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Silvia Carraro, Giorgio Piacentini, Marta Lusiani, Zeynep Seda Uyan, Marco
Filippone, Maurizio Schiavon, Attilio Loris Boner, Eugenio Baraldi

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EXHALED AIR TEMPERATURE IN CHILDREN WITH BRONCHOPULMONARY DYSPLASIA

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Keywords:	bronchopulmonary dysplasia, asthma, exhaled air temperature, biomarkers



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5 **EXHALED AIR TEMPERATURE IN CHILDREN WITH BRONCHOPULMONARY**
6 **DYSPLASIA**
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10 Silvia Carraro^{1,2},MD, Giorgio Piacentini^{1,3},MD, Marta Lusiani²,MD, Zeynep S Uyan²,MD,
11 Marco Filippone²,MD, Maurizio Schiavon⁴,MD, Attilio L Boner³,MD, Eugenio Baraldi²,MD.
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17
18 ¹These authors contributed equally to the article

19 ²Department of Pediatrics, University of Padova, Padova, Italy

20
21 ³Department of Pediatrics, University of Verona, Verona, Italy.

22
23 ⁴Center for Sports Medicine and Physical Activity, Department of Social Health, ULSS 16,
24 Padova, Italy
25
26
27

28
29 **Corresponding author**

30
31 Eugenio Baraldi, MD
32 Department of Pediatrics
33 Respiratory Medicine and Allergy Unit
34 University of Padova
35 Via Giustiniani 3
36 35128 Padova, Italy
37 Phone: +39 049 8213560
38 Fax: +39 049 8213502
39 **E-mail: baraldi@pediatria.unipd.it**
40
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52
53 **Institution where the study was performed:** Department of Pediatrics, University of
54 Padova
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58
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ABSTRACT

Background. Because they have similar functional and clinical profiles, bronchopulmonary dysplasia (BPD) survivors are often treated as asthmatic patients. In truth, very little is known about the possible biochemical and inflammatory mechanisms playing a part in BPD survivors' lungs. The aim of this study was to measure exhaled breath temperature in BPD survivors by comparison with asthmatic cases and healthy controls.

Methods. Three groups of age-matched adolescents (n=17 each), i.e. BPD survivors (gestational ages < 31 weeks, birth weights < 1500 g), asthmatic subjects and healthy controls, underwent exhaled breath temperature and exhaled nitric oxide measurements, and spirometry.

Results. Exhaled breath temperature was significantly lower in the BPD survivors (26.72°C [25.11-27.57]) than in the asthmatic patients (29.60°C [29.20-30.02], p<0.001), while no significant difference emerged by comparison with healthy controls (26.97°C [26.58-27.38]). Considering the whole study population, a significant correlation was found between exhaled breath temperatures and exhaled nitric oxide concentrations (R=0.42 p=0.004). Spirometry revealed an obstructive lung function pattern in both the asthmatic cases and the BPD survivors, with lower parameters in the latter.

Conclusions. Exhaled breath temperatures and exhaled nitric oxide concentrations are significantly lower in BPD survivors than in asthmatic cases, suggesting that different pathogenetic mechanisms characterize these two chronic obstructive lung diseases.

Keywords: bronchopulmonary dysplasia; asthma; exhaled air temperature; biomarkers

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INTRODUCTION

Bronchopulmonary dysplasia (BPD) is the most common form of chronic lung disease of prematurity. In childhood and adolescence, BPD survivors may have respiratory symptoms and lung function impairment that closely resemble those of asthmatic patients,¹ even if BPD patients present fewer acute exacerbations, poorer response to inhaled corticosteroids and less pronounced airway reversibility. A recent study reported that low-birth-weight survivors have significantly higher odds of hospitalization for respiratory diseases (wheezing, respiratory infections and respiratory failure) as young adults than their normal-birth-weight counterparts.²

Although there are no data to suggest an asthma-like inflammatory profile in the airways of BPD survivors, they are often empirically treated as if they were asthmatic (e.g. with glucocorticosteroids).^{3,4} Very little is known, however, about the possible biochemical and inflammatory mechanisms involved in the pathogenesis of the symptoms and airway obstruction in BPD survivors,³ and no data are available on the pathological features of these patients' lungs beyond infancy.³

It is common knowledge that asthma is a chronic inflammatory disease of the airways and several non-invasive biomarkers have been proposed for indirectly assessing different patterns of airway inflammation.⁵ Exhaled nitric oxide has been studied the most and it is considered a reliable marker of eosinophilic inflammation in the airways.^{6,7} Since "calor" is one of the cardinal signs of inflammation, some authors recently recommended considering exhaled air temperature as a potential marker of inflammation.⁸⁻⁹ Increased values of exhaled breath temperature have been reported in children and adults with asthma,⁸⁻¹¹ correlating significantly with the concentrations of exhaled nitric oxide.⁸⁻¹⁰ To our knowledge, no studies have evaluated exhaled air temperature in individuals with BPD.

