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ORIGINAL RESEARCH

Exhaled Breath Temperature as a Novel Marker of Future Development of COPD: Results of a Follow-Up Study in Smokers

Marina Labor[a,e], Žarko Vrbica[b,c], Ivan Gudelj[d], Slavica Labor[a,e], Iva Jurić[f], and Davor Plavec[e,g]

[a]Department of Pulmonology, University Hospital Center Osijek, Osijek, Croatia; [b]Department of Pulmonology and Immunology, General Hospital Dubrovnik, Dubrovnik, Croatia; [c]University of Dubrovnik, Dubrovnik, Croatia; [d]Department of Pulmonology, University Hospital Center Split, Split, Croatia; [e]Faculty of Medicine, J.J. Strossmayer University of Osijek, Osijek, Croatia; [f]Department of Cardiology, University Hospital Center Osijek, Osijek, Croatia; [g]Research Department, Children's Hospital Srebrnjak, Zagreb, Croatia.

ABSTRACT

Although only less than one-third of smokers develop COPD, early marker(s) of COPD development are lacking. The aim of this research was to assess the ability of an average equilibrium exhaled breath temperature (EBT) in identifying susceptibility to cigarette smoke so as to predict COPD development in smokers at risk. The study was a part of a multicenter prospective cohort study in current smokers (N = 140, both sexes, 40–65 years, ≥20 pack-years) with no prior diagnosis of COPD. Diagnostic workup includes history, physical, quality of life, hematology and highly sensitive CRP, EBT before and after smoking a cigarette, lung function with bronchodilator test, and 6-minute walk test. Patients without a diagnosis of COPD and in GOLD 1 stage at initial assessment were reassessed after 2 years. COPD was additionally diagnosed based on lower level of normal (LLN) lung function criteria. Utility of EBT for disease progression was analyzed using receiver operator curve (ROC) and logistic regression analyses. Change in EBT after smoking a cigarette at initial visit (ΔEBT) was significantly predictive for disease progression (newly diagnosed COPD; newly diagnosed COPD + severity progression) after 2 years ($p < 0.05$ for both). ΔEBT had an AUC of 0.859 ($p = 0.011$) with sensitivity of 66.7% and specificity of 98.1% for newly diagnosed COPD using LLN criteria. We conclude that EBT shows potential for predicting the future development of COPD in current smokers. This was best seen using LLN to diagnose COPD, adding further evidence to question the use of GOLD criteria for diagnosing COPD.

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Introduction

Although we understand that cigarette smoke is a major environmental risk factor involved in the development of chronic obstructive pulmonary disease (COPD), we still have not unveiled the epigenetic regulatory mechanisms of oxidative genes involved in its pathogenesis (1, 2). Only less than one-third of smokers develop symptomatic disorder (primarily COPD) during lifetime, suggesting that there is an individual genetic or epigenetic background, making them susceptible to cigarette smoke (2). Progressive nature of COPD inflicts significant disability and later also an early mortality, thus producing serious public health and economic impact for the health system and economy in general. Therefore, stopping or slowing down the progression of the disease is a main therapeutic goal (3). Therapeutic interventions in COPD patients have been shown to have significantly larger impact if they were started earlier in the course of the disease (4–6). An early diagnosis should allow an early intervention, thereby preventing the progression of COPD, alleviating the symptoms, improving the tolerance of exertion and general wellbeing, preventing complications and co-morbidities and early mortality (7). Finding a marker of susceptibility to cigarette smoke that can predict the future development of COPD, before the significant end-organ damage, is still an unmet need in the management of COPD. Despite the significant efforts of the scientific community during the last decade in trying to find such markers for COPD, we are still far from that goal. MARKO project (https://clinicaltrials.gov/ct2/show/NCT01550679) was started with this aim by recruiting patients with no previous diagnosis of COPD, but at risk for future development of disease, having significant age and cigarette smoke exposure (smokers/ex-smokers aged 40–65 years with smoking history of ≥20 pack-years).

