

# Diffusion lung capacity of carbon monoxide: A novel marker of airways remodeling in asthmatic children?

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## ABSTRACT

Asthma is universally considered a chronic inflammatory disorder of the airways. Several noninvasive markers, such as exhaled nitric oxide (FeNO) and exhaled breath temperature (PletM), have been proposed to evaluate the degree of airway inflammation and remodeling in asthmatic children. The aim of this study was to evaluate the relationship between diffusion lung capacity of carbon monoxide (DLCO) and these inflammatory markers in asthmatic children. We compared data of FeNO, PletM, and DLCO collected in 35 asthmatic children at admission (T0) and discharge (T1) after a period spent in a dust-mite-free environment (Misurina, Italian Dolomites, 1756 m). PletM showed a reduction from 29.48°C at T0 to 29.13°C at T1 ( $p = 0.17$ ); DLCO passed from 93 to 102 ( $p = 0.085$ ). FeNO mean value was 29.7 ppb at admission and 18.9 ppb at discharge ( $p = 0.014$ ). Eosinophil mean count in induced sputum was 4 at T0 and 2 at T1 ( $p = 0.004$ ). Spearman standardization coefficient beta was 0.414 between eosinophils and FeNO and  $-0.278$  between eosinophils and DLCO. Pearson's correlation index between DLCO and PletM was  $-0.456$  ( $p = 0.019$ ). A negative correlation between DLCO and PletM was found. However, DLCO did not show a significant correlation with FeNO and eosinophils in the airways. Additional studies are needed to clarify the role of DLCO as a potential tool in monitoring childhood asthma.

(Allergy Rhinol 3:e66–e73, 2012; doi: 10.2500/ar.2012.3.0033)

Asthma is a chronic inflammatory disease of the airways and many definitions of asthma have been proposed. The Global Strategy for Asthma Management and Prevention Report<sup>1</sup> defines asthma based on its pathology and functional consequences as “a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role.” In addition to the acute pattern of inflammation, asthma represents a chronic inflammatory remodeling process of the airways.<sup>2</sup>

A number of different markers have been proposed to evaluate the level of airway inflammation in asthmatic patients.<sup>3</sup> Several studies support the evidence that the levels of exhaled nitric oxide (FeNO) can reflect the degree of airway inflammation in asthmatic patients.<sup>4–6</sup> NO has been proposed as a noninvasive method to assess the degree of inflammation in asthmatic patients<sup>4,5</sup> and practical aspects of its applications have been proposed by the European Respiratory Society Task Force.<sup>7</sup> Values of FeNO of  $>35$  ppb are strongly suggestive for eosinophilic airways inflammation.<sup>8,9</sup>

It has also been shown that it is possible to evaluate the level of bronchial inflammation by the examination of sputum induced by inhalation of hypertonic sa-

line<sup>10,11</sup> to examine the number of cells, in particular eosinophils, in the airways. However, this method requires skilled personnel, some laboratory facilities to follow the protocol, and a degree of collaboration by the patient in performing the test.

More recently, exhaled breath temperature has been evaluated as a marker of inflammation of the airways<sup>12,13</sup> and moreover of the remodeling process,<sup>14,15</sup> considering that higher temperature is a feature of the inflammatory process that can reflect the higher number of vessels and consequently of blood flow in the airways of asthmatic children.

Another important aspect of the pathogenesis of asthma is the airways' remodeling due to the persistent inflammation. The remodeling process affects the airways' wall thus influencing the diffusion lung capacity.<sup>16</sup> This, in fact, is determined both by the thickness of the membrane ( $Dm$ ) and the volume of Hb in the lung capillaries ( $Vc$ ) ( $1/DLCO = (1/Dm) + (1/\theta Vc)$ ).<sup>16</sup>

The purpose of this study was to evaluate the possible relationship between diffusion lung capacity and noninvasive markers of inflammation, in particular, exhaled NO, eosinophils in the sputum, and airway temperature in a group of asthmatic children.

## METHODS

### Subjects

Thirty-five children, ranging in age from 9 to 17 years, with mild to moderate allergic asthma, were selected among all asthmatic children evaluated at the

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Published online December 12, 2012

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residential house "Istituto Pio XII" (Misurina, Belluno, Italy). Of these 35 patients only 18 performed all of the tests of the study. "Istituto Pio XII" is located at 1756 m in the Italian Dolomites. The climatic conditions in the area of Misurina are characterized by prolonged periods during which the ground is covered by snow, from October to May or June, very low humidity, and low temperature. These conditions are unfavorable to the house-dust mite. In fact, repeated dust collection from mattresses in the residential house detected a mean level of 0.04  $\mu\text{g/g}$  of dust.<sup>17</sup> Furthermore, the pollen season is very short during the summer.

