
- improvement

Change in classes at V7 will be compared between the treatment groups using 2 tests:

- Chi2 test
- Adjusted test: logistic regression, adjusting on treatment, baseline ACT score, time since disease onset, GINA classification at baseline and country

6.8 Rhinitis scale

6.8.1 Rhinitis scale score

Rhinitis scale score will be described at each visit. Evolution graphs will be provided.

Change from baseline in rhinitis scale score will be described in the same way. Comparison between treatment groups will be provided using 2 tests:

- Ttest
- Adjusted test: analysis of covariance regression (ANCOVA), adjusting on treatment, baseline rhinitis scale score, time since disease onset, GINA classification at baseline and country

6.8.2 Rhinitis scale items

Rhinitis is composed of 5 questions:

Question 1: Has the patient had rhinitis problem at any time yes/no.

Comparison between groups of amount of problems at V1 and V7 will be provided using 3 classes:

- worse
- no change
- improvement

Question 2: Has the patient had a rhinitis problem during the past year yes/no

Comparison between groups of amount of problems at V1 and V7 will be provided using 3 classes:

- worse

- no change
- improvement

Question 3: Has the patient had any problems with running/itching eyes in connection with rhinitis during the past year yes/no.

Comparison between groups of amount of problems at V1 and V7 will be provided using 3 classes:

- worse
- no change
- improvement

Question 4: Amount of months during the last year the patient has had with Rhinitis problem during the last year score 0-12.

Comparison of change in amount of months will be provided, using a ttest.

Question 5: During the last year, how much has rhinitis problems affected the patients daily activity: 0=not at all, 1=a bit (mild), 2=moderate, 3=a lot (severe).

Comparison between groups of amount of problems at V1 and V7 will be provided using 3 classes:

- worse
- no change
- improvement

6.9 Exacerbations

Analysis of systemic corticosteroids, and HCRU used will be used as a proxy for exacerbations.

Use of systemic corticocterooids, hospital day, emergency department visit and unscheduled visits at least once during the whole study will be described and analyzed. Comparison between treatment groups will be provided using 2 tests:

- Ttest
- Adjusted test: logistic regression, adjusting on treatment, time since disease onset, GINA classification at baseline and stratified on country

6.10 Eosinophils

Eosinophils will be described at V1 and V7.

Change from baseline in eosinophils will be described in the same way. Comparison between treatment groups will be provided using 2 tests:



- Ttest
- Adjusted test: analysis of covariance regression (ANCOVA), adjusting on treatment, baseline eosinophils, time since disease onset, GINA classification at baseline and country

7. MEDICATION

Assuming dates of start and stop of treatment are available and usable, medication will be categorized using 5 classes:

- ICS (inhaled corticosteroids)
- OCS (oral corticosteroids)
- LABA (long acting β -agonist)
- SABA (short acting β -agonist)
- LTRA (leukotriene receptor antagonist)
- Other

For each class, use of medication will be described using the following variables:

- Use of medication at each visit
- If use of medication, daily dose at each visit
- If use of medication, number of days under medication class between previous visit and current visit, starting from V2
- If use of medication at the previous visit, stopping of medication at the current visit
- Change in the GINA treatment steps V7-V1

8. RATIONALE FOR ADJUSTMENT ON COUNTRY RATHER THAN ON CENTRE

Using center for adjustment is the most appropriate method in multicentric studies. However the low number of patients by centers and the large number of center and disparity across center suggested that center adjustment might not be feasible. This is why the original version of the SAP proposed to adjust on countries rather than centers.

This was confirmed during the analysis. Further analysis showed that there were too many centers ($n=20$) and not enough patients per centers for sub-analysis (please refer to 3.2). Adjusting on centers would have caused a significant loss of power. Moreover, some models did not converge using this adjustment and a pooling of centers was necessary. This was linked to the large number of centers, and the disparity of number of patients per center. Most of sub-analysis would have not been possible (those where only a small selection of population was analyzed).

