[Original Research]

CHEST

Validation of the Exhaled Breath Temperature Measure

Reference Values in Healthy Subjects

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BACKGROUND: Exhaled breath temperature (EBT) is a new noninvasive method for the study of inflammatory respiratory diseases with a potential to reach clinical practice. However, few studies are available regarding the validation of this method, and they were mainly derived from small, pediatric populations; thus, the range of normal values is not well established. The aim of this study was to measure EBT values in an Italian population of 298 subjects (mean age, 45.2 ± 15.5 years; 143 male subjects; FEV_1, 97.2% ± 5.8%; FVC, 98.4% ± 3.9%) selected from 867 adult volunteers to define reference values in healthy subjects and to analyze the influence of individual and external variables on this parameter.

METHODS: EBT was measured with an X-halo PRO device to different ambient temperature ranging from 0°C to 38°C.

RESULTS: We report reference values of EBT in healthy white subjects who had never smoked. EBT values were strongly influenced by the external temperature and to a lesser extent according to sex.

CONCLUSIONS: In a large population of healthy subjects who never smoked, these data provide reference values for measuring EBT as a basis for future studies. Our results are contribute to the promotion of EBT from "bench" to "bedside." CHEST 2016; ■(■):■-■

KEY WORDS: airways inflammation; correction factor; exhaled breath temperature; noninvasive methods; reference value; validation

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ABBREVIATION: EBT = exhaled breath temperature

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Exhaled breath temperature (EBT) is a new noninvasive method for the analysis of airway inflammation. Because calor (heat) is listed as one of the cardinal signs of inflammation, the measure of EBT was developed as a marker of airway inflammation and therefore used in the study of inflammatory respiratory diseases. Several studies have been performed in asthma,[1-3] COPD,[4] lung cancer,[5] and cystic fibrosis.[6]

A new third-generation thermometer, the X-halo PRO (Delmedica Investments), is now commercially available for measuring EBT. The smaller version of X-halo is currently being developed for personal daily use as an "inflammometer."[7] The use of EBT devices is particularly attractive in patients with asthma who largely exhibit significantly higher EBT values compared with healthy subjects; these patients are therefore encouraged to use these exhaled thermometers in clinical practice for the maintenance of asthma control and in therapeutic management.

There are several potential advantages of EBT measurements with the X-halo device. Measurement is very easy for patients and requires only a few minutes; it is completely noninvasive and is therefore also suitable for children and patients with severe disease. The device is well accepted by patients and ethics committee, it is inexpensive, and it does not affect the underlying airway disease. Despite the potential of EBT, it has not yet reached the clinical setting, partly because of a lack of standardization and validation of the method.

Popov et al[7] evaluated the influence of room temperature, atmospheric pressure, and relative humidity on EBT measurements with X-halo in a group of healthy subjects and patients with asthma. They showed the reproducibility of EBT measurements. Vermeulen et al[8] confirmed the reproducibility of EBT in a group of 124 healthy children and children with asthma.

Some important data are available on the standardization and validation of EBT measurements necessary to advance the clinical applicability of this tool as a potential noninvasive marker of airway inflammation.[9] To validate EBT measurements, it is important to follow the validation used for other noninvasive methods, such as induced sputum and exhaled nitric oxide, which have earned a place in the routine management of asthma and COPD and are now included in guidelines.[2]

Data from previous studies on thousands of subjects have shown that EBT is a safe and noninvasive method. No contraindications exist to its measurement, and no adverse events have been reported. As for standardization of EBT measurements, the Delmedica Investments manual for users has some recommendations for its use and for cleaning of the device (http://www.x-halo.com). For validation of EBT measurements, several factors need to be addressed: (1) the reproducibility of the measurement; (2) comparison with other biomarkers; (3) differences between measurements in diseased and healthy subjects; (4) sensitivity to risk factors or treatment; and (5) reference normal values. Several studies reported good reproducibility of EBT measurements.[7,8] Some comparitive studies between EBT, exhaled nitric oxide,[8] and induced sputum have been previously reported.[8,10] Differences in EBT measurements in healthy subjects and patients with asthma,[1-3] COPD,[4] cystic fibrosis,[6] and lung cancer[5] have been reported. However, these studies included only small numbers of subjects; reference values in the general population are therefore not well defined. In addition, there are few studies assessing how the measurement may be changed physiologically with exercise and ventilation or with inhaled therapy.[11,12]

The present study was designed to measure EBT in a large population of healthy, never smoking adults to provide reference values for the healthy population. An additional goal was to analyze the influence of external variables on EBT measurements.