The degree of asthma severity was defined according to guidelines.<sup>1</sup> Data about home therapy<sup>18</sup> and asthmatic exacerbations<sup>1</sup> were collected by a structured questionnaire at the moment of admission. An informed consent was provided by parents of all children.

### Study Design

This study was performed with the data collected during the month they spent in the residential house, and each child underwent a prick test for inhalant and foods, measurement of FeNO, sputum induction, spirometry, measurement of the diffusion lung capacity for carbon monoxide (DLCO), and exhaled air temperature (Plet). Every test was repeated both at admission (T0) and discharge (T1) of the patient from the residential house.

### Prick Test

All patients were skin-prick tested with commercial extracts (Starallergens, Milan, Italy) of pollens present in the geographical area of the study: grasses, plantain, *Alternaria*, *Parietaria*, birch, olive, cypress, hazelnut, *Artemisia*, *Cladosporium*, *Aspergillus*, *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, and cat and dog dander. Depending on the clinical history of the patient, they were tested also for food allergens. All of the skin-prick tests were performed using disposable 1-mm-tip lancets. The positive control was histamine at 1 mg/dL. The negative control was diluent of extracts. Results were evaluated after 15 minutes. Wheals at least 3 millimeter greater in diameter than the wheal at the site of the negative control were considered positive.

### Pulmonary Function

Measurements of pulmonary function were done according to the American Thoracic Society guidelines<sup>19</sup> considering the best of three efforts starting with full force vital capacity maneuvers performed by a Vitalograph Compact spirometer (MasterScreen Body; Jaeger, Wuerzburg, Germany). The maneuvers were performed with the child standing up in front of the

spirometers and wearing a nose clip. Patients were instructed to avoid the use of bronchodilators at least 6 hours before the test.

### Diffusion Lung Capacity for Carbon Monoxide

Diffusion lung capacity was performed according to the American Thoracic Society guidelines<sup>20</sup> ( $V_{\text{max}}$  Encore 22 days System for Measuring Diffusion; Jaeger-Vyasis Healthcare, Inc., Höchberg, Germany) making the subject rapidly inhale a mixture of gas containing CO starting from residual volume and reaching total lung capacity. After a short period of apnea ( $10 \pm 2$  seconds) the subject was asked to exhale air and a sample of it was collected and analyzed for the CO concentration. The sample must have a minimum volume of 0.75–1.0 L, for current volumes  $<2$  L it was considered sufficient also a 0.50-L sample. Since there was a variability between measurements due to technical reasons, mean values were reported. At least two repeated measurements with a smaller difference than 3 mL of  $\text{CO} \times \text{min}^{-1} \times \text{mmHg}^{-1}$  or  $>10\%$  of the maximum value reached were considered. Patients were instructed to perform the test and were allowed to sit down during the entire maneuver, wearing a nose clip. Patients were prevented from using bronchodilators at least 6 hours before the test.<sup>21</sup>

### NO Measurement

Exhaled NO was measured by chemiluminescence analyzer (Logan LR 2149; Logan, Rochester, Kent, U.K.) before spirometry was performed.<sup>6,22</sup> The subjects were asked to perform a single slow exhalation through a mouthpiece, against a resistance and with a biofeedback used to maintain a 5- to 6 L  $\cdot \text{min}^{-1}$  steady flow. This method allows the soft palate to separate the nasopharynx from the oropharynx, hence, preventing the contamination of exhaled NO with nasal NO. This method has been shown to be successfully applicable both in adults and in children. The NO value was measured at the plateau of the end-exhaled reading and expressed in parts per billion according to the guidelines.<sup>8</sup> Values of NO considered in the data analysis were always measured in the last part of exhalation (plateau exhaled NO), taking the plateau of the end-exhaled  $\text{CO}_2$  reading as a representative of an alveolar sample.<sup>5,6,17,23,24</sup>