There are significant pathohistological changes of the airways associated with the duration and progression of COPD, and depending on the phenotype, a significant decrease in bronchial vascularity can be found (8–11). On the other hand, it has been shown that chronic inflammation of the airways induces vascular proliferation, as also seen in some COPD phenotypes (8). As a consequence of inflammation and remodeling/destruction of the airways/parenchyma, changes in the bronchial blood flow affect the heat and water vapor exchange in the airways, thereby affecting the exhaled breath temperature (EBT). Changes, such as the growth of submucosal capillary network, medial and intimal hyperplasia and/or exudation, can directly influence the airway wall thickness, thus influencing the heat exchange (12). It has been shown that EBT can be used as an individual measure of fluctuating changes in the balance of these processes (13). Recently, EBT has been proposed to reflect airways

CONTACT Davor Plavec plavec@bolnica-srebrnjak.hr Head of Research Department, Children's Hospital Srebrnjak, Srebrnjak 100, Zagreb, Croatia.
Marina Labor and Žarko Vrbica are equally contributing first authors.

inflammation, as a positive relationship was observed between EBT, bronchial blood flow and exhaled nitric oxide in asthmatic patients (14, 15). Also, it has been suggested as a new method to detect and monitor pathological processes in asthma, COPD and lung cancer (16).

Based on the pathophysiological changes in COPD and also previous studies showing significant change in EBT in patients with inflammatory respiratory disorders (including COPD), this study aimed at testing if measuring EBT in current smokers at COPD risk can show susceptibility to cigarette smoke and be predictive for future development of COPD and/or disease progression.

Methods

Study framework

This study is a part of the broader research project (Early detection of COPD patients in GOLD 0 (smokers) population – MARKO project). The whole protocol of the MARKO project can be found at https://clinicaltrials.gov/ct2/show/NCT01550679. In short, the MARKO project is a prospective, observational, non-interventional cohort study of patients (both sexes) at risk for the development of COPD (smokers/ex-smokers with a smoking history of ≥20 pack-years), without a previous diagnosis of COPD. The project was approved by Local Ethics Committees and carried out in accordance with the Declaration of Helsinki, GCP, and all relevant international and national legislation. The patients were approached by their general practitioners (GPs) during any (unrelated to respiratory problems) visit to GP's office if they were smokers or ex-smokers of the predefined age group for the study, together with the prescreening for inclusion/exclusion criteria using a structured interview. Eligible patients were given the informed consent document with enough time to read it and to discuss any relevant issues regarding the study before consenting. All participants signed a written consent before entering the study and before any procedure was performed.

Subjects

For this study, 146 consecutive patients from 26 GP offices were recruited into the study by their GPs based on the inclusion criteria: written consent; current smokers (because they had to smoke a cigarette as a part of a study protocol) of both sexes aged 40–65 years with a smoking history of at least 20 pack-years (calculated as a number of cigarettes smoked per day multiplied by the number of years of smoking divided by 20); and no previous diagnosis of COPD. Exclusion criteria were: any clinically relevant chronic disease (cardiovascular, cerebrovascular, diabetes, hepatitis, nephropathy, chronic dialysis, systemic disorder, cancer) significantly affecting QoL; ongoing immunosuppressive therapy; preceding acute respiratory disease 4 weeks before inclusion; hospitalization for any reason during past 3 months; myocardial infarction (MI), cerebrovascular infarction (CVI) or transient ischemic attack (TIA) during past 6 months; diagnosis of asthma; and an inability to perform the diagnostic protocol. Six patients were excluded from the analyses because their baseline EBT (EBTb) values were extremely low (<25°C), suggesting possible artifacts in the measurement so the data from 140 subjects were analyzed.

Study workup

After the structured history and examination at the GP's office and lung function testing with COPD-6 (4000 COPD-6^{TM} Respiratory Monitor, Vitalograph Ltd., Buckingham, UK), patients were referred to the tertiary care hospital having a designated team consisting of a pulmonologist, research nurse and lung function laboratory technician. A structured diagnostic workup comprising QoL questionnaires, structured history and physical examination, EBT before (EBTb) and after smoking cigarette (EBTc), lung function testing with bronchodilator (salbutamol), laboratory [hematology and high-sensitivity C-reactive protein (hs-CRP)], functional status using 6-minute walk test (6MWT), ending with the assessment for diagnosis and severity of COPD according to GOLD (17) was performed. Patients with no COPD and with COPD GOLD 1 stage [postbronchodilator (PB) ratio of forced expiratory volume in 1 second and forced vital capacity (FEV_1/FVC) < 0.7 and FEV_1 ≥ 80% predicted] after initial assessment were included in the follow-up in accordance with a predefined study protocol. These patients were reassessed by the same pulmonologist after 2 years for diagnosis and progression of COPD. In the case of acute respiratory disease, preceding or at the time of a follow-up visit, subjects were rescheduled to present at least 4 weeks after the resolution of this acute episode.

