Altitude healing effect in severe asthmatic children

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Altitude Healing Effect in Severe Asthmatic Children

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Conflict of interest
Authors declare no conflict of interest

Contribution of each author:
P. Quignon and P da Mata organized data collection, F. Faraj, S. Guibert and J. Léonardi performed medical evaluations, A. Loundoun performed the statistical analysis, J. Vitte performed immunologic analysis, D. Charpin set up the study, wrote and supervised the protocol and wrote the paper.
All authors have approved the final article

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Abstract

Background: The beneficial effect of a climatic treatment in children with asthma was established quite some time ago, but the mechanism of this beneficial effect has not been fully elucidated. We investigated the role of the cytokines of the TH2 pathway, reactive oxygen species (ROS) and reactive nitrogen species (RNS) over the course of a high-altitude climatic therapy.
Methods: A group of 67 children originating from various French towns suffering from uncontrolled severe asthma was sent via their medical specialists, to the Briançon climatic area. They were monitored over the course of an entire school year. During this time, they returned home for 15 days during the Christmas holidays. At each stage, assessment of asthma control, lung function examination (peak flow meter and spirometry), and measurement of exhaled NO, ROS and RNS in exhaled breath condensate (EBC), and the level of cytokines in the plasma of the TH2 pathway were carried out.
Results: The degree of asthma control improved at high altitude and worsened upon returning home. The average value of the peak expiratory flow also improved during the first 3 months but then worsened upon returning home, while the other spirometric parameters did not change. The level of expired NO and the scores for quality of life underwent a similar change. The level of RNS and ROS in the EBC did not change significantly. Besides, a marked and statistically significant decrease in the level of IL-13 and IL-10 was noted.
Conclusion: The beneficial effect of a climatic stay of children suffering from allergic asthma at altitude appears to be linked with less allergenic stimulation.

Keywords: Altitude, Asthma, Allergy, Avoidance, Children, Biological markers

1. Introduction

The benefit of an altitude climatic treatment on asthmatics was first observed as far back as1879 [1]. The first clinical studies indicating their efficacy took place at the end of the 1970(s) [2], but rigorous studies including the notion of asthma control were not published until between 1995 and 2014 [3-6]. All these publications primarily included asthmatic children allergic to house-dust mites. The Rijssenbeek-Nouwens et al. paper in 2012 [5], showing the efficacy of climatic treatment with non-allergic asthmatics as well, drew attention to the unequivocal mode of action [7,8].

Two different mechanisms have been proposed to explain the beneficial effect of altitude climatic treatment [7], i.e. an allergic mechanism, which is linked to a lower concentration of mite allergens and an irritative mechanism which is linked with a lower concentration of atmospheric pollutants or other non-specific asthma triggers in altitude. The role of type 2 immune responses in the pathophysiology of asthma was recognized in the early 1990s. Schematically, Th_2 cytokines (IL_4, $IL_{5,}$ IL-10 and IL_{13}) oppose Th_1 cytokines, particularly IL_{12} and interferon (IFN) gamma.

To clarify the respective roles of these two modes of action, we undertook a prospective clinical study comprised of iterative EBC measurements of 8-isoprostane, which is a key marker of oxidative stress by the reactive oxygen species (or ROS) and reactive nitrogen species (or RNS). We also determined the level of a number of cytokines specific for the TH_2 pathway, in conjunction with an assessment of conventional clinical parameters.

2. Materials and Methods

Since the 1970´s, the French National Health System has agreed to subsidize stays at altitude during the school year for severe asthmatic patients selected by their asthma specialist. Their care is provided by pediatric asthma centers including a comprehensive asthma management and French school programs.

2.1 Criteria for the selection of patients

The study included children who were:

- Undergoing altitude treatment at three pediatric centers receiving only asthmatic patients in Briançon, France, at an altitude of 1350 m above sea level. The study took place over two consecutive school years. Children staying at these facilities for more than 1 year could only be included in the study for one of the years. The children coming to these treatment facilities for the first time were considered to be "naïve"
- Aged between 6 and 17 years, of either gender.
- Asthmatics, for whom the diagnosis had been made for at least two years based on clinical criteria or on the reversibility of the bronchial obstruction of at least 12% and an increase in the FEV1 by 200 ml after inhalation of 4 puffs of salbutamol.
- Afflicted with "severe" bronchial asthma[9] for more than two years.
- Atopic or non-atopic, according to the Phadiatop® test (Thermo Fisher Scientific, Uppsala, Sweden).
- Staying at one of the 3 pediatric facilities in the Briançon area for a duration of at least 3 months.
- For whom the family provided informed consent to participate in the study.

2.2 Criteria for non-inclusion

Children exhibiting at least one of the following characteristics were not considered to be eligible for inclusion:

- Stay at high-altitude was less than 3 months,
- Asthma did not represent the dominant respiratory pathology,
- Asthma was not be severe.

2.3 Data collected

Clinical data collected from the medical monitoring and discharge file regarding: asthma control, treatment being undertaken, paraclinical examinations at the various stages of the stay and at discharge, two questionnaires on quality of life of the child and the adolescent [10], and questionnaire regarding asthma control [11-12]. The asthma control was classified into one of three categories, according to GINA's experts. The quality of life questionnaire covered three main aspects: symptoms (10 items), activity limitations (5 items), and emotional function (8 items), with higher scores indicating a better quality of life.