### Sputum Evaluation

Sputum was induced by inhalation of hypertonic saline solution by a standardized method. Hypertonic saline solution was nebulized using an ultrasonic nebulizer (UN202; Technomedica, Verona, Italy) for four periods of 5 minutes each, for a total time of 20 minutes. The starting concentration was 4% NaCl delivered for a total of 10 minutes, followed by a 5% NaCl

solution for a further 10 minutes. Before inhalation, patients were premedicated with inhaled salbutamol (2 puffs, 200  $\mu$ g), to inhibit possible bronchoconstriction. The forced expiratory volume in 1 second was recorded before and 10 minutes after salbutamol and then every 5 minutes during saline inhalation. If forced expiratory volume in 1 second fell >20% of the post-bronchodilator value or troublesome symptoms occurred, nebulization was discontinued.

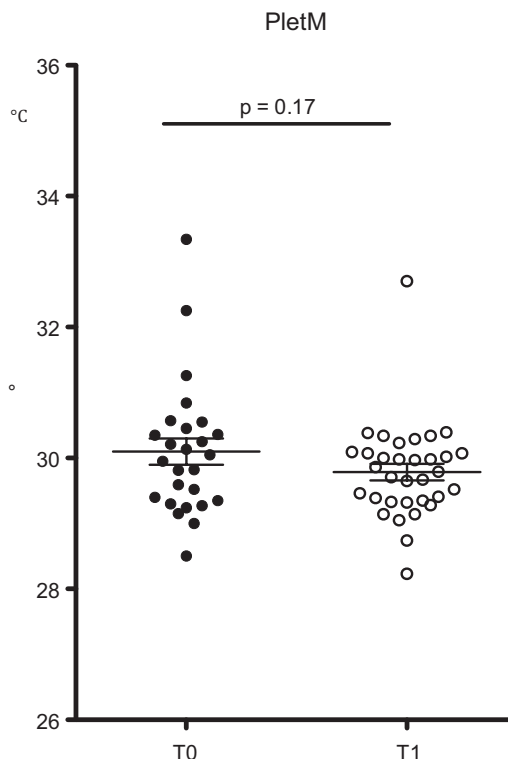
After each period of nebulization, subjects were instructed to rinse the mouth and then to cough sputum into a Petri dish, which was placed in the laboratory against a dark background, making possible the identification of the sputum portion in the watery saliva. Plugs arising from the lower respiratory tract were selected by visual inspection, suspended in dithiothreitol for 30 minutes at 37°C, and then centrifuged at 400  $\times$  g 10 minutes at room temperature. Supernatant was collected and frozen at -70°C for later eosinophil cationic protein analysis. The cell pellets were washed twice in 1 mL of 0.9% saline and the samples were transferred onto slides by cytocentrifugation (500 rpm for 5 minutes). Slides were fixed in alcohol 95% and then stained with May-Grünwald-Giemsa. The counts of eosinophils and epithelial cells were expressed as mean percentage values of total cells counted, excluding epithelial cells to correct for salivary contamination of the samples.

### Exhaled Breath Temperature

Exhaled breath temperature was measured after at least 1 hour of rest in a sitting position. The temperature was measured during a maximal slow exhalation with a mouth pressure of >5 cmH<sub>2</sub>O and flow at 5–6 L/min, using the Medical Mass Flow Sensor of the V<sub>max</sub> Spectra 229 Pulmonary Function Laboratory (VIASYS Healthcare, Yorba Linda, CA). Dynamic airway temperature and airflow were sensed from the mass flow sensor. Real-time signal display consisted of inspiratory and expiratory airway temperature, respiratory flow, and mouth (airway) pressure on a time axis. At the end of the test the software automatically calculated the end-expiratory maneuver plateau temperature.<sup>25</sup>

### Statistical Analysis

Statistical analysis has been performed using Graph Pad 5 for Mac (GraphPad Software, Inc., La Jolla, CA). Population characteristics and base variables are expressed as mean (SD), median (range), and number (percent). After evaluating sample distribution, Student's *t*-test was used to evaluate the difference between variables before and after admission. The correlation between levels of carbon monoxide diffusion (DLCO) and plateau of exhaled breath temperature



**Figure 1.** Difference of plateau of exhaled air temperature (PletM) values between the two measurements (T0 and T1 from the residential dust-mite-free house). Mean SD values are plotted in the graph ( $p = 0.17$ ).