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3 The aim of the present cross-sectional study was to evaluate exhaled breath temperature
4 (PLET, end-expiratory maneuver plateau temperature) in BPD survivors and to compare it
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6 with the temperature measured in age-matched asthmatic subjects and healthy controls.
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10 11 12 13 14 15 **MATERIALS AND METHODS**

16 17 **Study subjects**

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19 BPD survivors: 17 BPD survivors were considered (table), drawn from a population with
20 BPD born at gestational ages below 31 weeks and with birth weights under 1500 g
21
22 admitted to the Neonatal Intensive Care Unit between 1990 and 1994 and followed up
23
24 routinely thereafter at the Pediatrics Department in Padova. BPD was defined as oxygen
25
26 dependence persisting 28 days after birth in infants on mechanical ventilation (18 days on
27
28 average). At birth, the mean gestational age of the sample was 28.3 weeks (SD 1.9) and
29
30 the mean weight was 1011 g (SD 222).
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35
36 All the BPD survivors were clinically stable at the time of recruitment, 5 of them were on
37
38 low- to medium-dose inhaled steroid (ICS) treatment and two were being treated with
39
40 theophylline. Two of the BPD survivors were sensitive to common airborne allergens (dust
41
42 mites, dog and cat dander, grass and tree pollens).
43
44

45
46 Asthmatic subjects: 17 allergic asthmatic adolescents (table) who had been born at term
47
48 were enrolled from among those regularly attending our outpatients clinic. Their asthma
49
50 had been diagnosed by a pediatric respiratory physician based on their clinical history
51
52 (cough, shortness of breath, recurrent wheezing, chest tightness) and an increase in FEV₁
53
54 after salbutamol (400 µg) >12%, according to international guidelines.¹²
55
56

57
58 All the asthmatic patients were clinically stable at the time of recruitment, 6 of them were
59
60 on low- to medium-dose inhaled steroid (ICS) treatment (100-200 mcg/day budesonide
equivalent) and one of them was being treated with long-acting beta2 agonists too.

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3 Healthy controls: 17 age-matched healthy adolescents (table) with no history of pre-term
4
5 birth, atopy or respiratory diseases were recruited as a control group.
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7
8 For all three groups, subjects who had suffered any airway infections in the previous 4
9
10 weeks were excluded from the study.
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12 The Ethics Committee of our hospital reviewed and approved the protocol and all parents
13
14 gave their informed consent.
15

16
17 At recruitment, all the adolescents underwent physical examination, exhaled air
18
19 temperature measurement, FE_{NO} measurement and spirometry. All the measurements
20
21 were performed in the afternoon. FE_{NO} measurement was not possible in 3 subjects (1
22
23 with asthma and 2 with BPD)
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26

27 **Exhaled breath temperature (PLET)**

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29 Exhaled breath temperature (end-expiratory maneuver plateau temperature, PLET) was
30
31 measured as described elsewhere.¹³ The environmental conditions were controlled in
32
33 order to maintain a stable temperature of 23±1 °C and a relative humidity of 40-50%.
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35
36 The measurements were repeated three times. The inspiratory air stream temperature was
37
38 recorded during a complete inhalation to total lung capacity (TLC). The exhaled airway
39
40 temperature was measured during a slow exhalation to residual volume (RV) using the
41
42 Medical Mass Flow Sensor of the Vmax Spectra 229 Pulmonary Function Laboratory
43
44 (VIASYS Healthcare, Yorba Linda, CA, USA). Dynamic airway temperature and airflow
45
46 were measured with the Mass Flow Sensor. The response time of the Mass Flow Sensor
47
48 to a steep change in airway temperature was approximately 64 milliseconds, with a
49
50 thermal resolution of 0.01°C. Mouth pressure was measured at the airway opening using a
51
52 differential pressure transducer. Exhaled flow was directed through an alternate pathway
53
54 with a fixed resistor in place to control the expiratory air flow rate and maintain an airway
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56 pressure sufficient to keep the velum closed and prevent any contribution of warmed gas
57
58 from nasal sinuses. A mouth pressure of > 5 cm H₂O also helped the subject to stabilize
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3 the expiratory flow at 5-6 l/min. All the signals, including exhaled air temperature, mouth
4 pressure and exhalation flow rate, were transferred to a personal computer and analyzed
5 using software specifically developed for this purpose (Vmax Temperature, VIASYS
6 Healthcare, Yorba Linda, CA, USA). Real-time signal display consisted of inspiratory and
7 expiratory airway temperature, respiratory flow rate and mouth (airway) pressure on a time
8 axis. Flow and pressure limit indicators were presented on screen as a coaching aid.
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17 At the end of the test, the software automatically calculated the PLET, which was the
18 parameter used to compare the groups.
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20 21 22 23 **Fractional exhaled nitric oxide (FE_{NO}) and pulmonary function measurement**

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25
26 FE_{NO} was measured using the NIOX system (Aerocrine, Stockholm, Sweden), according
27 to the ERS/ATS guidelines for measuring FE_{NO} in children.¹⁴ Children inhaled NO-free air
28 to total lung capacity (TLC) and exhaled through a dynamic flow restrictor with a target
29 flow of 50 ml/sec for at least 6-7 seconds. No nose clip was used. The NIOX system was
30 calibrated using a 200 ppb NO tank (Lindegas Hoek Loos Speciality Gases, Amsterdam,
31 Netherlands) according to the manufacturer's instructions.
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40 Lung function was measured by means of a 10-liter bell spirometer (Biomedin, Padova,
41 Italy) and the best of three maneuvers was expressed as a percentage (%) of the
42 predicted reference values, according to the ERS/ATS guidelines.¹⁵
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46 47 **Statistical analysis**