Respiratory functional evaluation: comprising the flow-volume curve.

The measurement of FeNO in the exhaled breath was performed using a chemiluminescence device.

Blood analysis: complete blood counts, Phadiatop® (Thermo Fisher Scientific, Uppsala, Sweden) test which qualitative results as either "positive" or "negative", and 0.35 kU_A/L as the threshold for positivity.

Serum cytokines (IL_{1Ra} IL_5, IL-10, IL_{13} and CCL_{18}) levels were determined by a colorimetric sandwich ELISA method (Quantikine, R&D Systems). The detection range was from 6 to 20 pg/ml and the value expressed as pg/ml

Collection of exhaled breath condensates (EBC): a tube chilled by a freezer (R-Tube, Respiratory Research Inc., USA) was used for measurement of 8-isoprostane, a prominent ROS and RNS. The EBC was collected according to a standardized method [13] and was separated 20 min later into aliquots and stored at -80°C according to standardized procedures. The 8-isoprostane levels were measured in the EBC using a specific enzyme immunoassay kit (8-isoprostane EIA kit, Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's protocol. The RNS consisted in measurements of nitrite-nitrate levels as previously described[14]. The measurements were performed in duplicate and those with a coefficient of variation greater than 15% were excluded from the analysis. The threshold of detection for RNS (nitrates and nitrites) was 0.380 µM/L and the threshold for quantification was 1.378 µM

2.4 Execution of the study

Prior to starting the study, the Ethical committee provided approval (n°1245) and the Information Technology and Freedoms Commission were informed (file A00699-42). The parents provided a signed consent for their child's participation.

The children were admitted to the facility in September, and they then left for the Christmas holidays which lasted 2 weeks, returning to the facility after the holidays, and having their climatic stay ending in June (Figure 1). Upon leaving for the Christmas holidays, the children were advised to continue taking their medication, and this was verified when returned to the asthma facilities.

An outline of the modalities for medical monitoring during the study in shown in Figure 1. Upon admission to the facility and at the end of the school year, a clinical assessment comprising a questionnaire regarding asthma control, a questionnaire on quality of life, a lung function assessment, a blood sample, and the collection of EBC breath samples were carried out. Before leaving for the Christmas holidays and upon

returning after these holidays, a limited assessment was carried out, without a blood sample. Most of these examinations were carried out the Center for bioclimatic research in common to the three facilities. Adherence to the prescribed medications was supervised daily by nurses throughout the stay.

2.5 The number of subjects required

This was calculated following the hypothesis according to which at the inclusion 40% of the children had uncontrolled or incompletely controlled asthma and that this percentage reached 15% at the end of the climatic stay. In these conditions, with an alpha and a beta equal to 5%, 65 subjects would need to be included. Considering the patients missed to follow-up, we aimed to include 70.

2.6 Statistical analysis

Possible changes in the test of asthma control, parameters of the flow-volume curve, and biological parameters at each of the stages of the climatic stay were analyzed by a Wilcoxon signed-rank test applied to the paired measurements. Unpaired Wilcoxon and t-tests were used for the intergroup comparisons. The Generalized Estimating Equations (GEE) [14] was used to analyze repeated measurements. The GEE method extends standard regression analysis and accounts for the correlation between repeated measurements with binary or continuous outcome [15]). Because health outcomes at each step of the stay could be influenced by meteorological parameters (maximum, minimum and mean temperature, wind speed and relative humidity), air pollution data (PM_{10}, $PM_{2.5}$, NO_2, O_3) and pollen counts, those variables were included in the multivariate analysis

A value of $p<0.05$ was considered to be statistically significant. PASW Statistics version 17.0 (IBM SPSS Inc., Chicago, IL, USA) software was used for the statistical analyses.

The "before and after" and intergroup ("allergic vs. non-allergic") comparisons were applied to clinical scores, test of the asthma control, the scores for the quality of life, spirometric parameters, level of FeNO and biological parameters measured in peripheral blood and condensate of exhaled air. The overall results were studied for the group of 67 including children and then separately from those who were in their first year of climatic treatment (a "naive" group of 22 children) and those for whom the asthma was uncontrolled at the start of the stay (an "uncontrolled" group of 37 children). The group of non-allergic asthmatic children was too small (n=12, or 19 %) to be considered.

3. Results

The characteristics of the studied group are shown in Table 1.

Of the 64 patients, 51 were allergic, 22 were naïve and 37 had uncontrolled asthma. Among the "naïve" subgroup, 11 out of 22 had uncontrolled asthma. Among the 64 children, 45 (70%) were allergic to house-dust mites. Twelve children (18%) reported a food allergy. Regarding the severity of asthma, 53 of the 64 subjects had at least one exacerbation in the preceding year. This exacerbation led to an unexpected need for care in 21 of 53 subjects, with 7 attending the hospital emergency ward, and one patient who had to be admitted to the ICU. Thirty children (47%) acknowledged a fair compliance to long-term treatment for asthma.

3.1 Change in asthma control and consumption of medications

For the group considered as a whole, the percentage of children whose asthma was well-controlled was 36% upon admission, 66% before leaving for the Christmas

holidays, 15% upon returning from these holidays, and 88% at the end of the school year (Figure 2). These differences were all statistically significant.
In the "uncontrolled "subgroup, the change in the score of asthma control was identical. In the "naive" subgroup, improvement of the score was only significant over the first 3 months.
All of the patients took an inhaled corticosteroid therapy associated with a long-acting bronchodilator. The average inhaled dose of corticosteroids was 800 (± 494) µg at admission and 675 µg (± 338) when they left ($p < 0.001$).