(PletM), both of them measured at admission and discharge from the dust-mite-free environment, were tested by Spearman's Rank correlation coefficient according to data distribution. Differences have been valued as significant when  $p < 0.05$ .

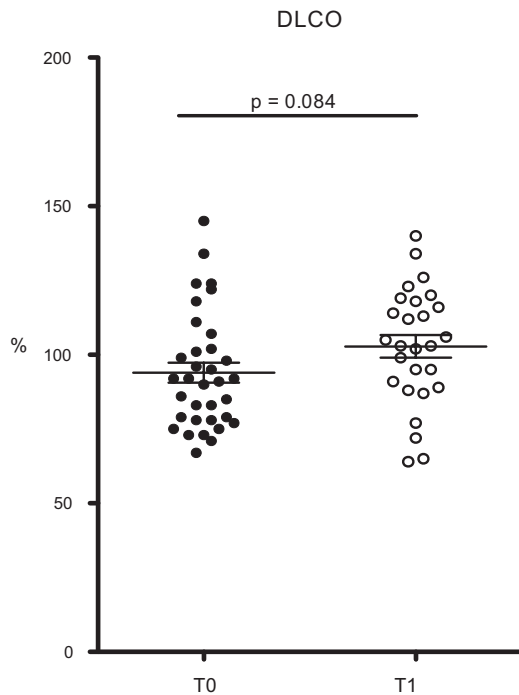
### RESULTS

In this study 35 asthmatic children were recruited: 32 were of white origin, 1 was African, and 2 were South American. Of the 35 patients, 32 (91.4%) had a parent or a sibling suffering from food (milk, fruit, nuts, and vegetables) allergy. Only six patients (17.1%) were allergic to some food. All of them were allergic to inhalant allergens such as house-dust mites, pollens, animals (cat or dog), and fungi.

All of them also presented some comorbidities: 33 of 35 (94%) also presented allergic rhinitis, 5 (14%) had atopic dermatitis, and 4 children (11.4%) also presented obesity, gastroesophageal reflux confirmed by a pH-metry test, emphysema, and glucose-6-phosphate deficit.

Patients suffering from cardiovascular, neurological, muscular, or other chronic pathologies or congenital defects were excluded from the study.

At admission children were receiving regular treatment with inhaled steroids, either fluticasone at 100–



**Figure 2.** Values of diffusion of lung capacity of carbon monoxide (DLCO) at the admission and discharge (T0 and T1). Mean and SD values are reported ( $p = 0.084$ ).

250  $\mu\text{g}/\text{day}$  or budesonide at 200–640  $\mu\text{g}/\text{day}$ ; 7 patients were treated also with long acting  $\beta_2$ -agonists and 10 with montelukast. Only 6 patients needed oral steroids at home to control of asthma. During the period of the study the clinical conditions allowed the gradually reduce or complete termination of inhaled corticosteroids in all patients. Only 2 patients received oral steroids during admission in the residential house.

Comparing data of tests performed at the moment of admission and those at discharge, parameters of inflammation as FeNO and eosinophils in induced sputum showed a significant reduction. FeNO mean value on admission was 29.7 ppb decreasing to 18.9 ppb on discharge ( $p = 0.014$ ). Eosinophils mean count in induced sputum was 4 at T0 and 2 at T1 ( $p = 0.004$ ).

In particular, we evaluate the difference of PletM and DLCO values in children on admission (T0) and discharge (T1) from the residential house.

Values of PletM showed a reduction from 29.48 to 29.13 $^{\circ}\text{C}$ , this difference was not statistically significant, with a value of  $p = 0.17$  (Fig. 1).

Values of DLCO passed from 93% on admission to 102% on discharge with a value of  $p = 0.085$ , a difference that was not statistically significant (Fig. 2). Removing the outlier, *viz.*, extreme values of DLCO that resulted in equal or smaller to SD, mean value of DLCO on admission was 87.79% and on discharge was 105.9% with a value of  $p < 0.0001$ .

A positive association resulted from data analysis, expressed with standardization coefficient  $\beta$ , between

**Table 1 Correlation between eosinophils, FeNO and DLCO**

	Eosinophils
	Standardization Coefficient $\beta$
FeNO	0.414
DLCO	-0.278

Values of standardization coefficient  $\beta$ .

FeNO = fractional exhaled nitric oxide; DLCO = diffusion lung capacity of carbon monoxide.