Subjects and Methods

Population

A total of 867 consecutive white Italian volunteers (mean age, 50.8 ± 16.3 years; 60% male; BMI, 27.11 ± 5.2 kg/m^2; 59% smokers; FEV$_1$, 89.3% ± 8.4%; FVC, 90.7% ± 4.8%; 90% living in industrialized areas and 10% in rural areas) were enrolled during public regional meetings ("Breath Days," Puglia, Italy) in shopping centers. The study was approved by the institutional ethics committee of the University of Foggia (institutional review board approval number 17/CE/2014). All subjects were informed of the purpose of breath temperature measurement and, after signing the informed consent form, anthropometric, physiological, and clinical data were collected at the time of recruitment; these data included date of birth, height, weight, sex, area of residence, work, hobbies, diagnosed diseases, drugs, smoking history, blood pressure, blood oxygen saturation, lung function (with Pony FX; Cosmed), axillary body temperature, and EBT measurement. All subjects with any respiratory, cardiac, or other diseases, as well as all smokers and former smokers, were excluded to obtain a new population of 298 healthy, never smoking subjects (Table 1).

EBT Measurement

EBT was measured with an X-halo PRO device (Delmedica Investments) according to previously validated methods.[13,14] Briefly, patients were requested to inhale freely through the nose and to exhale into the device at a rate and depth typical of their normal tidal-breathing rhythm.

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Ambient Temperature Measurement

In consideration that EBT measurements were conducted in three different places (outside, in-hospital, and at a shopping center) and that only when in a hospital is the temperature usually controlled (between 19°C and 24°C), we decided to measure and register the ambient temperature before each EBT measurement. An external thermometer (PCE-HT 110; PCE Instruments) with a range of measure of 0°C to 50°C was used, with precision variability of ±0.8°C and resolution of 0.1°C. EBT was measured to different ambient temperatures that ranged from 0°C to 38°C.

Statistical Analysis

To determine the variables that have influenced EBT measurements, a classification tree called E-CHAID was used; this automatic classification algorithm imposes a tree-like structure of the data. At each split, the algorithm looks for the predictor variable that, if split, most "explains" the category response variable. To decide whether to create a particular split based on this variable, the E-CHAID algorithm tests a hypothesis regarding dependence between the split variable and the categorical response (using the χ^2 test for independence). Using a prespecified significance level, if the test shows that the split variable and the response are independent, the algorithm stops the tree growth. Otherwise, the split is created, and the next- best split is searched. Using the result obtained by the classification tree, we estimated a regression model to study the relationship between external temperature and EBT. The level of significance was set at $P < .05$.

Results

Population

A total of 867 subject volunteers were enrolled consecutively and successively divided on the basis of the presence or absence of respiratory or other diseases and smoking habit to obtain 298 healthy, never smoking subjects. Anthropometric, functional, and clinical data of the healthy subjects enrolled are summarized in Table 1.

EBT in Healthy, Nonsmoking Subjects

The E-CHAID tree estimated by using the 298 healthy subjects found that the reference value of EBT in healthy, white, nonsmoking subjects was 30.459°C ± 2.955°C (Fig 1). External temperature was the main factor influencing EBT ($F = 58.991$; $df1 = 2$; $df2 = 295$; $P < .00001$).

Three groups of external temperatures influenced EBT in different ways. The first group considered the cases with external temperature ≤ 23°C. In this case, the average EBT was 28.268°C ± 2.872°C. The second group considered cases measured with an external temperature of 23°C to 28°C. In this case, EBT was 30.949°C ± 2.511°C. The third group showed that if the test is performed with an external temperature > 28°C, the EBT was 32.558°C ± 1.805°C.

The third level of the tree shows that sex influenced EBT for the first two groups. The female EBT was 1.486°C and 0.915°C lower, respectively, than the male EBT. Other factors analyzed, such as age, BMI, blood pressure, internal temperature, lung function, and area of residence, had an influence but did not reach statistical significance in the EBT.