3.2 Change in the Respiratory Function (Table 2)

Peak expiratory flow measurements

For the entire group, the peak expiratory flow significantly increased during the first 3 months of the stay, decreasing during Christmas holidays and increasing again, albeit in a non-significant manner, upon their return to altitude (Figure 3).

Change in the spirometric parameters

There were, however, no significant changes in the FEV_1 nor in the MMFR25/75**.**

3.3 Change in the fractional expired nitric oxide (FeNO) (Table 2)

The level of FeNO decreased significantly over the course of the first 3 months and then increased, in a non-significant manner, when the child left with their parents for Christmas holidays, followed by another decrease, although not significantly so, starting shortly after their return to the health facility that lasted until the end of the school year. The same changes were seen in the "naive" and in the "uncontrolled" subgroups.

3.4 Change in the scores for quality of life (Table 3)

The change in the score for quality of life in the children aged 5 to 11 years showed a trend for improvement, although not statistically significant in the first 3 months and, overall, between the start and the end of the stay. According to Juniper questionnaire, the change amounted to improvement during the first 3 months, a worsening upon returning from Christmas holidays, and an improvement at the end of the stay, although the observed differences were not statistically significant.

3.5 Change in the biological parameters

The **Phadiatop® test** was positive for 51 of the 63 children (81%) for whom it was performed.

Blood counts

No significant difference in red and white series were observed. However, among the 43 patients who had an evaluation of the percentage of eosinophils, 51% had more than 200 cells/mm3. In this latter group, for 82% their eosinophilia decreased (fewer than 200 cells) at T4 and for 18% these numbers increased (none of these differences were statistically significant).

Cytokine levels (Figure 4)

Comparison of the results of the samples taken on admission and upon leaving the treatment showed a significant decrease in the level of IL_{13} and IL_{10} an increasing trend in IL_{1RA} and an absence of variation for the level of IL_5 and of CCL_{18}.

Exhaled breath condensate (EBC)

The levels of RNS and 8-isoprostane were evaluated in T1, T2, T3 and T4. As far as isoprostane and RNS measurements, a trend in keeping with the clinical results was seen, although the differences were not statistically significant (Table 4).

Table 5 provides the results of the multivariate analysis which takes into account potential confounding factors

4. Discussion

This observational study of children afflicted with severe asthma revealed an improvement in asthma control, and a decrease in the average consumption of inhaled corticosteroids. The improvement was seen as of the 3rd month of the stay. Going home to a low elevation in an urban environment for Christmas holidays led to rapid deterioration of asthma control and of peak exhaled volume, without alteration of the level of 8-isoprostane and of nitrates/nitrites in the exhaled air, but with a statistically significant increase in the level of FeNO. This observation explains why medical compliance and indoor home environment - allergens, molds, and passive smoking – which can contribute to loss of asthma control- should be carefully checked.

These results were confirmed at the biological level by a very significant decrease in the levels of IL_{13}. The role of IL_{13} is not redundant with that of IL_4, which has a much smaller set of cellular targets. IL_{13} is, therefore, involved in the early stages of Th2, in the effector stages of asthmatic exacerbations, and also the late, chronic stages of bronchial remodeling responsible for the irreversible deterioration of respiratory function. IL-10 is a cytokine which regulates and suppresses the expression of proinflammatory cytokines during the recovery phases of infections and consequently reduces the damage caused by inflammatory cytokines. The decrease in the level of $IL_{10,}$ could be due to a pendulum effect: by means of a feedback effect, the decrease in allergic inflammation induced by IL_{13} leading to less potent induction of this regulatory and very potent anti-inflammatory cytokine. Thus, the biological results are in keeping with a reduction of allergenic stimulation. The concentrations of IL_1-RA, which acts as a major naturally anti-inflammatory protein and CCL_{18}, which effects mainly on the adaptative immune system did not change significantly. A similar conclusion was conveyed by a study performed in the Swiss alps where 26 asthmatic patients were followed up during a 3-week stay [16]: the number of eosinophils and the type-2 immune response significantly decreased after altitude treatment, while IL_5 and IL_{13} also significantly decreased. The study by Rijssenbeek-Nouwens et al [6] did showed that non-allergic asthmatic patients did quite as well in altitude than allergic asthmatic patients In this group of adult asthmatic patients, the improvement was likely related to air quality (decrease in airway irritants, low air pressure, low air density, low relative humidity) as well as a comprehensive management of the disease, including a better compliance to medications.

The level of 8-isoprostane in EBC did not changed over the course of the stay. In terms of RNS, the concentrations of nitrites/nitrates revealed a trend of an increase when the children went home for the Christmas holidays. This is at variance with the results of a pediatric study performed in Davos [17] where RNS levels in EBC decreased significantly at the end of a 4-week stay in altitude. Reasons for this discrepancy have to be elucidated. The level of FeNO decreased significantly during this period.