**Table 2 Correlation between DLCO and PletM**

	$\Delta\text{DLCO}$ Pearson's Correlation index
$\Delta\text{PletM}$ ( $p = 0.019$ )	-0.456

Values of Pearson's correlation index.

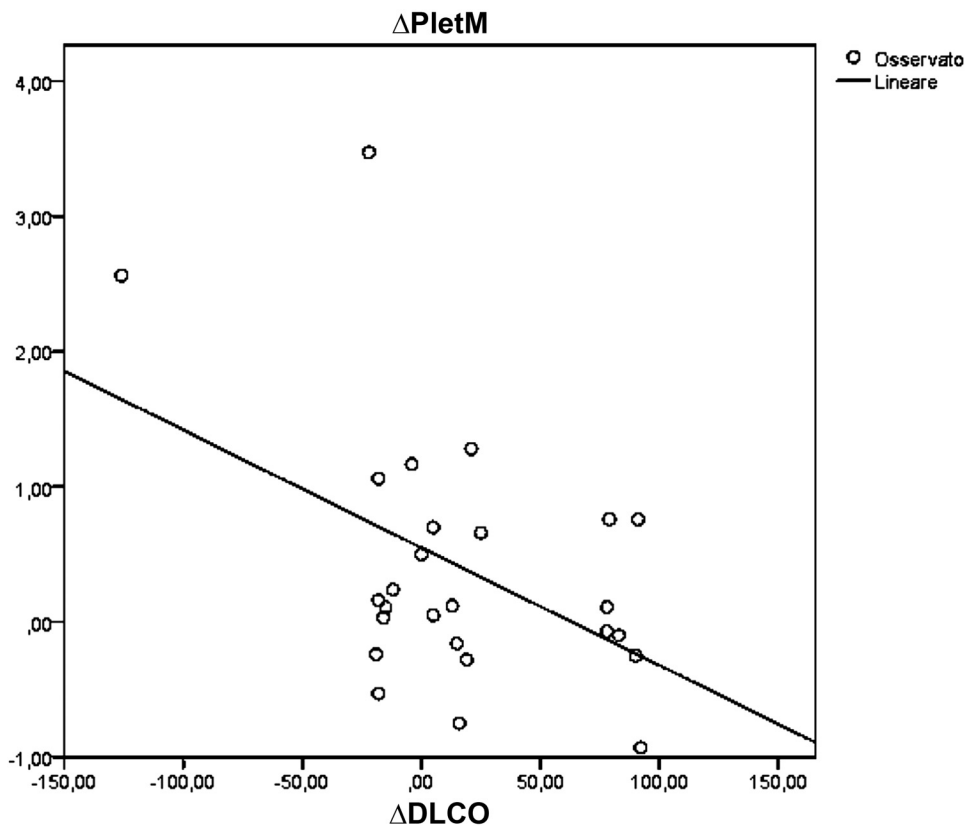
$\chi\Delta\text{DLCO}$  = difference values of diffusion lung capacity of carbon monoxide measured at T0 and T1;  $\Delta\text{PletM}$  = difference values of exhaled breath temperature measured at T0 and T1.

values of eosinophils and FeNO (0.414) and a negative association between eosinophils and DLCO (-0.278). At the time of admission to the residential house, inflammation parameters were higher compared with those on discharge, whereas DLCO followed an opposite trend with lower value on admission and higher on discharge (Table 1).

From data analysis there was no correlation between DLCO and FeNO and between other inflammation parameters, whereas a negative correlation expressed by Pearson's index was observed, between DLCO and temperature considering for each the difference between the value on admission and on discharge ( $\Delta\text{DLCO}$  and  $\Delta\text{PletM}$ ) as shown in Table 2. This correlation has a negative trend: while DLCO increases, PletM decreases, with  $p = 0.019$  (Fig. 3).

Both the number of exacerbations and the drugs used to achieve asthma control showed a significant reduction during the period in which the children stayed in the dust-mite-free environment of Misurina.

In fact, the mean number of exacerbations in the month before admission was 0.25, which fell to 0.092 during the period spent in the residential house ( $p < 0.0001$ ) as shown in Fig. 4. This clinical improvement has been confirmed by the reduction of drugs prescribed to control the pathology, in particular, reduction or complete suspension of steroids during the period children spent in the antigen-free environment starting from a mean dosage of 277.7  $\mu\text{g}/\text{die}$  of budesonide at home to 69.3  $\mu\text{g}/\text{die}$  during the period spent



*Figure 3. The negative correlation between the difference of DLCO values between admission and discharge from the residential house ( $\Delta$ DLCO) and the difference of PletM values between admission and discharge ( $\Delta$ PletM)*

in the clinical house. This difference was statistically significant ( $p < 0.0001$ ; Fig. 5).