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49
50 It has been calculated that a sample size of 17 subjects allows detecting a 1 °C difference
51 between groups of with a power of 80% at a 2-sided α -level of .05.
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55 Age and anthropometric data were expressed as the mean and standard deviation (SD)
56 (table).
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60 Since PLET, FE_{NO} and lung function data were not normally distributed (as demonstrated
by the Shapiro-Wilk test), they were expressed as medians and interquartile ranges (IQR),

1
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3 and analyzed by means of non-parametric tests. Differences between groups were
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5 analyzed using the Kruskal-Wallis test, followed (where significant) by the Mann-Whitney
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7 U-test for comparisons between groups. Correlations were evaluated using Spearman's
8
9 rank test. Statistical significance was set at a 2-sided alpha level of 0.05.
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12 13 14 15 **RESULTS**

16 17 **Exhaled breath temperature (PLET)**

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19 The Kruskal Wallis test revealed a significant difference in the PLET for the 3 groups
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21 ($p < 0.001$) (figure 1A). The between-group comparison found a significantly lower PLET in
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23 BPD survivors (26.72°C [25.11-27.57]) than in asthmatic patients (29.60°C [29.20-30.02],
24
25 $p < 0.001$), while no significant difference emerged between BPD survivors and healthy
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27 controls (26.97°C [26.58-27.38] $p = 0.23$). The asthmatic cases had a significantly higher
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29 PLET than the healthy controls ($p < 0.001$).
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33 34 **Exhaled nitric oxide (FE_{NO})**

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36 The Kruskal Wallis test found a significant difference in FE_{NO} for the 3 groups ($p = 0.003$)
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38 (figure 1B). The between-group comparison revealed a significantly higher FE_{NO} in
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40 asthmatic cases (29.9 ppb [15.0-49.2]) than in either healthy controls (11.7 ppb [8.5-16.8],
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42 $p = 0.008$) or BPD survivors (10.6 ppb [7.0-13.2], $p = 0.002$), with no significant difference
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44 between the latter two groups ($p = 0.28$).
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48 49 **Lung function**

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51 The Kruskal Wallis test disclosed significant differences in all the spirometric parameters.
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53 The between-group comparison showed that BPD children had lower FVC, FEV₁, FEF₂₅₋₇₅
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55 and FEV₁/FVC than either asthmatic cases or healthy controls; and the asthmatic cases
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57 had lower FEV₁, FEF₂₅₋₇₅ and FEV₁/FVC than healthy controls (table, figure 1C and 1D).
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Correlations

Considering the whole sample, a significant correlation emerged between PLET and FE_{NO} ($R=0.42$ $p=0.004$) (figure 2).

In BPD survivors there was a correlation approaching statistical significance between FVC and PLET ($p=0.058$ $R=0.47$), which was stronger considering the whole sample ($R=0.28$ $p=0.036$).

None of the other spirometric parameters correlated with PLET values in BPD survivors.

No significant correlations emerged between spirometric measurements and PLET in asthmatic cases or healthy controls.

DISCUSSION

This study shows that exhaled breath temperature is significantly lower in BPD survivors than in asthmatic patients.

BPD survivors have respiratory symptoms and lung function profiles that resemble those of asthmatic children¹⁶ and this explains why they are often considered and empirically treated as asthmatic patients during their childhood.³ Very little it is known about the processes underlying the obstructive lung function pattern in BPD survivors and there is no evidence to support any asthma-like inflammation in this condition.^{4,17,18}

Here we demonstrate that both PLET and FE_{NO} are lower in BPD survivors than in asthmatic children, suggesting that the two conditions have a different pathogenesis (figure 1A and 1B). In a previous study, we already showed that exhaled NO is lower in BPD survivors than in asthmatic cases;¹⁹ the present study further supports the lack of any asthma-like airway inflammation in BPD survivors. A possible limit in the interpretation of our results is the potential effect of ICS treatment and atopy on PLET values. In fact while all asthmatic patients were allergic, only two BPD patients were sensitized. In addition some patients in both groups were treated with ICS.