The main strength of this study lies in the accurate and homogenous monitoring of this group of children, by specialized doctors and paramedical professionals specialized in the treatment of asthma. Besides, potential confounding factors, namely meteorological variables, air pollution data and pollen counts, were taken into account in the statistical analysis. Another strength of the study lies in the monitoring of 8-isoprostane and NRS over the course of the stay. The variations in 8-isoprostane, a hallmark of ROS, and of the level of RNS over the course of the climatic stay were not significant. FeNO is recognized as an indicator of bronchial inflammation and of allergic sensitization. In our study, the FeNO varied significantly depending on the different stages of the stay.

The difficulties relate to several factors: recruitment of a substantial number of patients fulfilling the inclusion criteria, delay between arrival and the implementation of the investigations, arrival of the children at the centers in September at a time when the weather is still favorable regarding asthma control, associated pollen rhinitis leading to a loss of asthma control adversely interfering with the results of the climatic treatment. Moreover, we were not provided with any information about the indoor environment of the children´s homes and the compliance with medication while at home.

5. Conclusion

The biological results are in keeping with a reduction of allergenic stimulation to account for the beneficial effect of altitude climatic therapy in asthmatic children. Additional studies including a larger group of asthmatic children are needed to confirm our results.

However, besides a reduction of allergenic stimulations, there are several parameters which could also influence clinical and biological outcomes [7]. Observational studies are limited in their ability to dismantle their own contribution**.**

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HIGHLIGHTS

-Beneficial effects of climatic treatment in severe asthmatic children

-Few changes in oxidative and nitrogen stresses over the course of the stay

-Significant decrease in cytokines from the Th2 pathway

-Underlining the crucial role of low allergenic stimulations

Figures legends

-Figure 1: Schematic course of the study and examinations performed at each stage

-Figure 2: Change in asthma control over the course of the stay. +: $p<0.05$, ++: $p<0.01$

-Figure 3: Changes in peak-flow measurements over the course of the study. +: $p<0.05$, ++: $p<0.01$

-Figure 4: Changes in the serum cytokine levels over the course of the study. +$p<0.05$, +++: $p<.001$

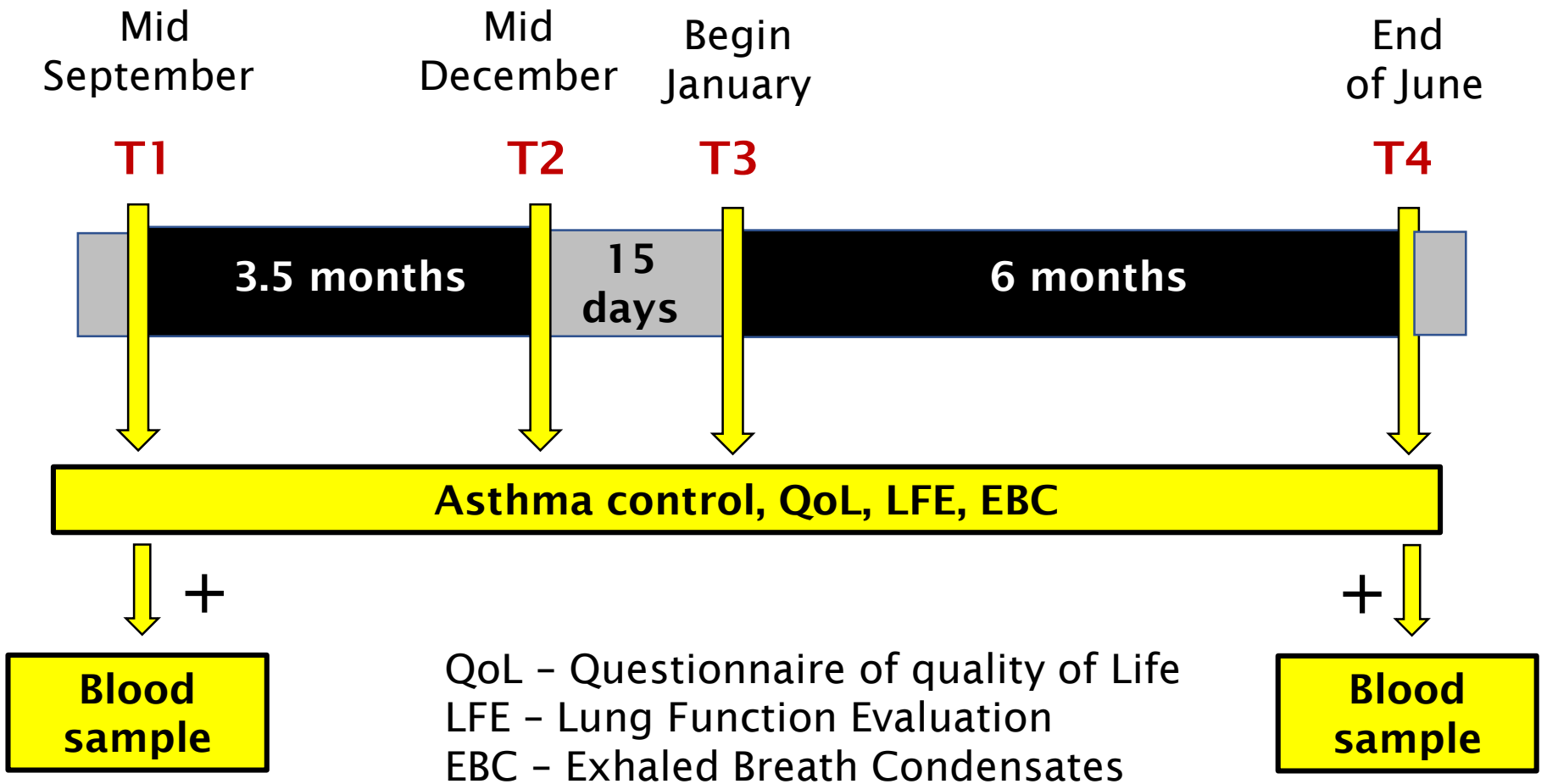

Mid September
Mid December
Begin January
End of June
T1
T2
T3
T4
3.5 months
15 days
6 months
Asthma control, QoL, LFE, EBC
+
+
Blood sample
Blood sample
QoL – Questionnaire of quality of Life
LFE – Lung Function Evaluation
EBC – Exhaled Breath Condensates