## DISCUSSION

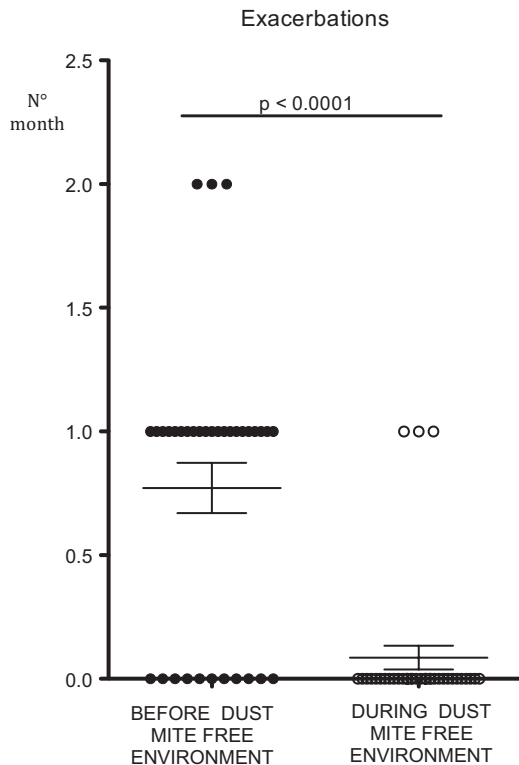
To the best of our knowledge, this is the first study on DLCO conducted in a population of asthmatic children. We found a negative correlation between diffusion lung capacity and exhaled breath temperature, but no significant correlation emerged with markers of airways inflammation (FeNO and eosinophils). Previous studies hypothesized a role of exhaled breath temperature in monitoring airway inflammation, thus reflecting an increased vascularity of airway wall.<sup>12</sup>

Exhaled temperature has also been found to correlate with metalloproteinase 9, which is considered a relevant marker of airway remodeling in asthma,<sup>26</sup> thus tempting to speculate a role of PletM in monitoring airway remodeling in asthmatic patients. Remodeling is known to represent a mechanism of tissue repair in chronic inflammation.<sup>1</sup> Nevertheless, although inhaled corticosteroids are more promptly and effectively controlling eosinophilic inflammation,<sup>1</sup> they have been reported to be less performing in gaining control of remodeling process.<sup>27,28</sup> These consideration are in keeping with the hypothesis that remodeling does not simply represent a direct consequence of inflammation and that several additional mechanisms may be involved, including changes in vascularization.<sup>28</sup>

Because of the correlation we have found between DLCO and PletM, we are tempting to speculate a possible role of diffusion lung capacity as a noninvasive marker of airway remodeling.

The diffusion process is highly complicated and it includes (1) bulk flow delivery of CO to the airways and alveolar spaces; (2) mixing and diffusion of CO in the alveolar ducts, air sacs, and alveoli; (3) transfer of CO across the gaseous to liquid interface of the alveolar membrane; (4) mixing and diffusion of CO in the lung parenchyma and alveolar capillary plasma; (5) diffusion across the red cell membrane and within the interior of the red blood cell; and (6) chemical reaction with constituents of blood Hb.<sup>29–35</sup> This process can be simplified in two transfer conductance properties: membrane conductivity and the binding of CO and Hb. The first reflects the diffusion properties of the alveolar capillary membrane; the latter is influenced by the chemical reaction rate and by the volume of Hb in alveolar capillary blood.