That is why we anticipated to chose country as alternative adjustment. This was shown in the analysis to be relevant.

9. APPENDIX

9.1 Appendix 1

Site	Pat No	Initials	PAQLQ=P	MAQLQ=A
1	1	WHG	x	
1	3	ABL		x
1	4	VMP	x	
1	5	PAK	x	
1	6	SLS		x
1	7	AVA	x	
1	8	ETS		x
1	9	HIH	x	
1	39	NAT		x
1	40	TKH		x
1	41	KEL		x
1	42	AWV		x
1	43	SNM	x	
1	44	CSE		x
1	145	NLA	x	
1	146	AFW		x
1	149	RMG		x
1	150	FRL		x
1	152	JBj		x
1	153	MGW		x
1	163	SCB	x	
1	164	FMM		x
1	165	SMW		x
1	166	MMN	x	

Site	Pat No	Initials	PAQLQ=P	MAQLQ=A
1	168	ETA	x	
1	169	IjW	x	
1	170	AAV		x
1	171	CEL		x
1	316	JCP		x
1	320	SHK		x
1	321	OG		x
1	322	AHT		x
1	323	GLF		x
1	324	AGA		x
1	370	TVM	x	
1	371	KLN		x
1	372	KAM	x	
1	373	OjW	x	
1	374	ZIZ		x
14	46	ROS		x
14	47	SAJ	x	
14	49	KLC		x
14	51	JOJ		x
14	53	SRO	x	
14	103	LOU		x
14	106	ROC		x
14	108	RCS		x
14	181	HAS	x	

1 **Nocturnal Temperature controlled Laminar Airflow for**
2 **treating atopic asthma: a randomised controlled trial**

3 **Robert J Boyle, clinical senior lecturer,¹ Christophe Pedroletti, consultant,²**
4 **Magnus Wickman, professor,³ Leif Bjermer, professor,⁴ Erkkka Valovirta,**
5 **professor,⁵ Ronald Dahl, professor,⁶ Andrea Von Berg, professor,⁷ Olof**
6 **Zetterström, professor,⁸ John O Warner, professor,¹ for the 4A Study Group**

7 ¹Department of Paediatrics, Imperial College London, United Kingdom, ²Department
8 of Woman and Child Health, Karolinska Institutet, Sweden, ³Institute of
9 Environmental Medicine, Karolinska Institutet and Sachs' Children's Hospital,
10 Stockholm, Sweden, ⁴Department of Respiratory Medicine and Allergology, Lund
11 University Hospital, Sweden, ⁵Terveystalo, Finland, ⁶Department of Respiratory
12 Diseases, Aarhus University Hospital, Denmark, ⁷Research Institute for Prevention of
13 Childrens' Allergy and Respiratory Diseases, Marien-Hospital, Wesel, Germany,
14 ⁸Allergy Centre, University Hospital of Linköping, Sweden.

15

16 Address for Correspondence:

17 John O Warner

18 Professor of Paediatrics

19 Imperial College London

20 Wright Fleming Building

21 Norfolk Place

22 London W2 1PG

23 Tel: +44 207 594 3990 Fax: +44 207 594 3984 Email: j.o.warner@imperial.ac.uk

24 ***METHODS***

25 **Mode of action of Protexo**

26 At night airborne particles are carried by a persistent convection current established
27 by the warm body, transporting allergens from the bedding area to the breathing
28 zone. The TLA device Protexo is designed to displace the body convection which
29 leads to persistent exposure to particles and allergen in bed. Ambient room air is
30 filtered, cooled by 0.5-0.8°C and distributed to the breathing zone by Protexo – the
31 reduced temperature allows the filtered air to descend slowly in a steady laminar
32 stream, displacing particulate and allergen rich air from the breathing zone (fig S4).
33 The method is able to break body convection without creating draught or
34 dehydration, and thereby reduces and controls particle and aeroallergen exposure in
35 the breathing zone (1). A recent study demonstrated >30-fold reduction of cat
36 allergen in the breathing zone with TLA compared to no treatment, and >3000-fold
37 reduction in all particles $\geq 0.5\mu\text{m}$ (>3700-fold reduction in particles $\geq 10\mu\text{m}$)¹.