Figure 1

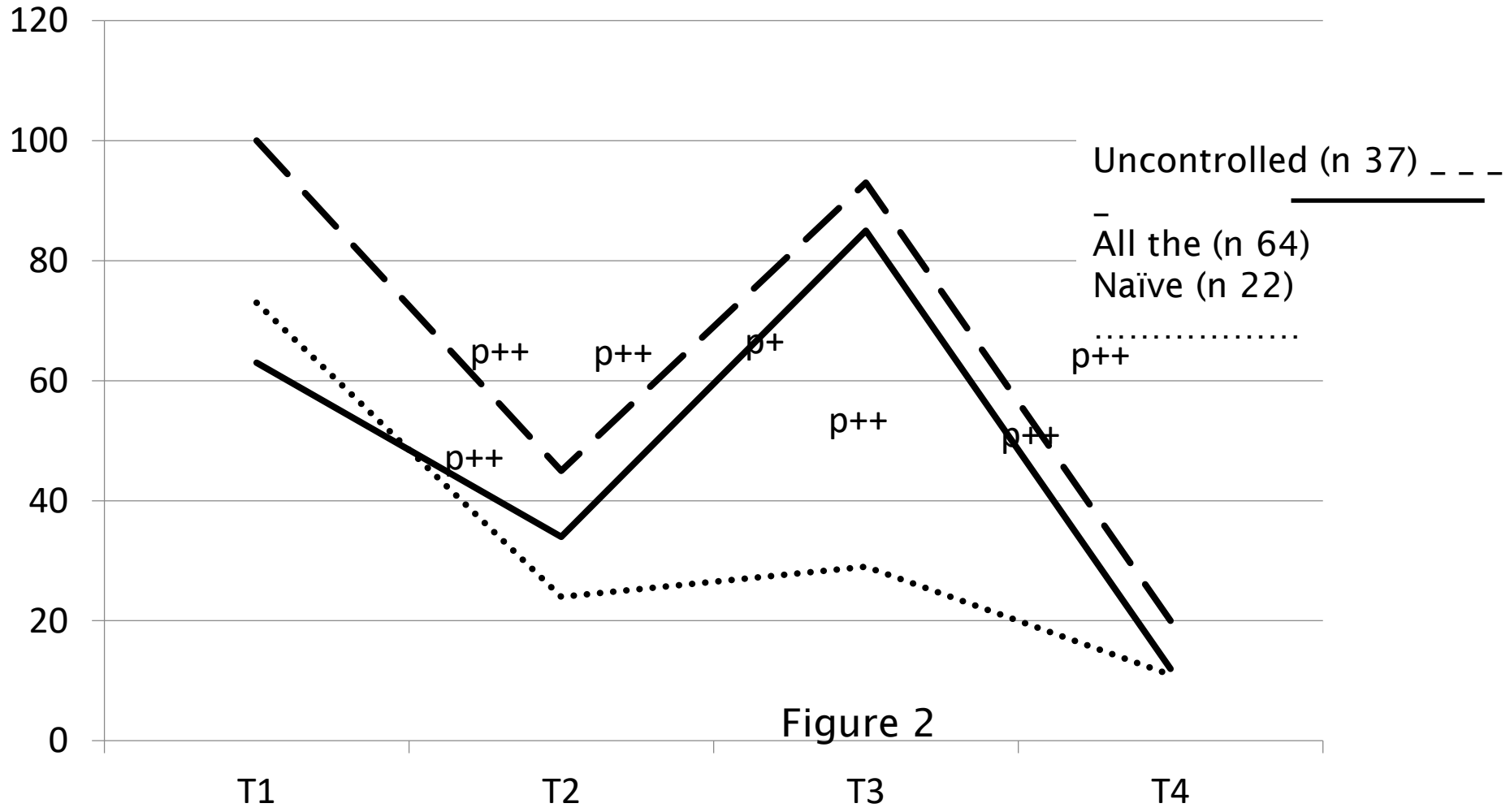

120
100
80
60
40
20
0
T1
T2
T3
T4
p++
p++
p+
p++
p++
p++
Uncontrolled (n 37) _ _ _ _
All the (n 64)
Naïve (n 22)

