Exhaled breath temperature, a new biomarker in asthma control: A pilot study*

Temperatura do ar exalado, um novo biomarcador no controle da asma: Um estudo piloto

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Abstract

Objective: To evaluate whether the exhaled breath temperature (EBT), measured by a noninvasive method, is an effective means of monitoring patients with uncontrolled asthma. **Methods:** A pilot study comprising nine patients (seven women and two men; mean age: 39 years) diagnosed with asthma at least one year prior to the beginning of the study and not having been under maintenance therapy for the last three months. In the first visit, the patients underwent spirometry and measurement of EBT. The patients were then instructed to use inhaled budesonide/formoterol (200/6 µg) every 12 h for six weeks. In addition, the patients with severe asthma (FEV_1 < 60% of predicted) were instructed to use oral prednisolone (40 mg/day) for five days. After six weeks, the patients underwent the same tests. **Results:** All of the patients reported an improvement in the symptoms of asthma, as confirmed by a statistically significant increase in FEV_1 from the first to the second visit (mean, 56.1% vs. 88.7% of predicted; $p < 0.05$). Five patients used oral prednisolone for the first five days of the treatment period. Six patients used additional doses of inhaled budesonide/formoterol (mean duration, 2.5 weeks). The EBT decreased significantly from the first to the second visit (mean EBT: 35.1°C vs. 34.1°C; $p < 0.05$). **Conclusions:** Uncontrolled asthma, especially during exacerbations, is followed by an increase in EBT, which decreases after appropriate asthma control, as demonstrated by an increase in FEV_1 and an improvement of the reported symptoms. These preliminary results suggest that EBT can be used as a parameter for the assessment of asthma control.

Keywords: Asthma; Biomarkers, pharmacological; Hydroxycorticosteroids.

Resumo

Objetivo: Avaliar se a temperatura do ar exalado (TAE), medida por um método não invasivo, é efetiva no monitoramento de pacientes com asma não controlada. **Métodos:** Estudo piloto com nove pacientes (sete mulheres e dois homens; média de idade: 39 anos) com diagnóstico de asma por pelo menos um ano e sem uso de tratamento de manutenção por pelo menos três meses antes do início do estudo. Na primeira visita, os pacientes foram submetidos à espirometria e à medida da TAE. Todos os pacientes foram orientados a iniciar tratamento com budesonida/formoterol (200/6 µg) inalatório a cada 12 h por seis semanas. Além disso, os pacientes com asma grave (VEF_1 < 60% do previsto) foram orientados a utilizar prednisolona oral (40 mg/dia) por cinco dias. Após seis semanas, os pacientes foram submetidos aos mesmos testes. **Resultados:** Todos os pacientes relataram melhora dos sintomas de asma; confirmada por um aumento significativo de VEF_1 da primeira para a segunda visita (média de VEF_1: 56,1% vs. 88,7% do previsto; $p < 0,05$). Cinco pacientes utilizaram prednisolona oral, mas somente nos cinco dias iniciais do tratamento. Seis pacientes utilizaram doses extras da medicação inalatória (média de tempo de uso de medicação adicional = 2,5 semanas). Houve uma diminuição significativa da TAE entre os momentos de avaliação (média de TAE: 35,1°C vs. 34,1°C; $p < 0,05$). **Conclusões:** A asma não controlada, sobretudo durante exacerbações, é acompanhada pela elevação da TAE, que se reduz após o controle adequado da asma, demonstrado pela melhora do VEF_1 e dos sintomas referidos. Esses resultados preliminares apontam para o monitoramento da TAE como um parâmetro possível na avaliação do controle da asma.

Descritores: Asma; Biomarcadores farmacológicos; Hidroxicorticosteroides.

* Study carried out in the Allergy, Clinical Immunology, and Rheumatology Section of the Department of Pediatrics, *Universidade Federal de São Paulo/Escola Paulista de Medicina* – UNIFESP/EPM, Federal University of São Paulo/Paulista School of Medicine – São Paulo, Brazil.

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Financial support: None.

Submitted: 9 February 2010. Accepted, after review: 17 August 2010.