38 **Airborne particle count measurements**

39 Home visits for clean zone validation according to EN-ISO14644-3:2005 standard
40 were performed by technicians at device installation, 3, 6 and 12 months follow-up.
41 Airborne particle count measurements were made using GT-321 Handheld Particle
42 Counters (Met One Instruments Inc, USA).

43 **Dust allergen collection and analysis**

44 Three months after device installation a vacuumed dust sample was collected from
45 participants' beds as previously described². Briefly, mattresses with undersheets left

46 on were vacuumed for 2 minutes using a vacuum cleaner with sampling nozzle
47 (ALK, Hørsholm, Denmark) according to a standard protocol. Protein was extracted
48 from 100mg dust in 2ml phosphate buffered saline with 0.05% Tween-20 for 2 h at
49 room temperature with rotation. Samples were centrifuged at 4500 rpm for 10 min
50 then 10,000 rpm for 10 min and supernatants stored at -20°C. Allergen levels were
51 determined using a sandwich ELISA kit for cat (Fel d 1) and dust mite (Der f 1 and
52 Der p 1) allergens according to the manufacturer's instructions (Indoor
53 Biotechnologies, Warminster, UK). Allergen concentrations were expressed as ng/g
54 dust with a detection limit of <50 ng/g.

55

55 **RESULTS**

56 **Treatment compliance and efficacy of blinding**

57 In the active group, 136/166 (72%) participants who completed the whole study used
58 their device on at least 80% of expected nights. In the placebo group this figure was
59 66/79 (71%). At the end of the study 165 patients answered the question which
60 treatment they believed they received, to assess efficacy of masking. In the active
61 group 52/105 (50%) believed they had received an active device; in the placebo
62 group 35/60 (58%) believed they had received a placebo device.

63 **Aeroallergen exposure and relationship with specific IgE levels**

64 The median particle count (particles \geq 0.5 μ m diameter) in patients' bedrooms at
65 device validation visits (installation, 3, 6 and 12 months) was 103,804 particles/ft³
66 (IQR 56,880 to 193,840; n=1064 measurements). Median counts in the breathing
67 zone a few minutes after turning the device on were 720 particles/ft³ (IQR 306 to
68 1,485) for TLA and 117,047 (68,197 to 215,921) for placebo. In view of the finding of
69 lesser increase in cat-specific IgE in active versus placebo treated patients in this
70 study, we also analysed dust samples aspirated from the mattresses of 132
71 participants (87 active, 45 placebo) at 3 months. Allergen detection rates are shown
72 in table S4 – Der p 1, Der f 1 and Fel d 1 were detected in 20%, 40% and 67% of
73 mattress dust samples respectively, and there was no significant difference in
74 detection rates between active and placebo treated patients. Among sensitized
75 participants, allergen-specific IgE levels were positively correlated with mattress dust

76 allergen levels for cat ($r=0.36$, $P=0.004$), and house dust mite allergens Der f 1
77 ($r=0.37$, $P=0.001$) and Der p 1 ($r = 0.57$, $P<0.001$; fig S5 A-C).

78 **Adverse Events**

79 Adverse events affecting $\geq 5\%$ of patients on ≥ 1 occasion were upper respiratory
80 tract infection (ICD-9 code 480-488) in 117 (61.9%) participants in active and 62
81 (66.7%) in placebo group; upper respiratory tract symptoms (ICD-9 code 490-496) in
82 54 (28.6%) in active and 22 (23.7%) in placebo group; general symptoms (ICD-9
83 code 780-789) in 43 (22.8%) in active and 19 (20.4%) in placebo group.