Outcomes

Primary outcome was to assess the predictive potential of the change in EBT after smoking a cigarette (ΔEBT) using three different outcome measures for the progression of disease after 2 years of follow-up in patients at risk (active smokers with smoking history of ≥20 pack-years without COPD or with a COPD in GOLD 1 stage after baseline assessment): (1) newly diagnosed COPD (ND COPD); (2) disease progression (DP; ND COPD + progression to a higher severity stage); and (3) higher rate of loss of lung function (LoLF).

Secondary outcomes were to assess the predictive potential of (1) baseline EBT (EBTb) and (2) EBT after smoking cigarette (EBTc) using three outcome measures of DP after 2 years of follow-up in patients at risk (as for the primary outcome).

Exploratory outcome was to assess the associations of outcome measures with EBT and patients' baseline characteristics [age, sex, smoking status, comorbidities, lung function, functional exercise capacity, laboratory parameters, and health-related QoL (HRQoL)].

Outcome measures (ND COPD, DP) were defined using the definition of COPD and COPD severity criteria in GOLD (17); (1) ND COPD – subjects with chronic respiratory symptoms (dyspnea, cough or sputum), a history of exposure to risk factors and a persistent airflow limitation (PB FEV_1/FVC < 0.7) at a control visit after 2 years if they were not diagnosed according to the same criteria at baseline visit; (2) DP – subjects with ND COPD + subjects that progressed from GOLD 1 severity stage at baseline visit to GOLD 2 or higher severity stage based on the % predicted value of FEV_1 at a control visit after 2 years. To assess if the progression of airflow limitation (PB FEV_1/FVC

going below 0.7 or PB FEV_1 going below 50% predicted after 2 years) was just the function of age and not the disease, more stringent criteria for airflow limitation using a lower level of normal (LLN) proposed by Global Lung Initiative (GLI) and American Thoracic Society/European Respiratory Society (ATS/ERS) were also used for both outcome measures (18, 19). For ND COPD, the criterion was PB FEV_1/FVC going below LLN after 2 years, and for DP it was PB FEV_1/FVC going below LLN or PB FEV_1 going below LLN (if FEV_1/FVC was below LLN at a baseline visit) after 2 years.

Outcome measure LoLF was defined not only as a loss of PB $FEV_1 > 70$ mL/year but also as a loss in FEV_1 defined as > 1 standardized residual (SR) units after 2 years of follow-up to correct the flaw coming from using % predicted values that retain sex, age and height bias (18, 20).

Health-related quality of life

HRQoL was assessed using two questionnaires; St. George Respiratory Questionnaire (SGRQ) and COPD Assessment Test (CAT). The SGRQ is a standardized self-administered airways disease-specific questionnaire divided into three subscales: symptoms (8 items), activity (16 items), and impacts (26 items). SGRQ scores were calculated using score calculation algorithms and missing data imputation (if total number of missing items was ≤ 10) using the Excel® SGRQ calculator. For each subscale and for the overall questionnaire, the scores range from zero (no impairment) to 100 (maximum impairment) (21). The CAT is a validated, short (8-item) and simple patient completed questionnaire, with good discriminant properties, developed for use in routine clinical practice to measure the health status of patients with COPD. Every item has a scale of 0–5 with scoring range from zero (no impairment) to 40 (maximum impairment) (22). Both QoL questionnaires were self-completed by patients before any other procedure was done.

Lung function

Spirometry was done by computerized pneumotachographs (Jaeger®, CareFusion, CA, USA) using the same procedure at all clinical sites (lung function labs at tertiary hospitals), harmonizing the procedure according to ATS/ERS guidelines (23). The best of three technically satisfactory efforts was recorded. Bronchodilator test was done with the repeated spirometry 20 minutes after the inhalation of 400 mcg of salbutamol using the inhalation chamber. Spirometric indexes [FVC, FEV_1, FEV_1/FVC, peak expiratory flow (PEF)] at baseline and after bronchodilator (PB) were recorded as absolute values and as percentage of predicted values according to Quanjer (18). The values were also expressed as SRs and an LLN was calculated for FEV_1 and FEV_1/FVC according to GLI (18, 24).