Introduction

Cellular infiltration of the mucosa, edema, epithelial damage, exaggerated production of mucus, and bronchospasm are well-known aspects of asthma. In clinical practice, the degree of inflammation, which is an important component of the monitoring of asthma patients, is noninvasively estimated by the symptoms reported by patients and by their recent history.[1] Therefore, patients who have recently experienced asthma attacks and those who complain of nocturnal symptoms, dyspnea, and cough after exercise, as well as those who experience exacerbations caused by everyday stimuli, are classified as having uncontrolled asthma, in accordance with the current guidelines for asthma management, which are based on the most recent revision of the Global Initiative for Asthma (GINA) guidelines.[2] It is reasonable to assume that, in such cases, bronchoscopy with biopsy would show infiltrate characteristic of bronchial inflammation, which can be eosinophilic—in patients with allergic asthma who respond to corticosteroids—or neutrophilic—in patients who do not respond well to corticosteroids and generally present with severe asthma.[3]

There seems to be a direct relationship between bronchial inflammation and the symptoms reported. However, there is a need for objective measurements. There are now many noninvasive methods for measuring inflammatory cells, mediators, or products (in blood, induced sputum, exhaled breath, and exhaled breath condensate).[4-6] However, bronchospasm, which is evaluated by pulmonary function testing (considered the gold standard and widely available), is the only other facet of asthma that can be objectively evaluated.

Recently, a quite simple and yet innovative hypothesis—that inflammation produces one of its cardinal signs (i.e., heat) also in the bronchial lumen—resulted in the publication of two studies that examined the measurement of exhaled breath temperature (EBT). One group of researchers from the United Kingdom[7] and another from Italy[8] noted, through different methods, that the EBT was higher in patients with asthma. We find it interesting that both groups also noted a correlation between EBT and the level of exhaled NO. In 2007, a study employing a new device for measuring EBT (X-halo®; Delmedica Investments Pte Ltd., Singapore) also demonstrated that EBT is higher in patients with uncontrolled asthma.[9] The use of this device has simplified the technique, since the air is exhaled directly into the device, the mechanism of which is the same as that of a thermos and maintains heat in its copper core until the temperature—which tends to suffer less interference from the external environment—stabilizes (Figure 1). The plateau measured also makes it unnecessary to calculate the difference in temperature or the peak temperature.

In the present study, we used the aforementioned device (second generation) to evaluate adolescent and adult patients with asthma before and after asthma control. The treatment given, which consisted of a variable regimen of inhaled corticosteroid and bronchodilator, aimed to control the symptoms effectively until the end of a six-week follow-up period.

Methods

The present study was conducted in the Allergy, Clinical Immunology, and Rheumatology Section of the Department of Pediatrics of the Federal University of São Paulo, in the city of São Paulo, Brazil, and included nine atopic patients aged 10-68 years who were diagnosed with uncontrolled asthma at the initial visit and were able to perform the procedures appropriately.

All of the patients were diagnosed with asthma in accordance with the American Thoracic Society criteria[10] and presented immediate skin reactivity to *Dermatophagoides pteronyssinus* antigen (code 110/2; IPI ASAC Brazil, São Paulo, Brazil). In addition, the patients met the following inclusion criteria:

- not having had respiratory infection in the four weeks prior to the initial visit
- not having cardiovascular disease
- being a nonsmoker
- being able to perform forced expiratory maneuvers, using the device, at each respiratory cycle, for 5-10 min
- being able to perform pulmonary function tests with reproducibility
- not being under treatment with medication at the first evaluation
- having uncontrolled asthma, as confirmed by altered pulmonary function test results

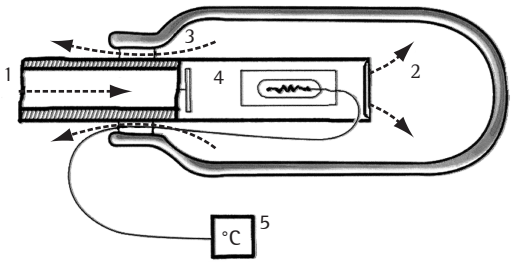

Figure 1 - Simplified schematic illustration showing the X-halo® device—1: air is exhaled into a valve mouthpiece; 2: air enters the chamber; 3: excess air is pushed out of the device; 4: copper tube ("metal heart"); and 5: temperature is recorded until it stabilizes.