According to the result of the segmentation tree, we estimated the following regression model:

$$EBT = \alpha + \beta \text{ external temperature} + \varepsilon$$

with $\alpha = 26.40$ and $\beta = 0.19$, with $E[\varepsilon] = 0$ and $VAR[\varepsilon] = 1$whit $R^2 = 0.2$, $F = 175.4$. The model shows that EBT was influenced by the external temperature. On average, when the external temperature increased 1°C,

TABLE 1] Anthropometric and Functional Data of Healthy, Neversmoking Subjects (N = 298)

Variable	Value
Age, y	
Median	48
Interquartile range	Q1 = 39, Q3 = 70
Male	143 (48%)
BMI, kg/m^2	
Median	27.7
Interquartile range	Q1 = 24.6, Q3 = 31.2
Blood pressure, mm Hg	
Median	120/79
Interquartile range	Q1 = 115, Q3 = 121.25 (pressure maximum); Q1 = 73.75, Q3 = 81.25 (pressure minimum)
FEV_1, % predicted	
Median	97.5
Interquartile range	Q1 = 95.75, Q3 = 100.25
FVC, % predicted	
Median	98
Interquartile range	Q1 = 96, Q3 = 100.75
FEV_1/FVC ratio, median	97.7
Sao_2, %	
Median	99
Interquartile range	Q1 = 98, Q3 = 99.5
Area of residence	
Rural	28 (9%)
Industrialized	270 (91%)

Q = quarter; Sao_2 = arterial oxygen saturation.

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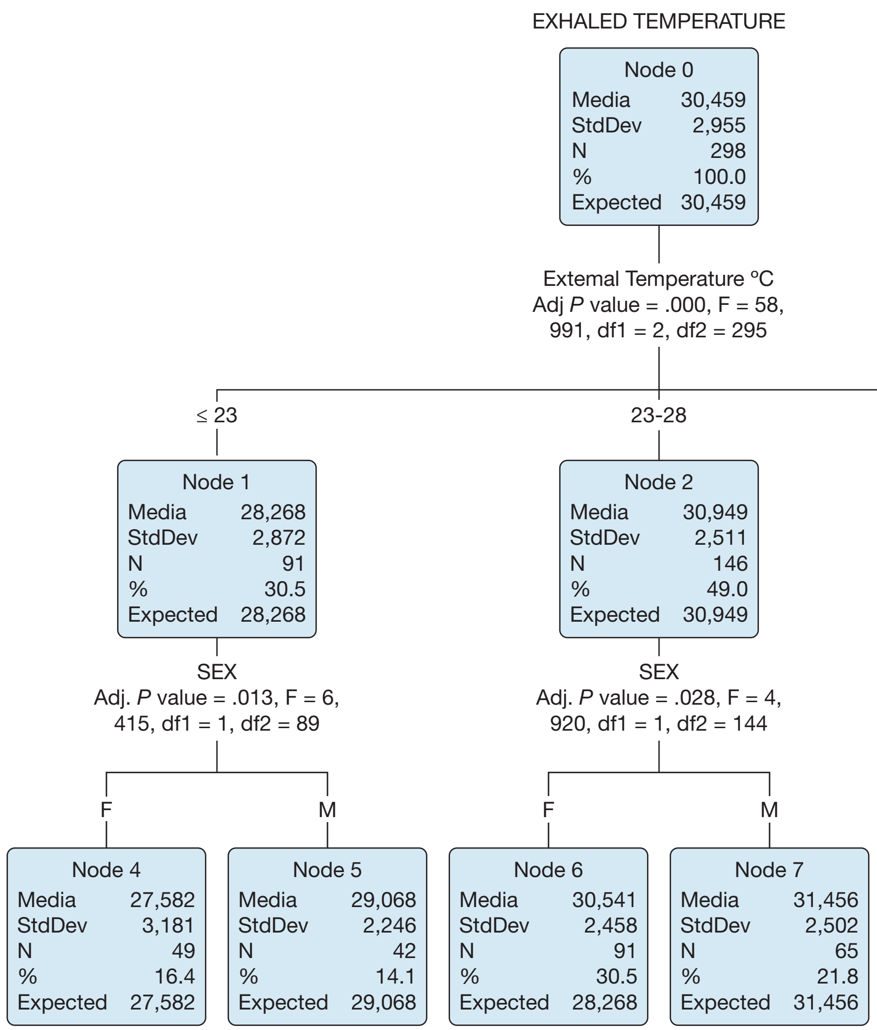

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Figure 1 – *Classification tree for 298 healthy subjects. F = female; M = male.*

the EBT increased 0.19°C. Thus, it is important to measure the external temperature and, if necessary, to apply a correction factor to the results obtained. Using this model, the reference value of EBT in healthy nonsmoker subjects was 30.66°C at 22°C of external temperature, which is common in hospital rooms. At 24°C, the value was 31.4°C; at 26°C, it was 31.40°C; and at 28°C, it was 31.8°C.