Exhaled breath temperature

EBT was measured using X-Halo® device (Delmedica Investments, Singapore) according to the previously validated method (25, 26). The device does not measure an instantaneous EBT but an average equilibrium temperature. Measurements using this device and type of breathing, compared to other methods, did not show the influence of environmental factors and lung volume on the measured EBT (14, 27). Patients were requested to inhale freely through their nose and to exhale through mouth into the device at a rate and depth typical of their normal tidal breathing pattern using a one-way valve (preventing the rebreathing through device) and were closely monitored by the staff during the measurement. The measurement was continued until the software of the instrument indicated that the measured value was stable, thus fulfilling the criteria of a previously described mathematical model; the instrument processed an incremental temperature curve in relation to the initial temperature of the air in the thermal chamber of the instrument and was able to capture the achievement of the temperature plateau within an error of $< 2\%$. The reproducibility of the EBT measurements performed by X-Halo® had been previously demonstrated to have an intraclass correlation coefficient of 0.99 (14). The tests were carried out at a room temperature of 19–25°C, and at relative humidity of 30–60% in the lung function lab where the atmosphere is controlled, measured and logged. If the measured EBT was out of the expected range (30–35°C) the measurement was repeated after the temperature of X-Halo® device decreased to room temperature. In such a case, the measurement was repeated (no more than 3 times) until 2 repeatable measurements were obtained (difference < 0.1°C) and an average of the two was recorded. Patients who had an EBT < 25°C, even after repeated measurements, were excluded from analysis ($n = 6$). EBT was measured twice on the same occasion (during the initial visit); (1) baseline measurement before any other procedure (lung function, bronchodilator test, 6MWT) at least 1 h after smoking cigarette (EBTb) and (2) 15 minutes after smoking cigarette (EBTc) and recorded with a precision of 1/100 of 1°C. Subjects also avoided food, beverages and medicine consumption at least 2 hour before measurement. No other procedure except cigarette smoking was performed between two EBT measurements. The change in EBT after smoking cigarette (ΔEBT) was calculated as the absolute difference between EBTc and EBTb ($\Delta EBT = EBTc - EBTb$).

Other procedures

Laboratory measurements were conducted in local clinical laboratories and included complete blood cell count, white blood cells differential count, hematocrit, hemoglobin and hs-CRP.

Functional exercise capacity was assessed using 6MWT according to the ATS guidelines and expressed as walked distance in meters and as % predicted according to Trooster et al. (28, 29).

Data analysis

Data analysis was done using STATISTICA version 12 (StatSoft, Inc., OK, USA) and MedCalc Statistical Software version 15.8 (MedCalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2015). Categorical data was presented as absolute and relative (%) numbers. Continuous variables were presented as mean and standard deviations (SD). Categorical data was

Table 1. Characteristics of patients at initial visit according to sex (N = 140).