The patients were also submitted to specific anamnesis. After the patients had given written informed consent, EBT was measured, and a pulmonary function test was performed with a MicroDL spirometer (Micro Medical Ltd., Kent, United Kingdom) and the program Spida 5 (Micro Medical Ltd.), the patients being in the orthostatic position and using a nose clip. We evaluated FEV_1, which was expressed as the percentage of the predicted value for gender and age in accordance with the guidelines of the Brazilian Thoracic Association.[11] For each pulmonary function measurement, we considered only the best one of the three curves obtained in three consecutive measurements.

Figure 2 - Photograph of the latest-generation X-halo® device with a frontal USB port and interchangeable mouthpiece. The bottom part can be unscrewed for cleaning after each measurement. The mouthpiece glows green when the device is ready for use.

The device used in order to determine EBT consisted of a portable exhaled breath collector, similar to a thermos, into which patients exhaled repeatedly, through a valve mouthpiece, for 5-10 min until the temperature, as measured by a digital thermometer, stabilized. At each new exhalation, the air that was collected in the compartment was expelled and was therefore replaced as the temperature of the central metal tube gradually increased.

After the initial evaluation, the patients were instructed to use budesonide (200 µg) and formoterol (6 µg), administered via the Turbuhaler® system, every 12 h, as well as to repeat the dose whenever symptoms appeared, in accordance with a concept involving the use of this combination of drugs for the relief and control of asthma.[12] In addition, the patients received a diary to record how many doses of oral medication and of the budesonide/formoterol combination they used.

The Wilcoxon test was used in order to compare the data obtained at the two evaluation time points.

Results

Although the device used in the present study is considered easy to use (patients only have to press a button and blow into the mouthpiece), six preselected patients diagnosed with asthma (after anamnesis and pulmonary function testing) were unable to measure EBT successfully. Of those six, five were children (two 7-year-old girls, two 8-year-old boys, ad one 9-year-old boy), and one was a 26-year-old woman. These patients were not included in the study because they were unable to sustain the constant expiratory flow needed in order to increase the temperature progressively until it stabilizes and thus maintain the digital thermometer active. When the temperature increase was not sufficiently rapid, the display showed an error message, indicating a failure (increased resistance to the expiratory flow making it difficult to perform the maneuver, principally for the children). This problem should be corrected in future models of the device. The latest version of the device (Figure 2) has a USB

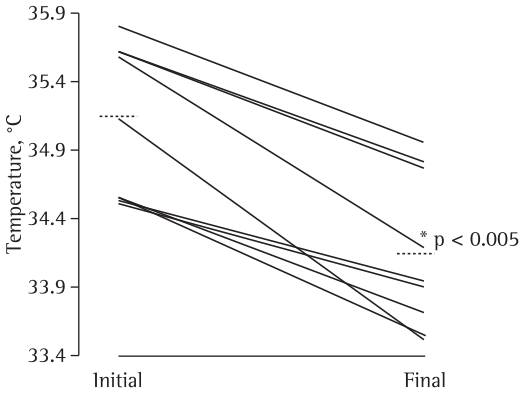

Figure 3 - Graph of the exhaled breath temperature (°C) at the beginning and at the end of the study. Each solid line represents one patient. The dashed line represents the mean value for the group. *Wilcoxon test.

port for computers and allows the visualization of the temperature curve by means of a program designed especially for that purpose.

The nine patients included in the present study were able to measure EBT adequately and completed the protocol. All of the patients had uncontrolled asthma, and five showed FEV_1 values < 60% of predicted, characterizing a severe asthma attack, according to the GINA consensus.[2] As shown in Table 1, these patients were treated with inhaled albuterol (400 µg), improved rapidly, and were instructed to start using oral prednisolone (40 mg/day). None of the patients evaluated had to use a new course of oral corticosteroids after the first five days of monitoring.

In the second visit, six weeks after the first, all of the patients reported an improvement in the symptoms of asthma, as confirmed by a statistically significant increase in the mean FEV_1 from the first to the second visit (56.1% vs. 88.7% of predicted; $p < 0.05$). Conversely, the mean EBT decreased significantly from the first to the second visit (35.1°C vs. 34.1°C; $p < 0.05$). Six patients used additional doses of inhaled budesonide/formoterol (Table 1).

