Usefulness of the Exhaled Breath Temperature Plateau in Asthma Patients

Astrid Crespo Lessmann^{a, b} Jordi Giner^a Alfons Torrego^{a, b} Eder Mateus^a
Montserrat Torrejón^a Alicia Belda^a Vicente Plaza^{a, b}

^aDepartment of Respiratory Medicine, Hospital de la Santa Creu i Sant Pau, Institut d'Investigació Biomèdica Sant Pau (IIB Sant Pau), and ^bDepartment of Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain

Key Words

Asthma · Inflammation · Exhaled breath temperature

Abstract

Background: Exhaled breath temperature (EBT) has recently been proposed as a noninvasive marker of bronchial inflammation in patients with asthma. However, the usefulness of EBT in everyday clinical practice is not well established. Results to date are contradictory and are mainly derived from small, pediatric populations. A comparison of results is further complicated by the use of different equipment and measurements. **Objective:** We performed a comprehensive study to determine whether EBT is related to asthma control, disease severity, bronchial obstruction, or bronchial inflammation. **Methods:** Sixty-nine patients on maintenance treatment for asthma were included in a cross-sectional study. At the same visit, we measured the EBT plateau (EBTp) using an X-halo Breath Thermometer (Delmedica, Singapore), the fraction of exhaled nitric oxide (FeNO), spirometry, and inflammatory cell count in induced sputum, and we administered the Asthma Control Test questionnaire. **Results:** No significant differences were found between EBTp measurements and the level of asthma control, disease severity, bronchial obstruction, FeNO levels, or inflammatory asthma phenotypes. We found a significant difference between

EBTp and gender. The EBTp was 34.07°C (SD 0.74) in women and 34.38°C (0.46) in men ($p = 0.038$). We also found a significant correlation between EBTp measurements and the induced sputum eosinophil count ($R = -0.348$, $p = 0.003$).

Conclusions: The results of this study do not support the usefulness of the EBTp in asthma management in routine clinical practice. Further research using standardized methods is needed to determine the potential use of the EBTp measurement in asthma management.

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Background

In recent years, there has been increasing interest in developing noninvasive methods for measuring inflammation in asthma, such as analysis of induced sputum cells and measurement of inflammatory markers in the exhaled breath condensate and in the fraction of exhaled nitric oxide (FeNO). Inflammation is a protective response characterized by several classic signs, one of which is heat. However, temperature has been little used in the exploration of inflammatory respiratory diseases.

This paper is part of the doctoral thesis of A.C.L.

Exhaled breath temperature (EBT) has been proposed as a noninvasive marker of airway inflammation in asthma [1]. The basis for this proposal is that inflammation of the bronchial mucosa increases local vascularization, consequently increasing bronchial temperature. This increase could be measured in exhaled air [2–5]. EBT has also been related to markers of inflammation such as FeNO levels, eosinophil counts, forced expiratory volume in 1 s (FEV₁), and bronchial challenge tests [1, 6–9], but results to date have been contradictory. Furthermore, most studies have been performed in small, pediatric populations with varying equipment and measurements.

The use of EBT as a marker of inflammation in asthma has been explored in different ways. Paredi et al. [1] calculated the exhaled air temperature increase ($\Delta e^{\circ}\text{T}$) by measuring the time it took the temperature to reach 63% of the final reading in a slow and controlled expiratory maneuver. Piacentini et al. [4] measured the EBT with a high-performance temperature indicator connected to a thermocouple during a controlled expiratory maneuver. They reported the peak expiratory temperature and the plateaus valued at the end of the expiration. Popov et al. [6] designed and marketed a simple, potentially individual device to measure the EBT plateau (EBTp). They took measurements at 1-min intervals until no further increments were noted.

The present work was designed to analyze the usefulness of the EBTp [6] for asthma patients. Our aim was to correlate EBTp measurements with asthma control, asthma severity, bronchial obstruction, and bronchial inflammation.

Subjects and Methods

Study Design

We performed an observational cross-sectional study of data collected from January 2012 to July 2013.

Legal and Ethical Aspects

The study design complied with the principles of the Declaration of Helsinki (18th World Medical Assembly, 1964) and was approved by the Clinical Research Ethics Committee at the Hospital de la Santa Creu i Sant Pau in Barcelona (COD 26/2012). The patients gave written informed consent prior to participation in the study. No data were recorded that could identify individual patients; their identities were known only by their physicians and were not disclosed to any third parties. The ClinicalTrials.gov identifier is NCT02064686.