Figure 2

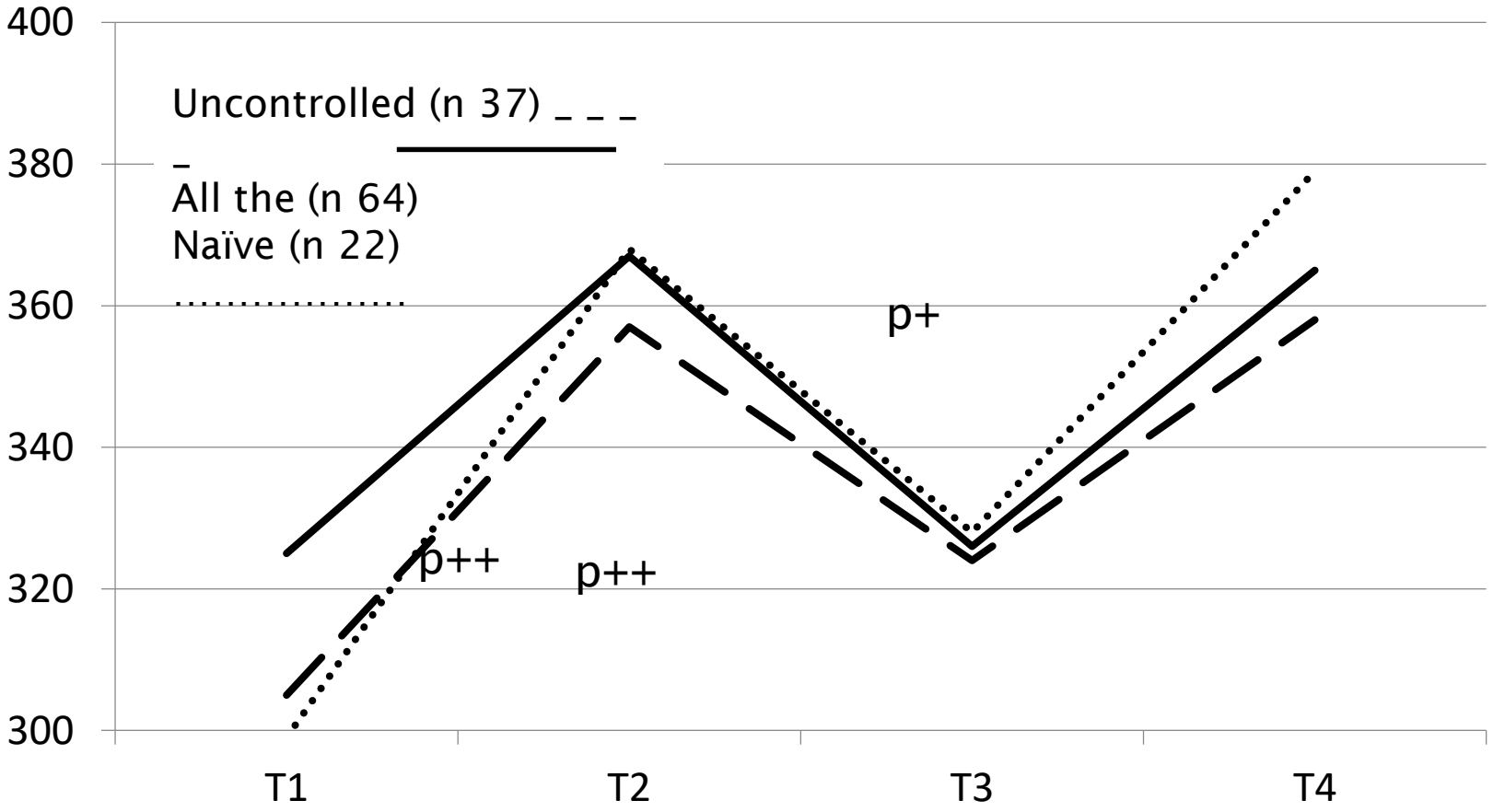
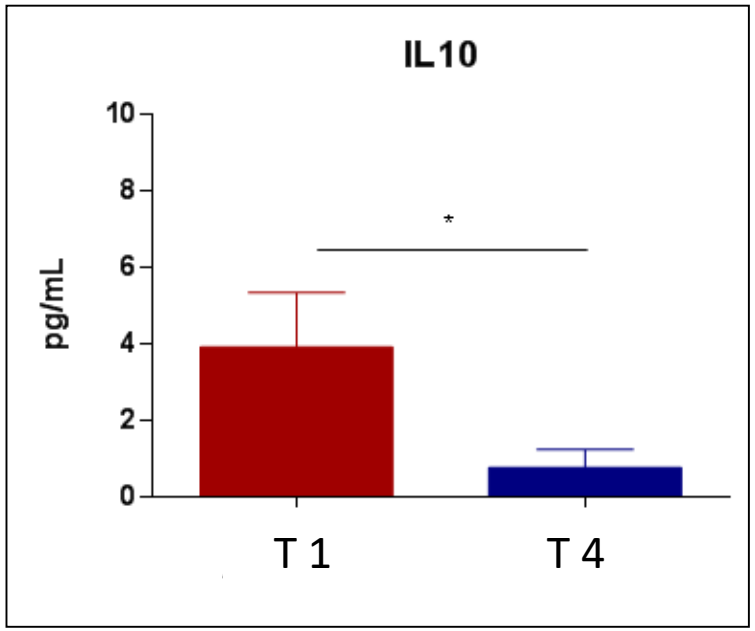
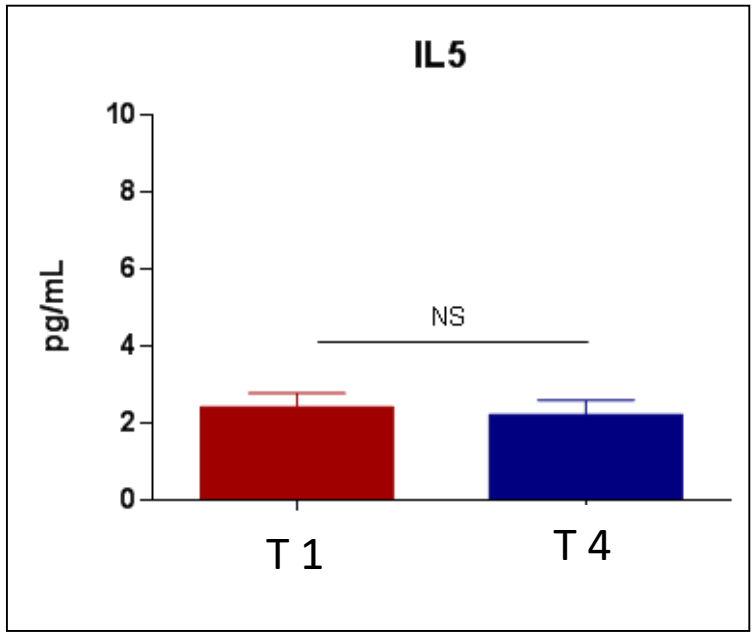
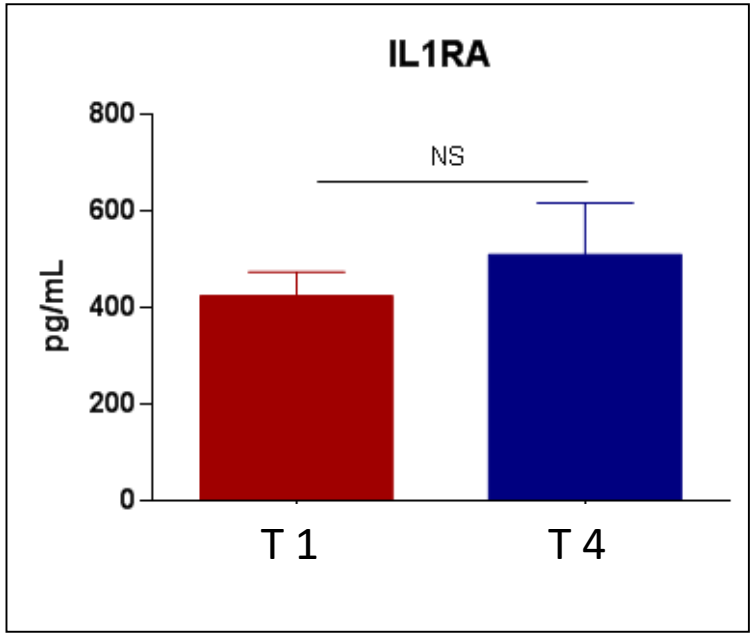