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3 In keeping with previous studies conducted on asthmatic patients,⁸⁻⁹ PLET and FE_{NO}
4 correlated significantly. It is worth noting that the scatter plot (figure 2) shows a clear
5 separation between asthmatic cases and BPD survivors, again suggesting that different
6 pathogenetic mechanisms underlie these two chronic obstructive lung diseases.
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12 As previously reported,⁸⁻¹⁰ asthmatic subjects have higher levels of both FE_{NO} and PLET,
13 which are thought to reflect eosinophilic airway inflammation.^{6,20} The higher exhaled breath
14 temperature in asthmatic subjects also seems to suggest an increased vascularity and
15 bronchial blood flow.¹⁰ In BPD survivors, on the other hand, PLET and FE_{NO} are
16 significantly lower than in asthmatic patients and even tend to be lower than in healthy
17 controls, although without reaching the statistical significance (figure 1A and 1B). This
18 trend could be explained by the anatomical alterations that characterize BPD lungs,
19 namely alveolar simplification, variable degree of interstitial fibrosis^{21,22} and dysmorphic
20 vascular growth.²³ These anatomical alterations might presumably be responsible for an
21 impaired heat exchange between the bronchial vessels and the airway lumen, giving rise
22 to normal-to-low values of exhaled air temperature in BPD survivors.
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38 As previously reported,²⁴ we confirm here that BPD survivors have a lung function pattern
39 with airflow limitation (figure 1C). An autoptic study demonstrated thickening of the airway
40 walls and an increase in smooth muscle, suggesting that these aspects may have a role in
41 airway obstruction and hyper-responsiveness in BPD survivors.²⁵ Moreover BPD survivors
42 have a significantly reduced forced vital capacity (figure 1D). This last features might be
43 explained by the impaired growth of the BPD survivor's lungs, which would lead to reduced
44 pulmonary volumes. Previous studies have already documented in BPD survivors an
45 impaired lung function.^{24,26,27} It may be that lung function impairment in BPD survivors is
46 not just the static outcome of the initial damage, but due to an ongoing
47 remodeling/damaging process. Although our findings go against any suggestion of an
48 asthma-like eosinophilic inflammatory profile, other inflammatory pathways (e.g.
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3 neutrophilic or lymphocytic) and/or remodeling mechanisms may be involved. Elucidating
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5 this pathophysiological substrate could have an impact on the long-term management of
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7 BPD subjects.
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10 In conclusion, we showed that exhaled breath temperature (PLET) and exhaled nitric
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12 oxide (which are considered biomarkers of airway inflammation) are not increased in BPD
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14 survivors. Further studies are needed to assess the role of other non-invasive biomarkers,
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16 as measured for instance in exhaled breath condensate, to shed light on the pathogenic
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18 processes affecting the BPD survivor's lung and thus pave the way to the development of
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20 new, specifically targeted drugs.
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35 software for exhaled air evaluation.
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FIGURE LEGENDS

Figure 1. Panel A shows PLET values in the 3 groups; panel B shows FE_{NO} values in the 3 groups; panel C shows FVC values in the 3 groups; panel D shows FEV1 values in the 3 groups. **p<0.001 and *p<0.01 vs healthy controls; #p<0.05 and §p<0.01 vs asthmatic subjects; γ p<0.001 vs BPD survivors

Figure 2. Correlation between FE_{NO} values and PLET values in BPD, asthmatic and healthy subjects (R=0.42 p=0.004).

For Peer Review

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5 **EXHALED AIR TEMPERATURE IN CHILDREN WITH BRONCHOPULMONARY**
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10 Silvia Carraro^{1,2},MD, Giorgio Piacentini^{1,3},MD, Marta Lusiani²,MD, Zeynep S Uyan²,MD,
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12
13
14
15
16

17
18 ¹These authors contributed equally to the article

19 ²Department of Pediatrics, University of Padova, Padova, Italy

20
21 ³Department of Pediatrics, University of Verona, Verona, Italy.

22
23 ⁴Center for Sports Medicine and Physical Activity, Department of Social Health, ULSS 16,
24 Padova, Italy
25
26
27

28
29 **Corresponding author**
30

31 Eugenio Baraldi, MD
32 Department of Pediatrics
33 Respiratory Medicine and Allergy Unit
34 University of Padova
35 Via Giustiniani 3
36 35128 Padova, Italy
37 Phone: +39 049 8213560
38 Fax: +39 049 8213502
39 **E-mail: baraldi@pediatria.unipd.it**
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52 **Institution where the study was performed:** Department of Pediatrics, University of
53 Padova
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55 **Financial support:** The work had no financial support

56 **Running title:** Exhaled air temperature in BPD
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ABSTRACT

Background. Because they have similar functional and clinical profiles, bronchopulmonary dysplasia (BPD) survivors are often treated as asthmatic patients. In truth, very little is known about the possible biochemical and inflammatory mechanisms playing a part in BPD survivors' lungs. The aim of this study was to measure exhaled breath temperature (~~an indirect marker of airway inflammation~~) in BPD survivors by comparison with asthmatic cases and healthy controls.

Methods. Three groups of age-matched adolescents (n=17 each), i.e. BPD survivors (gestational ages < 31 weeks, birth weights < 1500 g), asthmatic subjects and healthy controls, underwent exhaled breath temperature and exhaled nitric oxide measurements, and spirometry.

Results. Exhaled breath temperature was significantly lower in the BPD survivors (26.72°C [25.11-27.57]) than in the asthmatic patients (29.60°C [29.20-30.02], p<0.001), while no significant difference emerged by comparison with healthy controls (26.97°C [26.58-27.38]). Considering the whole study population, a significant correlation was found between exhaled breath temperatures and exhaled nitric oxide concentrations (R=0.42 p=0.004). Spirometry revealed an obstructive lung function pattern in both the asthmatic cases and the BPD survivors, with lower parameters in the latter.

Conclusions. Exhaled breath temperatures and exhaled nitric oxide concentrations are significantly lower in BPD survivors than in asthmatic cases, suggesting that different pathogenetic mechanisms characterize these two chronic obstructive lung diseases.