Discussion

Various noninvasive biological markers have been proposed for the evaluation of bronchial inflammation, which is a fundamental aspect of the pathophysiology of asthma. For lack of a universal, practical method (as is pulmonary function testing for the evaluation of bronchospasm), these markers have not been used as a monitoring parameter in routine clinical practice.

Analysis of induced sputum, which has played an increasingly important role in screening protocols, is a safe technique[4] that has great potential for the study of mediators in the bronchial tree. Analysis of induced sputum has been used in order to evaluate mediators such as IL-18, which has thus been shown to be decreased in asthma patients who smoke.[13] This inverse correlation between IL-18 and smoking has yet to be fully elucidated. In parallel with an increase in IL-8, the presence of neutrophils[14] can provide clues to the understanding of refractory asthma. However, analysis of induced

Table 1 - Age, gender, exhaled breath temperature, FEV_1, and use of medication for asthma control.

Patients	Age (years)	Gender	Exhaled breath temperature (°C)		FEV_1 (% of predicted)		Oral medication	Mean number of doses of budesonide+formoterol
			Initial	Final	Initial	Final		
1	12	M	35.58	34.18	72	98	Not used	1 dose every 12 h
2	68	F	34.56	33.71	38	63	Prednisolone (5 days)	1.5 doses every 12 h
3	32	M	35.81	34.95	62	93	Not used	1 dose every 12 h
4	42	F	35.13	33.51	67	98	Not used	1.8 doses every 12 h
5	48	F	34.55	33.54	41	95	Prednisolone (5 days)	1.5 doses every 12 h
6	41	F	34.50	33.91	50	103	Prednisolone (5 days)	1.4 doses every 12 h
7	55	F	34.51	33.90	78	91	Not used	1 dose every 12 h
8	43	F	35.63	34.76	41	84	Prednisolone (5 days)	2.1 doses every 12 h
9	10	F	35.62	34.81	56	73	Prednisolone (5 days)	1.2 doses every 12 h
Mean	-	-	35.10	34.14*	56.1	88.4*	-	-
Median	-	-	35.13	33.91*	56.0	93.0*	-	-

*p < 0.005 vs. initial (Wilcoxon test).

sputum requires specific knowledge and techniques in order to be effectively applied. To date, this test has principally been used for counting eosinophils, which play a central role in allergic inflammation. In a meta-analysis of the use of induced sputum analysis in adults, the technique was shown to be useful in determining the use of drugs for asthma prevention, reducing the frequency of severe events in patients with symptoms that are more severe, that is, in those suspected of having bronchial inflammation that is more severe.[15]

Oxidative stress is intimately associated with the inflammatory process and results from an imbalance between oxidants and antioxidants, particularly when the cell membrane is affected, in a phenomenon designated lipid peroxidation. Malondialdehyde is a by-product of this reaction and can be detected, for instance, in workers with occupational respiratory disease by using another method for quantifying the products of respiration: analysis of exhaled breath condensate.[16] Patients with asbestosis also present with increased inflammatory markers, such as hydrogen peroxide and elevated total protein levels.[17] In this technique, the air that is exhaled undergoes freezing or cooling. The resulting material (condensate) is believed to reflect the characteristics of the epithelial lining fluid and the bronchial mucosa. Exhaled breath condensate has been analyzed in studies of interstitial lung disease, cancer, and acute respiratory failure; however, special attention should be paid to the degree and velocity of cooling of the fluid in exhaled breath, since the measurements can vary.[18] In cases of COPD and asthma, the analysis of exhaled breath condensate allows the evaluation of substances such as prostanoids and eicosanoids. In asthma patients, there are no changes in the levels of prostaglandins $F_{2\alpha}$ and E_2,[19] whereas these levels are elevated in COPD patients.[20] It is possible that the opposite is true for leukotriene E4, elevated levels of which have been reported in asthma patients but not in COPD patients.[19] Regarding 8-isoprostane, which is another oxidative stress marker, studies involving children have produced contradictory results.[21] Although nitrotyrosine and adenosine have also been studied, neither has been shown to be a gold standard marker.[22] It is possible that a "combination of markers"[6] is required in order to differentiate asthma patients from normal individuals (or from those with controlled asthma).