400
380
360
340
320
300
Uncontrolled (n 37) _ _ _ _
All the (n 64)
Naïve (n 22)
.....
p+
p++
p++
T1
T2
T3
T4

Figure 3


IL10
pg/mL
10
8
6
4
2
0
*
T 1
T 4


IL5
pg/mL
10
8
6
4
2
0
NS
T 1
T 4


IL1RA
pg/mL
800
600
400
200
0
NS
T 1
T 4

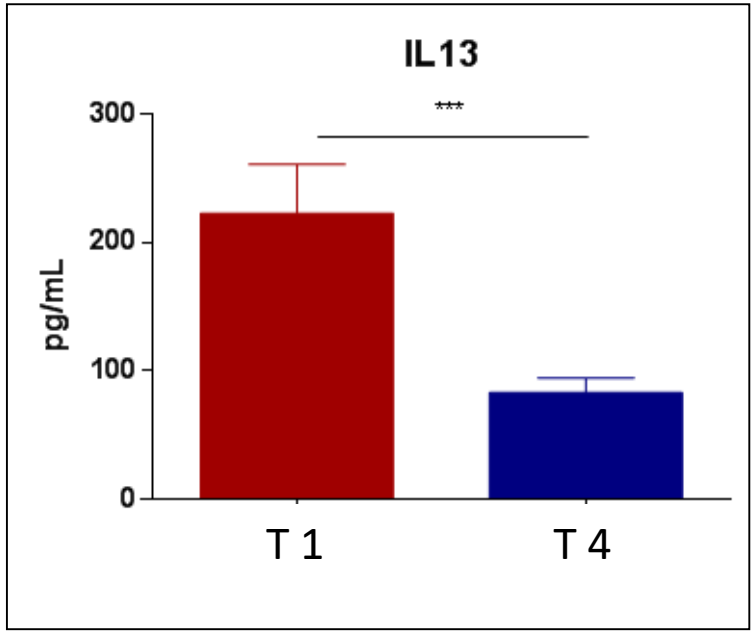

IL13
pg/mL
300
200
100
0
***
T 1
T 4

Figure: 4

At inclusion	Mean	S.D.	Min.	Max.
Age (yrs)	13.4	2.6	7	17
Height (cm)	157	30	133	185
BMI	22.4	5.9	13.7	39
FEV1 (l)	2.61	0.71	1.36	5.13
Eosinophils (G/l)	0.43	0.47	0.12	1.51