Keywords: bronchopulmonary dysplasia; asthma; exhaled air temperature; biomarkers

INTRODUCTION

Bronchopulmonary dysplasia (BPD) is the most common form of chronic lung disease of prematurity. In childhood and adolescence, BPD survivors may have respiratory symptoms and lung function impairment that closely resemble those of asthmatic patients,¹ **even if BPD patients present fewer acute exacerbations, poorer response to inhaled corticosteroids and less pronounced airway reversibility** ~~though they do not share the same origins.~~ A recent study reported that low-birth-weight survivors have significantly higher odds of hospitalization for respiratory diseases (wheezing, respiratory infections and respiratory failure) as young adults than their normal-birth-weight counterparts.²

Although there are no data to suggest an asthma-like inflammatory profile in the airways of BPD survivors, they are often empirically treated as if they were asthmatic (e.g. with glucocorticosteroids).^{3,4} Very little is known, however, about the possible biochemical and inflammatory mechanisms involved in the pathogenesis of the symptoms and airway obstruction in BPD survivors,³ and no data are available on the pathological features of these patients' lungs beyond infancy.³

It is common knowledge that asthma is a chronic inflammatory disease of the airways and several non-invasive biomarkers have been proposed for indirectly assessing different patterns of airway inflammation.⁵ Exhaled nitric oxide has been studied the most and it is considered a reliable marker of eosinophilic inflammation in the airways.^{6,7} Since "calor" is one of the cardinal signs of inflammation, some authors recently recommended considering exhaled air temperature as a potential marker of inflammation.⁸⁻⁹ Increased values of exhaled breath temperature have been reported in children and adults with asthma,⁸⁻¹¹ correlating significantly with the concentrations of exhaled nitric oxide.⁸⁻¹⁰ To our knowledge, no studies have evaluated exhaled air temperature in individuals with BPD.

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3 The aim of the present **cross-sectional** study was to evaluate exhaled breath temperature
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5 (PLET, end-expiratory maneuver plateau temperature) in BPD survivors and to compare it
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7
8 with the temperature measured in age-matched asthmatic subjects and healthy controls.
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10 11 12 13 14 15 **MATERIALS AND METHODS**

16 17 **Study subjects**

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19
20 BPD survivors: 17 BPD survivors were considered (**table**), drawn from a population with
21
22 BPD born at gestational ages below 31 weeks and with birth weights under 1500 g
23
24 admitted to the Neonatal Intensive Care Unit between 1990 and 1994 and followed up
25
26 routinely thereafter at the Pediatrics Department in Padova. BPD was defined as oxygen
27
28 dependence persisting 28 days after birth in infants on mechanical ventilation (18 days on
29
30 average). At birth, the mean gestational age of the sample was 28.3 weeks (SD 1.9) and
31
32 the mean weight was 1011 g (SD 222).
33

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35
36 All the BPD survivors were clinically stable at the time of recruitment, 5 of them were on
37
38 low- to medium-dose inhaled steroid treatment and two were being treated with
39
40 theophylline. Two of the BPD survivors were sensitive to common airborne allergens (dust
41
42 mites, dog and cat dander, grass and tree pollens).
43
44

45
46 Asthmatic subjects: 17 allergic asthmatic adolescents (**table**) who had been born at term
47
48 were enrolled from among those regularly attending our outpatients clinic. Their asthma
49
50 had been diagnosed by a pediatric respiratory physician based on their clinical history
51
52 (cough, shortness of breath, recurrent wheezing, chest tightness) and an increase in FEV₁
53
54 after salbutamol (400 µg) >12%, according to international guidelines.¹²
55
56

57
58 All the asthmatic patients were clinically stable at the time of recruitment, 6 of them were
59
60 on low- to medium-dose inhaled steroid treatment (100-200 mcg/day budesonide
equivalent) and one of them was being treated with long-acting beta2 agonists too.

1
2
3 Healthy controls: 17 age-matched healthy adolescents (table) with no history of pre-term
4
5 birth, atopy or respiratory diseases were recruited as a control group.
6

7
8 For all three groups, subjects who had suffered any airway infections in the previous 4
9
10 weeks were excluded from the study.
11

12 The Ethics Committee of our hospital reviewed and approved the protocol and all parents
13
14 gave their informed consent.
15

16
17 At recruitment, all the adolescents underwent physical examination, exhaled air
18
19 temperature measurement, FE_{NO} measurement and spirometry. All the measurements
20
21 were performed in the afternoon. FE_{NO} measurement was not possible in 3 subjects (1
22
23 with asthma and 2 with BPD)
24
25

26 27 **Exhaled breath temperature (PLET)** 28

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30 Exhaled breath temperature (end-expiratory maneuver plateau temperature, PLET) was
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32 measured as described elsewhere.¹³ The environmental conditions were controlled in
33
34 order to maintain a stable temperature of 23±1 °C and a relative humidity of 40-50%.