Variable*	All (N = 140)	Women (n = 76)	Men (n = 64)	Statistics†
Age (years)	53.0 ± 6.4	53.5 ± 5.9	52.4 ± 6.9	$t = 1.042$; $p = 0.299$
BMI (kg/m^2)	26.58 ± 3.97	25.89 ± 3.97	27.40 ± 3.83	***t* = 2.274; *p* = 0.025**
Pack-years	38.5 ± 17.9	33.2 ± 15.0	44.7 ± 19.3	***t* = 3.970; *p* < 0.001**
FVC (%Pred, SR)	111.38 ± 15.41, 0.84 ± 1.14	113.35 ± 16.54, 0.96 ± 1.18	108.93 ± 13.63, 0.70 ± 1.07	$t = 1.870$; $p = 0.064$, $t = 1.370$; $p = 0.173$
FEV_1 (%Pred, SR)	100.42 ± 13.60, 0.03 ± 0.96	100.54 ± 14.12, 0.04 ± 0.98	100.27 ± 13.04, 0.03 ± 0.95	$t = 0.116$; $p = 0.908$, $t = 0.061$; $p = 0.951$
FEV_1/FVC (ratio, SR)	0.749 ± 0.064, −0.51 ± 0.94	0.759 ± 0.060, −0.47 ± 0.97	0.738 ± 0.066, −0.55 ± 0.91	$t = 1.918$; $p = 0.057$, $t = 0.492$; $p = 0.623$
PB FVC (%Pred, SR)	109.94 ± 14.51, 0.74 ± 1.07	111.92 ± 15.03, 0.83 ± 1.07	107.54 ± 13.60, 0.63 ± 1.06	$t = 1.773$; $p = 0.078$, $t = 1.113$; $p = 0.268$
PB FEV_1 (%Pred, SR)	101.24 ± 13.96, 0.09 ± 0.97	101.12 ± 14.38, 0.08 ± 0.98	101.38 ± 13.54, 0.10 ± 0.97	$t = 0.104$; $p = 0.918$, $t = 0.128$; $p = 0.898$
PB FEV_1/FVC (ratio, SR)	0.763 ± 0.062, −0.28 ± 0.91	0.771 ± 0.054, −0.25 ± 0.88	0.753 ± 0.068, −0.32 ± 0.95	$t = 1.661$; $p = 0.099$, $t = 0.398$; $p = 0.691$
ΔPB FEV_1 (%)	1.28 ± 4.99	1.04 ± 3.59	1.54 ± 6.19	$t = 0.477$; $p = 0.635$
6MWT (%Pred)	64.25 ± 13.49	67.44 ± 11.31	60.39 ± 14.95	***t* = 2.878; *p* = 0.005**
EBTb (°C)	33.33 ± 2.30	32.94 ± 2.67	33.80 ± 1.67	$Z = 1.108$; $p = 0.268$
EBTc (°C)	33.22 ± 2.10	32.79 ± 2.56	33.81 ± 0.96	$Z = 1.207$; $p = 0.227$
ΔEBT (°C)	− 0.07 ± 1.42	− 0.13 ± 1.45	0.01 ± 1.40	$Z = 0.755$; $p = 0.755$

Legend: *all values are presented as mean ± SD; †statistical significance was tested using Student's *t*-test (*t* value) or Mann-Whitney U-test (*Z* value); BMI – body mass index (calculated as weight in kg divided by the squared height in m); FVC – forced vital capacity as % predicted (%Pred) or as standardized residuals (SR); FEV_1 - forced expiratory volume in 1 second as % predicted (%Pred) or as standardized residuals (SR); FEV_1/FVC as ratio or as standardized residuals (SR); PB – postbronchodilator value; Δ – % change after bronchodilator; 6MWT – 6-minute walk test as % predicted (%Pred); EBT – exhaled breath temperature; EBTb – baseline EBT; EBTc – EBT after smoking cigarette; ΔEBT – change in EBT after smoking cigarette.

compared between subgroups using chi-square test and continuous variables using Student's *t*-test or Mann-Whitney U-test, analysis of variance (ANOVA) or Kruskal-Wallis ANOVA after assessing the criteria for the use of parametric tests. Utility of EBT (EBTb, EBTc and ΔEBT) for DP was analyzed using receiver operator curve (ROC) analysis, and data was presented as AUC (with 95% confidence intervals, CIs) together with associated criterion, sensitivity, specificity and positive (PPV) and negative predictive (NPV) values. Association of outcome measures with EBT and baseline characteristics was tested using logistic regression analysis using stepwise approach. $P < 0.05$ was considered statistically significant for all analyses.

Results

Description of the cohort

One hundred and forty patients (76 women) aged 40–65 years at entry were included in this cohort study. Women had a mean (SD) age of 53.5 (5.9) years and men had a mean age of 52.4 (6.9) years ($p = 0.419$) with a mean smoking history of 33.2 (15.0) years and 44.7 (19.3) pack-years—men having a significantly greater cumulative exposure ($t = 3.970$; $p < 0.001$). The only functional index significantly different between sexes was 6MWT expressed as the % of predicted values significantly lower in men (mean ± SD; 60.39 ± 14.95% vs. 67.44 ± 11.31%, $t = 2.878$; $p = 0.005$), and no significant difference was found for lung function or EBT ($p > 0.05$ for all, Table 1). No significant association was found between EBTb and ΔEBT with age (Spearman R = −0.143 and −0.069, $p = 0.092$ and $p = 0.448$, respectively; Figures 1 and 2) and with lung function indexes (baseline and postbronchodilator) in a univariate analysis ($p > 0.05$ for all). Seventy-one (50.7%) patients had 1–3 co-morbidities and 62 (44.3%) were using 1–4 medications as a chronic treatment. After the initial pulmonologist assessment, 81 (57.9%) patients could be classified as symptomatic smokers (FEV_1/FVC ≥ 0.70) and 22 (15.7%) as COPD GOLD 1 stage (Table 2).