84 **REFERENCES**

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86 Laminar Airflow device on personal breathing zone aeroallergen exposure. (*under*
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89 dust of farm children, Rudolf Steiner school children and reference children--the
90 PARSIFAL Study. *Allergy* 2005;60(5):611-8.

Table S4. Level of asthma medication use during TLA treatment

	Baseline Active	Baseline Placebo	3-12 months Active	3-12 months Placebo	Difference in medication*	P value
Inhaled corticosteroids	0.72 (0.46)	0.77 (0.47)	0.74 (0.53)	0.77 (0.49)	0.03 (0.04)	0.38
Short acting β -2 agonist	0.20 (0.40)	0.22 (0.39)	0.19 (0.25)	0.22 (0.41)	0.02 (0.02)	0.39
Long acting β -2 agonist	0.51 (0.51)	0.53 (0.48)	0.51 (0.48)	0.55 (0.47)	-0.01 (0.03)	0.77
Leukotriene receptor antagonist	0.29 (0.46)	0.24 (0.41)	0.31 (0.53)	0.28 (0.43)	-0.00 (0.02)	0.88

All medication doses are expressed as mean (sd) proportion of the 'Defined Daily Dose', according to World Health Organisation Drugs Statistics Methodology guidelines. * Difference = mean (SE) of [(Active during 3-12 months) – (Active at baseline)] – [(Placebo during 3-12 months) – (Placebo at baseline)]. During the whole study period, systemic corticosteroids for ≥ 3 days were administered on ≥ 1 occasion to 25/189 (13.2%) patients in active and 12/93 (12.9%) patients in placebo group (P=0.94), and the mean (sd) number of systemic corticosteroid courses administered per patient was 0.17 (0.53) in active and 0.24 (0.83) in placebo group (P=0.50).

Table S5. Allergen detection in mattress dust samples

	Active	Placebo	P value	Total
Der p 1	18/87 (21%)	8/45 (18%)	0.69	26/132 (20%)
Der f 1	36/87 (41%)	17/45 (38%)	0.69	53/132 (40%)
Fel d 1	57/87 (66%)	32/45 (71%)	0.52	89/132 (67%)

Data shown are the number (%) of mattress samples with detectable levels of house dust mite (Der p 1, Der f 1) or cat (Fel d 1) major allergens. Mattress dust samples were taken at 3 months from study participant bedrooms at UK and some Swedish sites. Detection limit for all allergens = 50ng/g mattress dust. P values are calculated using chi-squared test.