Table 1.Characteristics of the study group

	T1		T2		T3		T4
PEFR (l /min)	322±97	***	362±101	*	330±94	NS	362±98
FEV1 (l/s)	2.60±0.7	NS	2.64±0.8	NS	2.7±0.8	NS	2.64±0.7
MMFR 25/75	87±41	NS	75±35	NS	80±35	NS	80±36
FeNO (ppb)	30	*	18	NS	24	NS	20

Table 2: Trends in spirometric and FeNO measurements over the course of stay in altitude
*: $p<0.05$, ***: $p<0.001$

	T1		T2		T3		T4
QoL questionnaire	113±26	*	121±25	NS	121±24	NS	128±23
				*			
Juniper questionnaire	9.2±6.0	NS	8.7±7.0	NS	9.3±7.0	NS	7.0±0.6

Table 3. Results of quality of life and Juniper questionnaires over the course of the stay *: $p<0.05$

	T1		T2		T3		T4
EBC NOx	5.4±4.1	P=0.07	4.6±3.6	P=0.06	5.4±4.1	NS	5.2±3.6
EBC Isopr	1.1±2.1	NS	0.9±2.6	NS	0.5±0.9	NS	0.9±1.8
Serum NOx	17.0±10.4	p=0.36					15.9±12.9

Table 4: Trend in EBC NOx and Isoprostane concentrations and Serum NOx concentration. (Serum isoprostane could not be evaluated). NS: Non significant difference

Table 5. Generalized Estimating Equations coefficients, and 95% confidence intervals (CI) in univariate and multivariate analysis of outcomes

	Univariate analysis				Multivariate analysis			
Parameters	β	Std. Error	95% CI	P	β	Std. Error	95% CI	P
Asthma control								
Visit 1 vs Visite 4	2.57	0.42	1.75 to 3.39	<0.001	-6.32	5.39	-16.90 to 4.26	0.241
Visit 2 vs Visite 4	1.34	0.39	0.57 to 2.11	0.001	-6.61	4.85	-16.12 to 2.89	0.173
Visit 3 vs Visite 4	3.74	0.68	2.40 to 5.08	<0.001	3.93	0.86	2.25 to 5.61	<0.001
Quality of life								
Visit 1 vs Visit 4	-15.24	2.96	-21.05 to -9.44	<0.001	-17.52	11.37	-39.80 to 4.76	0.123
Visit 2 vs Visit 4	-6.55	2.92	-12.26 to -0.83	0.025	-8.53	9.73	-27.61 to 10.55	0.381
Visit 3 vs Visit 4	-7.19	2.69	-12.45 to -1.92	0.007	-7.31	2.76	-12.73 to -1.89	0.008
Juniper scoring								
Visit 1 vs Visit 4	1.94	0.83	0.32 to 3.57	0.019	0.87	3.11	-5.24 to 6.97	0.781
Visit 2 vs Visit 4	1.42	0.87	-0.29 to 3.14	0.103	0.49	2.50	-4.41 to 5.38	0.845
Visit 3 vs Visit 4	2.50	0.89	0.76 to 4.24	0.005	2.44	0.91	0.66 to 0.42	0.007
Peak expiratory flow rate l/min								
Visit 1 vs Visit 4	-39.10	9.38	-57.48 to -20.72	<0.001	-66.41	37.75	-140.40 to 7.58	0.079
Visit 2 vs Visit 4	1.95	12.17	-21.90 to 25.81	0.873	-21.78	34.73	-89.86 to 46.28	0.531
Visit 3 vs Visit 4	-32.12	10.85	-53.39 to -10.85	0.003	-33.52	11.42	-55.90 to -11.13	0.003
FeNO (ppb)								
Visit 1 vs Visit 4	10.72	4.55	1.78 to 19.67	0.019	15.84	19.22	-21.83 to 53.50	0.410
Visit 2 vs Visit 4	-1.91	3.04	-7.88 to 4.05	0.529	2.49	15.93	-28.74 to 33.71	0.876
Visit 3 vs Visit 4	5.62	6.06	-6.27 to 17.50	0.354	5.97	6.54	-6.85 to 18.80	0.361
Isoprostane, pg/ml								
Visit 1 vs Visit 4	0.18	0.33	-0.48 to 0.83	0.598	2.21	1.85	-1.41 to 7.58	0.232
Visit 2 vs Visit 4	-0.03	0.40	-0.82 to 0.75	0.933	1.73	1.92	-2.04 to 5.49	0.368
Visit 3 vs Visit 4	-0.40	0.24	-0.87 to 0.08	0.103	-0.28	0.25	-0.77 to 0.22	0.277
NOX , µM/l								
Visit 1 vs Visit 4	0.22	0.68	-1.11 to 1.55	0.746	2.74	4.03	-5.16.40 to 10.64	0.497
Visit 2 vs Visit 4	-0.47	0.61	-1.67 to 0.72	0.439	1.72	3.68	-5.50 to 8.93	0.641
Visit 3 vs Visit 4	0.26	0.72	-1.15 to 1.67	0.717	0.41	0.64	-0.85 to 1.67	0.526
IL 10, pg/ml								
Visit 1 vs Visit 4	3.32	1.27	0.83 to 5.81	0.009	23.00	19.03	-14.30 to 60.31	0.227
IL 13, pg/ml								
Visit 1 vs Visit 4	142.79	44.36	55.84 to 229.74	0.001	-297.63	155.79	-602.96 to 7.707	0.056