A number of physiological changes can affect the DLCO values. As the lung inflates, the DLCO value tends to increase as well as during exercise, supine position, and during Muller maneuver (inspiratory efforts against a closed glottis), while Valsalva maneuver (expiratory efforts against a closed glottis) can reduce DLCO. The measurements of CO uptake are also affected by the distribution of ventilation with respect to

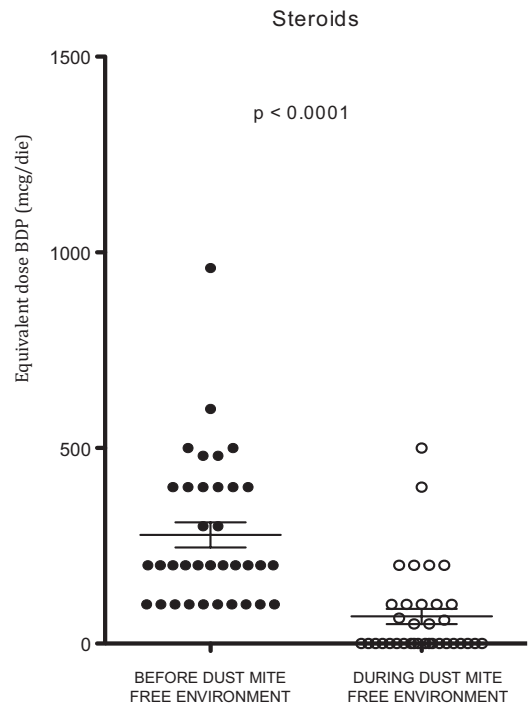


**Figure 4.** Asthma exacerbation before the admission in the dust-mite-free environment and at the moment of the discharge ( $p < 0.0001$ ).

membrane thickness or vascularization.<sup>16</sup> Also, pathological changes affect DLCO values, which is particularly important in diseases such as emphysema,<sup>16</sup> where the inhaled CO may only go to the better ventilated regions of the lung and the subsequently measured CO uptake will be determined primarily by uptake properties of those regions. DLCO values also change in those who suffer from asthma.<sup>36</sup>

In our study DLCO has not shown a significant correlation with the trend of inflammation parameters, such as FeNO and eosinophils. A possible explanation may be that modification of lung diffusion from one side, and inflammation on the other side, can reflect the degree of impairment and damage of two different sites of airways: peripheral airways for DLCO and central airways for inflammation parameters. Diffusion (DLCO), in fact, reflects changes of those parts of airway that contribute to gas exchange, a process that starts from the eighth generation of bronchial airways, considered peripheral airways. On the other side NO measurements and eosinophils count are noninvasive tests used for the monitoring of inflammation in central airways.<sup>37</sup>

Our results give basis for further studies in children aimed to clarify the possible correlation between NO measured in the central airways ( $c_{alv}$ ) and gas diffusion. Alveolar NO measured with multiple flow method



**Figure 5.** Significant diminution for inhaled steroids comparing the period before and during the time spent in the residential house (dust-mite free;  $p < 0.0001$ ).

shows a correlation with parameters used to investigate peripheral airways, especially in patients with severe asthma.<sup>38</sup>

In our study FeNO and eosinophils showed a significant decrease during the period spent in the residential house, thus confirming their usefulness as markers of airways inflammation,<sup>39–42</sup> in relationship with the particular climate of the clinical house.<sup>43,44</sup> The correlation existing between inflammatory parameters such as eosinophils in induced sputum and FeNO have been widely studied in literature.<sup>45</sup>

Inflammatory cells, such as eosinophils, have an inducible oxide nitric synthetase activated by proinflammatory cytokines such as TNF- $\alpha$  and IL-1 $\beta$ . NO, in turn, suppresses Th1 and IFN $\gamma$  production, thus eliciting Th2 lymphocyte proliferation. These cells are thus responsible for the production of cytokines like IL-5, able to attract and to increase the number of eosinophils in the airways, thus explaining the relationship between these two markers. This concept has been further underlined by other studies.<sup>46</sup>

The temperature of exhaled air (PletM) has also been studied as a marker of airway inflammation. In fact, typical inflammation of asthma leads to an increase of vascularization and cell metabolism with a resulting increase of temperature that is proportional to disease severity. Other studies correlate exhaled air temperature (PletM) with NO levels (FeNO),<sup>13</sup> and with quantity of eosinophils in induced sputum,<sup>47</sup> thus suggesting a possible role in monitoring inflammation.

## CONCLUSIONS

In this study it was not possible to include a control group, because no healthy child was spending a period of time in the residential house Istituto Pio XII and the number of subjects is low because of the necessity to enroll only those who were able to perform the tests. Despite that these factors may in part limit the interpretation of the results, it is possible to speculate that measurement of DLCO can mirror the process of airways wall and vessels remodeling. Nevertheless, additional studies are needed to better define the relationship between DLCO, airways inflammation, and remodeling in childhood asthma even with the help of animal models.

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