FIGURE LEGENDS

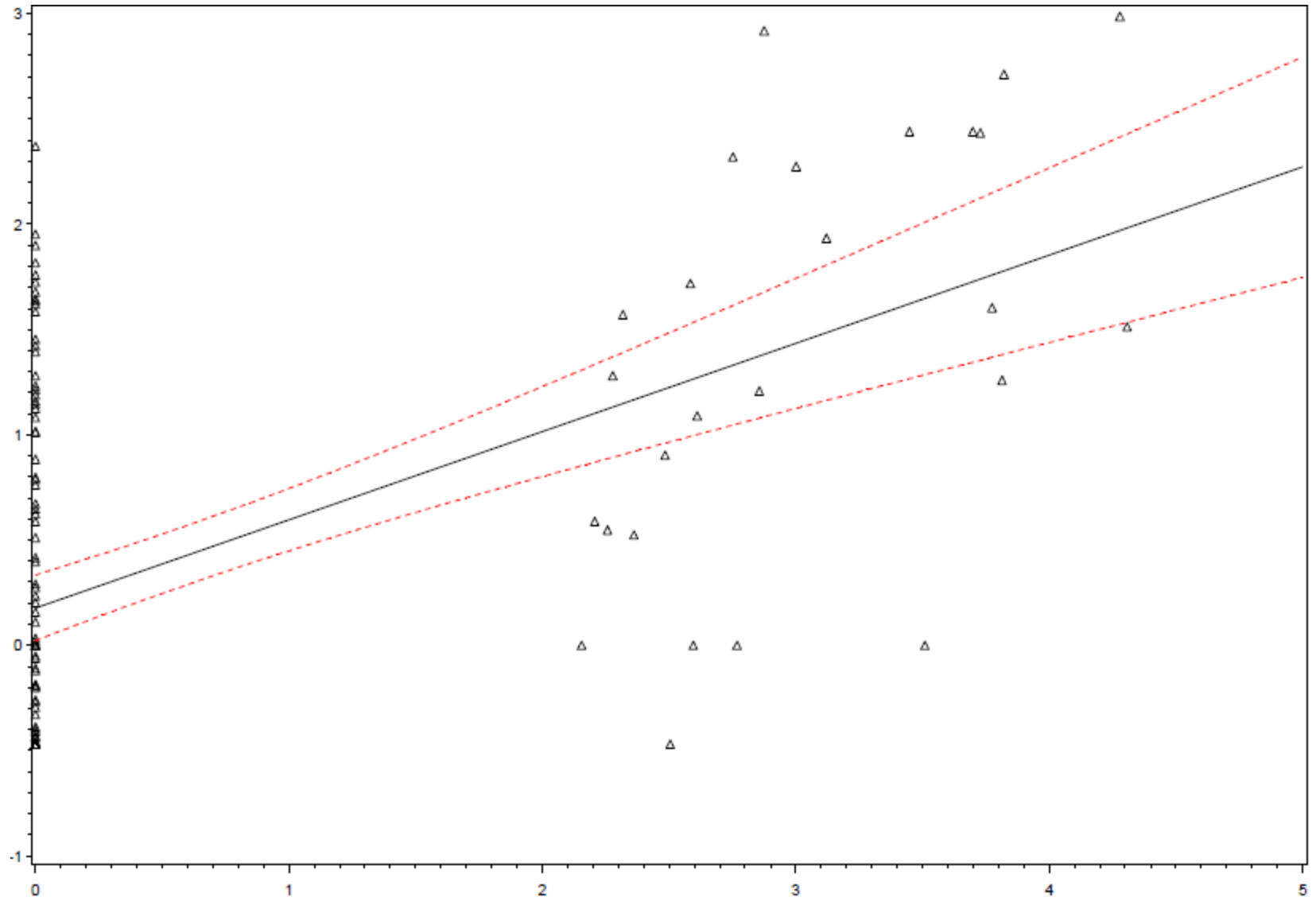
Fig S4. Temperature controlled Laminar Airflow Device - the device draws in ambient air, filters and cools it by 0.5-0.8°C, then distributes it to the breathing zone of a recumbent patient.

Fig S5. Relationship between mattress dust allergen levels and specific IgE levels to the same allergens in study patients. The data show the relationship between \log_{10} allergen levels as ng/g of mattress dust in samples taken at 3 months (x axis) and \log_{10} specific IgE levels in serum samples taken at baseline for dust mite allergens Der p 1 (A), Der f 1 (B) and cat allergen Fel d 1 (C). Among sensitized participants, allergen-specific IgE levels were positively correlated with mattress dust allergen levels for Der p 1 ($r = 0.57$, $P < 0.001$), Der f 1 ($r = 0.37$, $P = 0.001$) and Fel d 1 ($r = 0.36$, $P = 0.004$).

Fig S6. Mean \pm SEM change in AQLQ during treatment in TLA (blue) and Placebo (red) groups. Proportion (%) of participants in TLA and Placebo groups who completed the study and had a significant treatment response at different timepoints during the study is also shown. Significant treatment response was defined as an improvement in AQLQ ≥ 0.5 points from the time of randomisation.



Log₁₀ Der. pteronyssinus-specific IgE concentration (KU/L)



Log₁₀ Der p 1 concentration in mattress dust