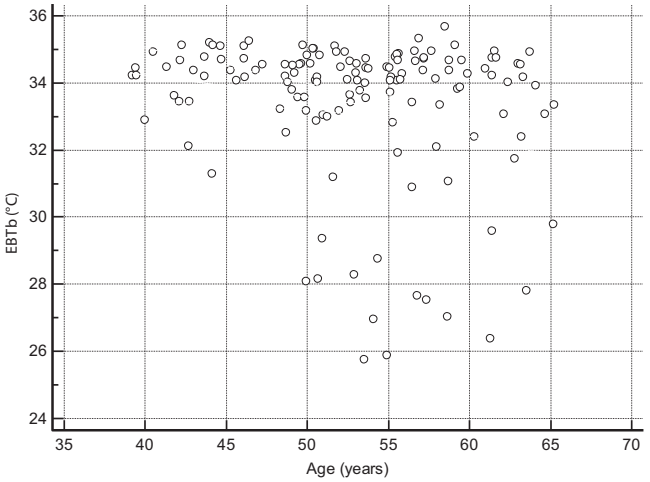

Figure 1. Association of EBTb with age (N = 140). Figure presents the association between the age (years) and the baseline exhaled breath temperature (EBTb), measured at the initial visit (Spearman R = −0.143, $p = 0.092$).

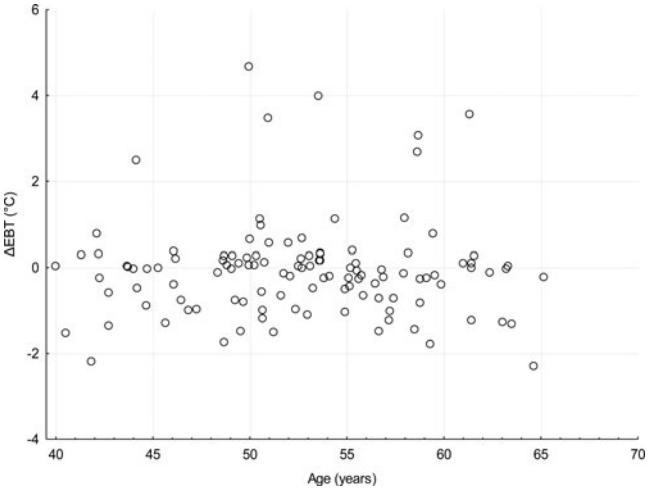

Figure 2. Association of ΔEBT with age (N = 140). Figure presents the association between the age (years) and the change in exhaled breath temperature after smoking cigarette (ΔEBT), measured at the initial visit (Spearman R = −0.069, $p = 0.448$).

Table 2. Characteristics of patients at the initial visit according to the pulmonologist assessment following GOLD guidelines (N = 140).