35
36 The measurements were repeated three times. The inspiratory air stream temperature was
37
38 recorded during a complete inhalation to total lung capacity (TLC). The exhaled airway
39
40 temperature was measured during a slow exhalation to residual volume (RV) using the
41
42 Medical Mass Flow Sensor of the Vmax Spectra 229 Pulmonary Function Laboratory
43
44 (VIASYS Healthcare, Yorba Linda, CA, USA). Dynamic airway temperature and airflow
45
46 were measured with the Mass Flow Sensor. The response time of the Mass Flow Sensor
47
48 to a steep change in airway temperature was approximately 64 milliseconds, with a
49
50 thermal resolution of 0.01°C. Mouth pressure was measured at the airway opening using a
51
52 differential pressure transducer. Exhaled flow was directed through an alternate pathway
53
54 with a fixed resistor in place to control the expiratory air flow rate and maintain an airway
55
56 pressure sufficient to keep the velum closed and prevent any contribution of warmed gas
57
58 from nasal sinuses. A mouth pressure of > 5 cm H₂O also helped the subject to stabilize
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3 the expiratory flow at 5-6 l/min. All the signals, including exhaled air temperature, mouth
4 pressure and exhalation flow rate, were transferred to a personal computer and analyzed
5 using software specifically developed for this purpose (Vmax Temperature, VIASYS
6 Healthcare, Yorba Linda, CA, USA). Real-time signal display consisted of inspiratory and
7 expiratory airway temperature, respiratory flow rate and mouth (airway) pressure on a time
8 axis. Flow and pressure limit indicators were presented on screen as a coaching aid.
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17 At the end of the test, the software automatically calculated the PLET, which was the
18 parameter used to compare the groups.
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20 21 22 23 **Fractional exhaled nitric oxide (FE_{NO}) and pulmonary function measurement**

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25
26 FE_{NO} was measured using the NIOX system (Aerocrine, Stockholm, Sweden), according
27 to the ERS/ATS guidelines for measuring FE_{NO} in children.¹⁴ Children inhaled NO-free air
28 to total lung capacity (TLC) and exhaled through a dynamic flow restrictor with a target
29 flow of 50 ml/sec for at least 6-7 seconds. No nose clip was used. The NIOX system was
30 calibrated using a 200 ppb NO tank (Lindegas Hoek Loos Speciality Gases, Amsterdam,
31 Netherlands) according to the manufacturer's instructions.
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40 Lung function was measured by means of a 10-liter bell spirometer (Biomedin, Padova,
41 Italy) and the best of three maneuvers was expressed as a percentage (%) of the
42 predicted reference values, according to the ERS/ATS guidelines.¹⁵
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47 48 **Statistical analysis**

49
50 It has been calculated that a sample size of 17 subjects allows detecting a 1 °C difference
51 between groups of with a power of 80% at a 2-sided α -level of .05.
52

53
54 Age and anthropometric data were expressed as the mean and standard deviation (SD)
55 (table).
56

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59 Since PLET, FE_{NO} and lung function data were not normally distributed (as demonstrated
60 by the Shapiro-Wilk test), they were expressed as medians and interquartile ranges (IQR),

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2
3 and analyzed by means of non-parametric tests. Differences between groups were
4
5 analyzed using the Kruskal-Wallis test, followed (where significant) by the Mann-Whitney
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7 U-test for comparisons between groups. Correlations were evaluated using Spearman's
8
9 rank test. Statistical significance was set at a 2-sided α level of 0.05.
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12 13 14 15 **RESULTS**

16 17 **Exhaled breath temperature (PLET)**

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19 The Kruskal Wallis test revealed a significant difference in the PLET for the 3 groups
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21 ($p < 0.001$) (figure 1A). The between-group comparison found a significantly lower PLET in
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23 BPD survivors (26.72°C [25.11-27.57]) than in asthmatic patients (29.60°C [29.20-30.02],
24
25 $p < 0.001$), while no significant difference emerged between BPD survivors and healthy
26
27 controls (26.97°C [26.58-27.38] $p = 0.23$). The asthmatic cases had a significantly higher
28
29 PLET than the healthy controls ($p < 0.001$).
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31
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33 34 **Exhaled nitric oxide (FE_{NO})**

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36 The Kruskal Wallis test found a significant difference in FE_{NO} for the 3 groups ($p = 0.003$)
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38 (figure 1B). The between-group comparison revealed a significantly higher FE_{NO} in
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40 asthmatic cases (29.9 ppb [15.0-49.2]) than in either healthy controls (11.7 ppb [8.5-16.8],
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42 $p = 0.008$) or BPD survivors (10.6 ppb [7.0-13.2], $p = 0.002$), with no significant difference
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44 between the latter two groups ($p = 0.28$).
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48 49 **Lung function**

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51 The Kruskal Wallis test disclosed significant differences in all the spirometric parameters.
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53 The between-group comparison showed that BPD children had lower FVC, FEV₁, FEF₂₅₋₇₅
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55 and FEV₁/FVC than either asthmatic cases or healthy controls; and the asthmatic cases
56
57 had lower FEV₁, FEF₂₅₋₇₅ and FEV₁/FVC than healthy controls (table, figure 1C and 1D).
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Correlations

Considering the whole sample, a significant correlation emerged between PLET and FE_{NO} ($R=0.42$ $p=0.004$) (figure 2).

In BPD survivors there was a correlation approaching statistical significance between FVC and PLET ($p=0.058$ $R=0.47$), which was stronger considering the whole sample ($R=0.28$ $p=0.036$) whereas none of the other spirometric parameters correlated with PLET values in BPD survivors.

No significant correlations emerged between spirometric measurements and PLET in asthmatic cases or healthy controls.