Study Population

The study group consisted of 69 patients on maintenance treatment for asthma. All were nonactive smokers aged ≥ 18 years. According to the Global Initiative for Asthma (GINA) criteria [10],

we defined asthma as a history of variable respiratory symptoms and evidence of variable expiratory airflow limitation. All patients had a positive bronchodilator test or daily peak expiratory flow variability $>20\%$, or a positive methacholine challenge test documented in their case history. High-dose inhaled corticosteroids (ICS) were defined according to the GINA 2014 criteria [10], i.e. when the patients had treatment with budesonide or equivalent ICS at ≥ 800 $\mu\text{g/day}$.

Patients were enrolled from an outpatient asthma unit at a tertiary referral hospital. Exclusion criteria were respiratory tract infection or asthma exacerbation in the 30 days prior to inclusion, treatment with oral steroids or immunomodulator drugs, programmed hospitalization during the study, and a cognitive disorder affecting comprehension of the study.

After signing the informed consent form, the patients answered the Asthma Control Test (ACT) questionnaire [11] and underwent the following examinations on the same day: FeNO, spirometry, induced sputum, extraction of 10 ml of peripheral venous blood for later analysis, and the skin prick test with standardized allergenic extracts (based on the modified test of Pepys) [12]. Total IgE was measured by EliA (ImmunoCAP, Phadia 250). Induced sputum was analyzed within 2 h. The body mass index (BMI) was recorded as well.

EBT Plateau

The EBTp was measured using the X-halo Breath Thermometer (Delmedica, Singapore) according to the method validated by Popov et al. [6]. The patients were instructed to breathe tidally, inhaling freely through the nose and exhaling into the device. The maneuver was continued until the software of the instrument indicated that the measured value was stable. The EBTp was defined as 20 s with changes $<0.01^{\circ}\text{C}$. For all patients, the EBT was measured in the morning (9.00–10.00 h) after a minimum of 2 h of fasting. The environmental temperature was controlled to maintain a stable condition of $24 \pm 1^{\circ}\text{C}$ and a relative humidity of 40–50% [7].

Asthma Control

To objectively assess the level of asthma control, we used the ACT. This is a simple 5-question questionnaire that measures the degree of control that asthmatic patients assign to their illness over the previous 4 weeks. The answers to each question are assigned 1–5 points, resulting in a total score ranging from 5 (worst possible control) to 25 (best possible control). A score >20 corresponds to ‘controlled asthma’ and <19 points to ‘not well-controlled asthma’ [11].

Asthma Severity

We classified asthma severity retrospectively, based on the level of treatment required to control symptoms and exacerbation using the GINA classification. Mild asthma was asthma that could be controlled with step 1 or 2 treatment, while severe asthma required step 4 or 5 treatment [10].

Bronchial Obstruction

Spirometry was performed using a Dataspir 500 (Sibelmed SA, Barcelona, Spain) following the recommendations of the European Respiratory Society [13] and the Spanish Respiratory Society (SEPAR) [14]. Patients were classified into two groups: those with an FEV₁ level $\geq 80\%$ and those with an FEV₁ level $<80\%$.

Table 1. Demographic and clinical characteristics of the 69 asthma patients

		p value
Sex, % women	62.3	p = 0.113*
Age, years	49.7 (15.6); 47 (22–80)	p = 0.752*
BMI	26.9 (4.9); 26.8 (16.6–45.3)	p = 0.838*
Duration of disease, years	11.7 (9.45); 9 (1–35)	p = 0.604*
Ex-smokers, %	31 (8.5 p/y)	p = 0.108*
Rhinitis, %	69.6	p = 0.628*
Nasal polyposis, %	21.7	p = 0.424*
Positive skin prick test, %	68.1 (60% to house dust mites, 13% to pollen and 27% to other allergens)	p = 0.082*
Asthma controlled (ACT $\geq 20^a$), %	62.3	p = 0.521*
Patients with budesonide (or equivalent ICS) ≥ 800 $\mu\text{g/day}$, %	33.3	p = 0.371*
Patients with ICS/LABA combinations, %	55.1	p = 0.565*
Patients with positive bronchodilator test, %	25.6	p = 0.425*
Asthma severity, %		p = 0.749*
Mild (step 1–2)	34.8	
Moderate (step 3)	18.8	
Severe (step 4–5)	46.4	
FEV ₁ , % of reference values	82.7 (19.3); 88 (36–117)	p = 0.255*
FEV ₁ /FVC	67.5 (13.6); 70 (32.2–94.4)	p = 0.734*
Total IgE, IU/ml	361.9 (633.1); 124 (2–3,500)	p = 0.881*
Induced sputum, %		
Eosinophil counts	7.2 (11.2); 3.1 (0–65.8)	p = 0.328*
Neutrophil counts	42.9 (23.2); 40.2 (4.9–85.7)	p = 0.190*
Inflammatory asthma phenotype, %		p = 0.364*
Paucigranulocytic	33.33	
Eosinophilic	50.72	
Neutrophilic	15.95	
FeNO, ppb	38.4 (32.7); 27 (3–174)	p = 0.589*
EBTp, °C	34.19 (0.66); 34.33 (32.09–36.2)	p = 0.038*