No adverse events were reported during or after measurement of EBT.

Discussion

The present study reports reference values for EBT in 298 healthy, never smoking subjects. We found that EBT was strongly influenced by the external temperature and less by sex.

Almost 200 articles are currently available in the literature on the use of EBT in asthma and other respiratory diseases. However, few of these studies have assessed EBT measurements in healthy subjects, which are needed if this noninvasive technique is to be useful in the clinic or for measurement of airway inflammation. There are no studies providing reliable reference values of EBT in healthy subjects. For this reason, the main aim of our study was to measure EBT in a large normal population to define the reference EBT values in healthy, never smoking subjects. We enrolled 867 apparently healthy volunteers who agreed to participate in this study. From this population, all subjects with respiratory, cardiac, or other diseases and all smokers or ex-smokers were excluded, thus yielding a selected population of 298 healthy subjects to be used for analysis. From this large healthy population, which is a strength of the study, we report a reference value for EBT that researchers and clinicians might use to identify subjects with increased EBT that could signify inflammation.

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A second aim of this validation study was to explore the influence of ambient temperature and anthropometric, physiological, and clinical variables (age, sex, height, weight, blood pressure, axillary temperature, and lung function) on EBT values. Popov et al[7] first addressed some of these issues but in small numbers of patients. In contrast to Popov et al, we found, in this large population of 298 healthy subjects, that external (ambient) temperature strongly influenced EBT values. In our daily practice, we had noted that EBT, when measured in ambient temperatures up to 28°C, was significantly higher, and we therefore decided to perform our measurements at various ambient temperatures ranging from 0°C to 38°C. The study was performed not only in hospital wards but also outside during public exhibitions and events in shopping centers. As expected, the ambient temperature strongly influenced the EBT, especially at < 20°C and > 28°C. This testing of EBT to the high external temperature found in southern Italy could be the reason for our discordant data compared with those of Popov et al.[7] EBT measurements are usually performed in the hospital, where the temperature is normally controlled between 19°C and 24°C. In addition, the instructions of Delmedica Investments for using the X-halo device suggest measuring EBT between 19°C and 24°C assuming no influence of the external temperature on EBT values. However, there are wards in which the temperature is not automatically adjusted where wider variations in ambient air temperature are frequent. Furthermore, if EBT is measured at home, greater temperature variations may occur. Although the measurement of EBT in controlled ambient temperature is the best option, we report a correction factor for ambient temperature that can normalize, in any situation, the EBT measurement. This correction factor calculated with a complex statistical method is easy to apply and in its simplest form, operators might increase the EBT value measured of 0.19°C for each degree of external temperature above and below 22°C. We believe that this correction factor is very important for the promotion of EBT from a research setting to clinical practice.

Another factor that influenced EBT in our analysis was sex. Male subjects seemed to have a higher EBT compared with female subjects, especially in the group with an external temperature of 23°C and in the group with an external temperature of 23°C to 28°C. Higher external temperature made sex less important in influencing EBT values. We suggest that higher blood pressure and cardiac diseases, which are usual in male subjects compared with female subjects, may influence EBT values. Another factor that may play an important role in sex differences is the sex hormones, with their selective inflammatory or antiinflammatory actions. The female sex hormones (estradiol and progesterone) in particular have been shown to have an antiinflammatory effect that modulates cellular and immune responses, which might consequently influence basal EBT in female subjects, resulting in lower values compared with those of male subjects.[15] Crespo Lessmann et al[10] previously also reported differences in EBT according to sex.

In agreement with the data from the studies of Popov et al,[7] we found no influence by other variables, such as height, weight, area of residence, work, blood pressure, blood oxygen saturation, lung function, and axillary temperature, on EBT. However, Bijnens et al[9] previously reported a strong relationship between EBT and factors such as age, BMI, and residential proximity that in our study did not significantly influence the segmentation tree of the EBT.

Conclusions

For the first time, we report reference values of EBT in a large population of healthy, never smoking subjects. We also found that EBT was strongly influenced by external temperature, and we therefore built a correction factor for EBT values in consideration of external temperature. Given the importance that EBT could obtain in the study of respiratory diseases and of the role that it could gain in clinical practice, further studies in this field are needed.

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