DISCUSSION

This study shows that exhaled breath temperature is significantly lower in BPD survivors than in asthmatic patients.

BPD survivors have respiratory symptoms and lung function profiles that resemble those of asthmatic children¹⁶ and this explains why they are often considered and empirically treated as asthmatic patients during their childhood.³ Very little is known about the processes underlying the obstructive lung function pattern in BPD survivors and there is no evidence to support any asthma-like inflammation in this condition.^{4,17,18}

Here we demonstrate that both PLET and FE_{NO} are lower in BPD survivors than in asthmatic children, strongly suggesting that the two conditions have a different pathogenesis (figure 1A and 1B). In a previous study, we already showed that exhaled NO is lower in BPD survivors than in asthmatic cases;¹⁹ the present study further supports the lack of any asthma-like airway inflammation in BPD survivors.

A possible limit in the interpretation of our results is the potential effect of ICS treatment and atopy on PLET values. In fact while all asthmatic patients were allergic, only two BPD patients were sensitized. In addition some patients in both groups were treated with ICS.

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3 In keeping with previous studies conducted on asthmatic patients,⁸⁻⁹ PLET and FE_{NO}
4 correlated significantly. It is worth noting that the scatter plot (figure 2) shows a clear
5 separation between asthmatic cases and BPD survivors, again suggesting that different
6 pathogenetic mechanisms underlie these two chronic obstructive lung diseases.
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12 As previously reported,⁸⁻¹⁰ asthmatic subjects have higher levels of both FE_{NO} and PLET,
13 which are thought to reflect eosinophilic airway inflammation.^{6,20} The higher exhaled breath
14 temperature in asthmatic subjects also seems to suggest an increased vascularity and
15 bronchial blood flow.¹⁰ In BPD survivors, on the other hand, PLET and FE_{NO} are
16 significantly lower than in asthmatic patients and even tend to be lower than in healthy
17 controls, although without reaching the statistical significance (figure 1A and 1B). This
18 trend could be explained by the anatomical alterations that characterize BPD lungs,
19 namely alveolar simplification, variable degree of interstitial fibrosis^{21,22} and dysmorphic
20 vascular growth.²³ **These anatomical alterations might presumably be responsible for an
21 impaired heat exchange between the bronchial vessels and the airway lumen, giving rise
22 to normal-to-low values of exhaled air temperature in BPD survivors.**
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38 As previously reported,²⁴ we confirm here that BPD survivors have a lung function pattern
39 with airflow limitation (figure 1C). An autoptic study demonstrated thickening of the airway
40 walls and an increase in smooth muscle, suggesting that these aspects may have a role in
41 airway obstruction and hyper-responsiveness in BPD survivors.²⁵ Moreover BPD survivors
42 have a significantly reduced forced vital capacity (figure 1D). This last features might be
43 explained by the impaired growth of the BPD survivor's lungs, which would lead to reduced
44 pulmonary volumes.
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54 Previous studies have already documented an impaired lung function in BPD survivors **an
55 impaired lung function and characterized by an accelerated decline.**^{24,26,27} ~~In a follow-up
56 study, Doyle et al.²⁸ reported significant airflow obstruction in BPD survivors at 18 years of
57 age, with an accelerated decline in their FEV₁/FVC ratio, suggesting an earlier~~
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3 deterioration in their respiratory health than in the general population. Lung function
4 declines physiologically in adulthood and senescence, albeit without reaching levels
5 associated with disability.²⁹ This decline could be more of a problem in BPD survivors,
6 whose maximal lung function is underdeveloped. It may be that lung function impairment in
7 BPD survivors is not just the static outcome of the initial damage, but due to an ongoing
8 remodeling/damaging process. Although our findings go against any suggestion of an
9 asthma-like eosinophilic inflammatory profile, other inflammatory pathways (e.g.
10 neutrophilic or lymphocytic) and/or remodeling mechanisms may be involved. Elucidating
11 this pathophysiological substrate could have a **great** impact on the long-term management
12 of BPD subjects.
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27 In conclusion, we showed that exhaled breath temperature (PLET) and exhaled nitric
28 oxide (which are considered biomarkers of eosinophilic airway inflammation) are not
29 increased in BPD survivors. Further studies are needed to assess the role of other non-
30 invasive biomarkers, as measured for instance in exhaled breath condensate, to shed light
31 on the pathogenic processes affecting the BPD survivor's lung and thus pave the way to
32 the development of new, specifically targeted drugs.
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50 **Acknowledgement**

51 The authors thank VIASYS Healthcare, Yorba Linda, CA, USA, for providing hardware and
52 software for exhaled air evaluation.
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For Peer Review

FIGURE LEGENDS

Figure 1. Panel A shows PLET values in the 3 groups; panel B shows FE_{NO} values in the 3 groups; panel C shows FVC values in the 3 groups; panel D shows FEV1 values in the 3 groups. **p<0.001 and *p<0.01 vs healthy controls; #p<0.05 and §p<0.01 vs asthmatic subjects □□ p<0.001 vs BPD survivors