Values are expressed as percentages or 'means (SD); medians (min–max)'. p/y = Pack-years; LABA = long-acting β -adrenoceptor agonists; FVC = forced vital capacity. * Significant value (comparison between genders).

^a Median (min–max) ACT 22 (9–25).

Bronchial Inflammation

Fraction of Exhaled Nitric Oxide

FeNO measurements were performed using an NO Vario Analyzer device (FILT GmbH, Berlin, Germany); the patients performed a maximal inspiration followed by a controlled expiratory maneuver at a flow of 50 ml/s [15]. FeNO concentration was expressed in parts per billion (ppb), and the reference values were taken from the literature [16].

Inflammatory Asthma Phenotypes

Cell counts in induced sputum were determined using the method of Pizzichini et al. [17]. Briefly, mucus plugs were manually selected and weighed, incubated (15 min at room temperature) in 4 times the weight (in ml) of the selected plug (in mg) in 0.1% dithiothreitol (Calbiochem, San Diego, Calif., USA), washed with 4 times the plug weight (in ml) in Dulbecco's PBS and gravity filtered through a 41- μm -pore nylon net filter (Millipore, Billerica,

Mass., USA). After dithiothreitol homogenization, each specimen was aliquoted into two parts of equal volume. Total cell counts were performed using a Neubauer hemocytometer. Visually identifiable squamous epithelial cells were not counted or included in the total cell count. Samples that did not produce adequate sputum cell numbers ($<1,000 \times 10^6$ cells/g) were excluded. Cell viability was determined by light microscopic assessment using trypan blue exclusion staining. After centrifuging the cell preparation, we obtained a cell pellet and a supernatant. The cell pellet was used for differential cell counts (macrophages, eosinophils, neutrophils, lymphocytes, and bronchial epithelial cells) performed on May-Grünwald-Giemsa-stained preparations. A differential leukocyte analysis of nonsquamous cells (Diff-Quik stained) was performed on a minimum of 400 cells. Differential cell counts are expressed as the percentage of total nonsquamous nucleated cells. Reference values of the cell counts were taken as previously described in the literature [18].

Fig. 1. Correlation between EBTp and eosinophil percentage in induced sputum.

Patients with a neutrophil count $>61\%$ were classified as having neutrophilic asthma, those with eosinophil counts $>3\%$ were classified as having eosinophilic asthma, and those with neutrophil counts $\leq 61\%$ and eosinophil counts $\leq 3\%$ were classified as having paucigranulocytic asthma [17–19].

Statistics

Descriptive baseline values are presented as percentages and frequencies for qualitative data, and means and SD for quantitative data. The comparison between the two groups was performed by analysis of the t test for quantitative or ordinal variables. The comparison between the groups according to the induced sputum inflammatory phenotypes was conducted using analysis of variance for the primary and the other quantitative variables. Results are presented as mean values and SD.

Categorical variables were compared with contingency tables using the χ^2 test. Results are given as numbers of cases and percentages. Correlation analysis was performed by Pearson's r . In all cases, the level of statistical significance was 5% ($\alpha = 0.05$). Statistical analysis was performed using SPSS software (version 18.0) for Windows (SPSS Inc., Chicago, Ill., USA).

Results

Table 1 shows the demographic and asthma characteristics of the 69 patients included in the study.

Asthma Control

The patients were classified into two groups according to the level of asthma control. Asthma was controlled in 43 patients (62.31%; ACT ≥ 20) and not well controlled in

26 (37.69%). EBTp levels showed no significant differences between groups: 34.29°C (SD 0.61) in the controlled versus 34.02°C (0.72) in the not-well-controlled asthma group ($p = 0.104$). No significant correlations were observed between EBTp and ACT levels ($R = 0.179$, $p = 0.142$).