When the air exhaled from the lungs is investigated using methods other than the analysis of exhaled breath condensate, one marker stands out: NO, a molecule that is found in the entire body in immune reactions and in neurotransmission. The NO synthase enzyme induces the production of L-arginine. In the analysis of exhaled NO, serial measurements seem to be more useful than are isolated measurements,[23] since the values of the latter in normal individuals and in those with asthma can overlap, as typically occurs for the two-tailed curves obtained in population-based studies. Numerous studies have investigated the exhaled NO fraction, which is closely related to eosinophilic inflammation.[5] In addition, the use of corticosteroids in COPD and asthma patients[24] is accompanied by a reduction in the exhaled NO fraction, which is not widely evaluated due to the lack of portability and cost of the devices needed in order to measure it.

An increasing number of studies have investigated the analysis of EBT, which is a new, direct, noninvasive method for detecting a biomarker that might be useful in medical practice.[7] In the various tissues, inflammation causes the classic signs of pain, heat, edema, hyperemia, and function loss. At least with regard to increased production of local heat and hyperemia, inflammation (from the Latin *inflammatio*, meaning "to catch fire") also seems to induce equivalent changes in the bronchial walls of patients with asthma, possibly due to vasodilation. In fact, increased bronchial vascularization and blood flow have previously been demonstrated.[25] Although the first studies of the physiology of heat exchange in the respiratory tree date from the 1960s and studies of the evolution of the observation of the differences in the temperature in each bronchial segment—as well as the relationship between these differences and the inhaled breath temperature—date from the 1980s,[26] the first studies involving direct measurements of EBT were not conducted until the beginning of the 21st century.[7,8]

The first studies of exhaled air, which were published in 2002,[7,8] compared the measurements of two markers: temperature and NO. The initial impression of the authors of those studies was

that the new marker was intimately associated with the bronchial inflammatory process. Another group of authors[27] investigated eosinophils in the induced sputum of children who moved to a virtually mite-free environment, the Italian Alps, and lived there for three months. In those children, the number of eosinophils, which play a fundamental role in the development of allergic bronchial inflammation, decreased (as did EBT). This decrease, however, was not statistically significant, perhaps due to the small number of patients (n = 13). Two other studies have demonstrated that asthma control and the consequent decrease in EBT are correlated with a smaller quantity of eosinophils in bronchial secretion.

In the present pilot study, patients who were suffering from asthma attacks and had not been treated previously were evaluated with the objective of improving their condition with the treatment given and demonstrating a possible difference in EBT before and after the treatment, since asthma control indirectly reflects bronchial inflammation.[28] It can be argued that subtle differences in bronchial inflammation (or in cases of patients with a stable profile) will seldom yield statistically significant data in comparisons between patients with and without asthma or between patients with controlled asthma and those with uncontrolled asthma.

We demonstrated that the clinical control of asthma was correlated with a decrease in EBT and, conversely, with an increase in FEV_1. However, as occurs in measurements of exhaled NO, the values overlapped (Figure 3). Therefore, isolated measurements have no clinical application. We can speculate that EBT is a biomarker that is indicated for individual control. In addition, EBT might be useful as an early indicator of loss of asthma control.

A potential application of EBT in the investigation of bronchial remodeling was tested by screening for matrix metalloproteinase 9 (MMP-9) in the induced sputum of 26 children with asthma who were sensitive to dust mites.[29] The authors of that study found that MMP-9, which is an important marker of remodeling, correlated with EBT. It is unknown to what extent this relationship can be considered valid, given that increased bronchial vascularization causes changes in the EBT of asthma patients.

In a study conducted in 2007, a new device was used—the same as that used in the present study—and was shown to be extremely easy to use.[9] The device dispenses with the use of mouthpieces, special sensors, and calculations to determine study points on the EBT increase curve.[30] In order to differentiate patients with asthma from those without, measurement of the EBT plateau, as performed by this device, seems to be more reliable. Another advantage is that the device can maintain the temperature in an isolated chamber, with less interference from the external temperature. Rigorous control of the external temperature was a concern in studies using other methods.[7,8]

Due to the small number of patients investigated in the present study, we still cannot affirm that the measurement of EBT is a valid biological marker for the evaluation of asthma control. However, this measurement might constitute a new line of research that, after further studies, might produce a method to be used in routine clinical practice, especially if the device employed is portable and easy to use.

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