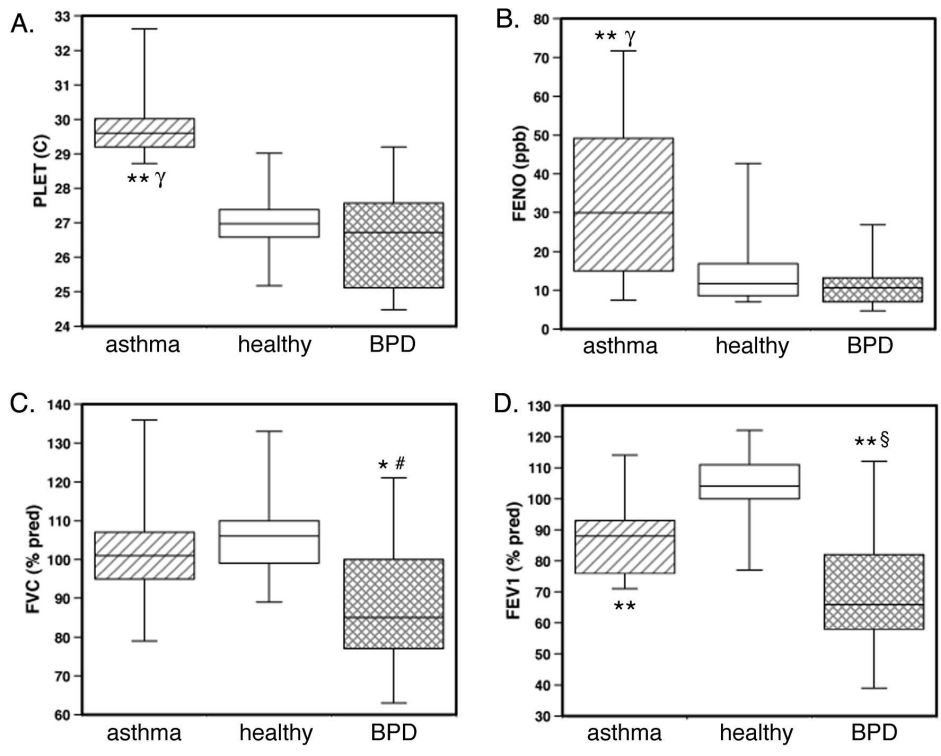
Figure 2. Correlation between FE_{NO} values and PLET values in BPD, asthmatic and healthy subjects (R=0.42 p=0.004).

For Peer Review

	Healthy	Asthma	BPD
Number (males)	17 (11)	17 (10)	17 (7)
Age (y)	14.4 (1.8)	13.3 (2.1)	14.5 (2.0)
Height (cm)	163.6 (7.5)	159.5 (9.1)	161.5 (11)
Weight (Kg)	56.5 (9.4)	57.9 (12.9)	53.9 (12.2)
FVC (%pred)	106 [99-110]	101 [95-107]	85 [77-100] [#]
FEV ₁ (%pred)	104 [100-111]	88 [76-93] ^{**}	66 [58-82] ^{**§}
FEF ₂₅₋₇₅ (%pred)	121 [107-133]	75 [57-87] ^{**}	46 [40-67] ^{###}
FEV ₁ /FVC	91 [88-93]	78 [76-84] ^{**}	72 [67-80] ^{**}

Table. Demographic characteristic and lung function measures of study subjects. Anthropometric data are expressed as mean (SD). Spirometric data are expressed as median and IQR. ^{**}p<0.001 and ^{*}p<0.01 vs healthy controls; [#]p<0.05 and [§]p<0.01 vs asthmatic subjects.

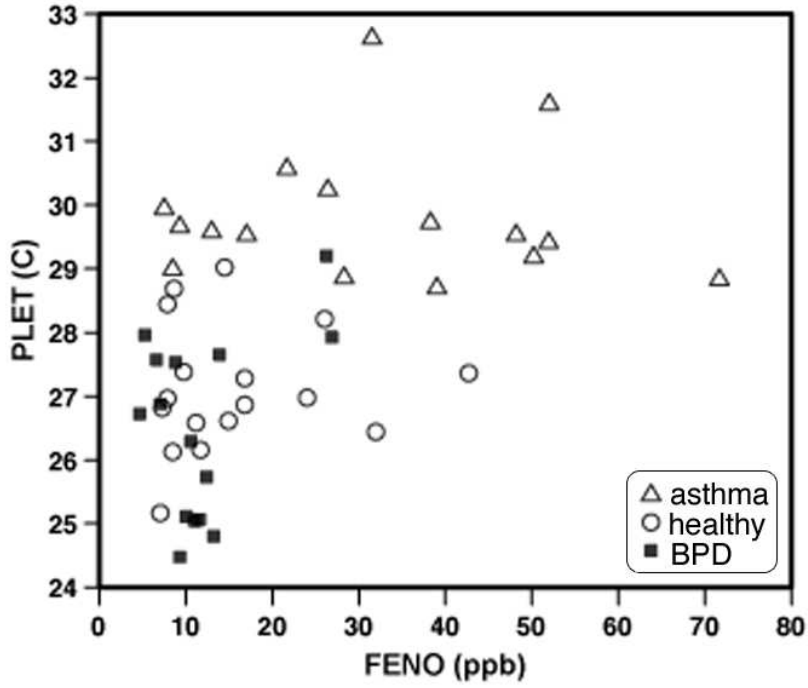
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Review