Asthma Severity

The patients were classified into two groups according to disease severity: 24 (34.78%) had intermittent or mild and 45 (65.22%) moderate or severe persistent asthma. The EBTp was 34.33°C (0.28) in the intermittent-mild and 34.11°C (0.78) in the moderate-severe group. No differences were observed between groups ($p = 0.200$).

Bronchial Obstruction

According to the FEV₁ values (if FEV₁ was $<80\%$ of the predicted normal value), 28 patients (40.58%) had a bronchial obstruction and 41 (59.42%) did not. The EBTp was 34.30°C (0.43) in the first and 34.02°C (0.88) in the second group. These differences were not statistically significant ($p = 0.128$). We found no significant correlation between EBTp measurements and FEV₁% ($R = 0.165$, $p = 0.176$) in any of the samples analyzed.

Bronchial Inflammation

Fraction of Exhaled Nitric Oxide

Eighteen patients (26.08%) had a high FeNO (≥ 50 ppb), and 51 (73.92%) had normal FeNO values (<50 ppb). The EBTp was 34.26°C (0.57) in the first and 34.17°C (0.69) in the second group. The difference was not significant ($p = 0.604$). No significant correlation was found between EBTp measurements and FeNO values ($R = -0.07$, $p = 0.568$).

Inflammatory Asthma Phenotypes

Twenty-three patients (33.33%) had a paucigranulocytic phenotype, 35 (50.72%) had an eosinophilic phenotype, and 11 (15.95%) had a neutrophilic asthma phenotype. The EBTp observed per group was 34.22°C (0.78) for the paucigranulocytic asthma patients, 34.12°C (0.53) for the eosinophilic asthma patients, and 34.34°C (0.78) for the neutrophilic asthma patients. No significant differences in EBTp measurements were found between the inflammatory asthma phenotype groups ($p = 0.607$). However, a significant negative correlation was shown between eosinophil counts in the induced sputum and EBTp measurements ($R = -0.348$, $p = 0.003$) (fig. 1).

Fig. 2. Comparison of EBTp parameters between subgroups: ACT <20 vs. ≥20; FEV₁ below vs. above the median value; FeNO ≥50 vs. <50 ppb; ICS high vs. middle/low dose; age below vs. above the median value; men vs. women. val. ref. = Reference value.

Confounding Variables

There was no significant correlation between the EBTp and age ($R = -0.052$, $p = 0.670$), BMI ($R = 0.091$, $p = 0.459$), ambient temperature ($R = -0.075$, $p = 0.586$), or body temperature ($R = 0.156$, $p = 0.262$). There was a significant difference in EBTp according to gender: the EBTp was 34.07°C (0.74) in women and 34.38°C (0.46) in men ($p = 0.038$).

Figure 2 compares EBTp values between subgroups: ACT <20 versus ≥20; FEV₁ below versus above the median value; FeNO ≥50 versus <50 ppb; ICS high versus middle/low dose; age below versus above the median value, and men versus women.

Discussion

In this study in adult patients with asthma, we did not find any relationship between EBTp and asthma control, disease severity, airway obstruction, or bronchial inflammation. The rationale for EBT as a new, simple, and non-invasive biomarker of airway inflammation is based on the inflammatory nature of asthma, characterized by increased vascularization that may lead to increased heat loss in exhaled air [1, 4]. EBT measurements have been performed in various studies using different nonstan-

dardized methods, but their clinical usefulness is still uncertain. In one of the first published studies that compared a group of asthma patients ($n = 18$) with healthy volunteers ($n = 16$), Paredi et al. [1] found a faster $\Delta e^{\circ}T$ in patients with asthma, but no differences in EBTp. They also failed to find a correlation between EBTp and FeNO. In another study, using a thermometer connected to a high-performance thermocouple, Piacentini et al. [4] found a significant correlation between plateau temperature measured at the end of expiration and FeNO, but no correlation between peak expiratory temperature and FeNO.

Results regarding the use of an X-halo Breath Thermometer to measure EBTp to control asthma vary among studies. Popov et al. [6] found that the EBT was increased in 14 not-well-controlled asthmatics, but that it decreased after anti-inflammatory treatment had been started. In a group of 100 patients, García et al. [20] observed higher levels of EBTp in not-well-controlled asthmatics than in well-controlled asthma patients and healthy subjects. However, using the same equipment, we could not confirm these findings, observing no correlation between EBTp and levels of asthma control. The discrepancies between these three studies could be explained by the lower proportion of not-well-controlled asthma patients in our study (37.69%).

In addition, we did not find any association between EBTp level and severity of airflow obstruction. Previous studies have shown discrepant results regarding this association. Peroni et al. [21] found no correlation with EBTp in 50 children with asthma treated with ICS after exercise-induced bronchoconstriction, while in asthmatic children not treated with ICS they found an increase in EBTp and a decrease in FEV₁ after the same exercise. Svensson et al. [22] did not find changes in EBTp in asthma patients before or after two bronchoconstriction tests (eucapnic voluntary hyperventilation and methacholine challenge). These differences between studies could be explained by the anti-inflammatory effect of ICS. Most of the patients included in our study were on this medication.

Exhaled temperature has previously been related to airway inflammation and bronchial blood flow [3, 5]. However, in a widely characterized sample of asthma patients, our study failed to demonstrate a correlation between EBTp and other markers of bronchial inflammation. This discrepancy has been observed in other studies. In a group of 19 asthmatic patients, for example, Paredi et al. [3] determined the $\Delta e^{\circ}T$ and found no correlation between FeNO levels and EBT – nor did Svensson et al. [23] observe any association between FeNO and EBTp in a group of 20 patients with mild asthma. However, unlike Paredi et al. [3], Piacentini et al. [4] found EBTp was associated with FeNO (41 children with allergic asthma) and with the percentage of eosinophils in induced sputum (16 children). Nevertheless, this association was lost when the $\Delta e^{\circ}T$ method was used. In the induced sputum from the 69 participants in our study, we observed a negative correlation between EBT and the proportion of eosinophils in sputum. One explanation for this discrepancy between studies could be that we studied three inflammatory phenotypes (paucigranulocytic, eosinophilic, and neutrophilic phenotypes), while the other authors investigated only eosinophilic phenotypes. Other explanations for the different findings could be the variability of the methods used to measure EBT, the lack of standardization of these methods, the differences in asthma populations, the effect of treatments, and the possibility that surrogate markers reflect different parts of the inflammatory cascade.

Higher EBT values have been associated with potential confounders such as male gender, younger age, physical activity, higher BMI, and higher room air temperature [7, 23–25]. We found no correlation between EBTp and age, BMI, room temperature, or humidity, but men showed higher EBTp values than women. The lack of a correlation of EBTp with age and BMI was probably due to the homogeneity of the group. The lack of a correlation of

EBTp with room temperature and humidity was most likely because these variables were always stable in our laboratory ($24 \pm 1^{\circ}C$, relative humidity 40–50%).

Our study has several limitations. First, we did not use other EBT methods such as $\Delta e^{\circ}T$ or peak EBT. Second, unlike some studies, we did not include children with asthma. Our study has several strengths, however. It is a comprehensive study in a large sample using multiple techniques to assess various aspects of the disease, while other studies to date have focused on specific or partial aspects. Furthermore, all clinical, functional, and inflammatory measurements were made on the same day for all patients.

Conclusions

We found no correlation between EBTp and asthma control, severity of disease, bronchial obstruction, or bronchial inflammation. According to our results, EBTp does not seem to provide useful clinical information as a single-point measurement. Further research regarding the potential use of EBTp measurements in asthma management should incorporate large series, longitudinal designs, and measurement standardization.

Authors' Contributions

All authors made substantial contributions to the study and critically revised and approved the manuscript. Specifically, A.C.L. and V.P. generated the hypothesis, designed the study, enrolled patients, and wrote the first draft of the manuscript; A.C.L. collected clinical data on the patients, built the database, and performed the statistical analysis; J.G. performed the FeNO and EBT measurements; A.T. contributed to the data interpretation and writing of the manuscript; E.M. processed the sputum and performed the inflammatory cell counts, and M.T. and A.B. conducted sputum induction and spirometry.

Acknowledgements

We thank Carolyn Newey for linguistic and editorial assistance. This work was supported by: the Catalan Foundation of Pneumology (FUCAP), Barcelona; the Spanish Society of Pneumology and Thoracic Surgery (SEPAR), and the Social Research Private Foundation (MIA) of the Hospital de la Santa Creu i Sant Pau, Barcelona.

Financial Disclosure and Conflicts of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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