Variable*	Classification of patients				
	Asymptomatic (n = 37)	Symptomatic (n = 81)	COPD GOLD 1 (n = 22)	Statistics†	COPD LLN (n = 13)
Age (years)	52.2 ± 6.4	53.6 ± 6.4	52.1 ± 6.7	F = 0.876; $p = 0.419$	51.4 ± 5.7
Pack-years	36.6 ± 19.1	38.9 ± 17.9	39.6 ± 16.9	F = 0.255; $p = 0.775$	37.9 ± 15.9
PB FVC (SR)	0.76 ± 1.01	0.69 ± 1.08	0.86 ± 1.14	F = 0.219; $p = 0.804$	0.51 ± 1.24
PB FEV_1 (SR)	0.18 ± 0.98	0.25 ± 0.86	− 0.62 ± 1.06	**F = 8.246; $p < 0.001$**	− 1.09 ± 1.12
PB FEV_1/FVC (SR)	− 0.26 ± 0.76	0.08 ± 0.71	− 1.60 ± 0.44	**F = 53.879; $p < 0.001$**	− 1.95 ± 0.30††
6MWT (%Pred)	63.74 ± 13.37	64.04 ± 14.26	66.15 ± 10.60	F = 0.186; $p = 0.813$	57.83 ± 12.43††
CAT score	5.2 ± 4.6	10.1 ± 6.7	13.1 ± 8.8	**H = 21.520; $p < 0.001$**	11.9 ± 5.8
SGRQ symptom score	12.3 ± 17.6	21.3 ± 16.3	30.5 ± 25.6	**H = 17.566; $p < 0.001$**	26.2 ± 19.0
SGRQ activity score	23.6 ± 15.2	26.0 ± 17.6	26.6 ± 19.9	**H = 15.628; $p < 0.001$**	23.8 ± 16.6
SGRQ impact score	3.7 ± 8.1	7.2 ± 9.1	11.5 ± 11.2	**H = 11.293; $p = 0.004$**	10.7 ± 9.4
SGRQ total score	8.2 ± 10.7	15.2 ± 10.4	19.2 ± 13.9	**H = 19.686; $p < 0.001$**	17.2 ± 10.0
EBTb (°C)	32.56 ± 3.66	33.98 ± 1.64	32.89 ± 3.47	F = 0.353; $p = 0.703$	33.96 ± 1.08
EBTc (°C)	32.66 ± 2.81	33.74 ± 1.71	32.82 ± 3.29	F = 1.248; $p = 0.291$	33.64 ± 0.88
ΔEBT (°C)	0.10 ± 2.00	− 0.24 ± 0.63	− 0.07 ± 1.48	F = 0.001; $p = 0.999$	− 0.25 ± 1.33

Legend: *all values are presented as mean ± SD; †statistical significance for comparison between three groups according to GOLD was tested using analysis of variance (ANOVA, *F* value) or Kruskal-Wallis ANOVA (*H* value); ††significantly different to COPD GOLD 1 ($p < 0.05$); COPD: chronic obstructive pulmonary disease; GOLD: Global Initiative for Chronic Obstructive Lung Disease; PB – postbronchodilator value; FVC – forced vital capacity as standardized residuals (SR); FEV_1 – forced expiratory volume in 1 second as standardized residuals (SR); FEV_1/FVC as standardized residuals (SR); 6MWT – 6-minute walk test as % predicted (%Pred); CAT – COPD assessment test; SGRQ – St. George Respiratory Questionnaire; EBT – exhaled breath temperature; EBTb – baseline EBT; EBTc – EBT after smoking cigarette; ΔEBT – change in EBT after smoking cigarette.

Subgroups comparisons at initial visit

Subgroup (after the initial pulmonologist assessment) comparisons at initial visit are presented in Table 2. No significant differences were found for age, smoking exposure or functional exercise capacity between the subgroups ($p > 0.400$ for all). Also no significant difference was found for FVC (as SR, $p = 0.804$) but a significant difference was found for FEV_1 and FEV_1/FVC (expressed as SR, $p < 0.001$ for both) with worst results found as expected in GOLD 1 subgroup (as FEV_1/FVC < 0.7 was used to define this group). Comparable results were found for HRQoL data with the worst results found in GOLD 1 subgroup ($p < 0.01$ for all). No significant differences were found between these subgroups for EBT results (EBTb, EBTc and ΔEBT) ($p > 0.200$). In Table 2 we also presented the data for the subgroup of subjects diagnosed as COPD according to LLN criteria. Upon comparison with the COPD GOLD 1 subgroup, expected significantly lower values were found in this subgroup for FEV_1/FVC (expressed as SR, $p < 0.05$) and for the 6MWT (expressed as % predicted, $p < 0.05$). Other variables (age, smoking habit, FVC and FEV_1, HRQoL, EBT) were not significantly different between these two subgroups.

Study outcomes

Reassessment after 2 years of follow-up (average follow-up was 2 years and 54 days) for the predefined outcomes of DP showed that out of 118 patients without COPD, 7 (5.9%) patients had an ND COPD (rate per 100 person-years of 2.767, 95% CI 1.210–5.473) with additional 4 [11 (7.9%) altogether] that had progressed from COPD GOLD 1 stage to GOLD 2 stage (DP rate per 100 person-years of 3.667, 95% CI 1.928–6.373), and 60 (42.9%) had an increased rate of LoLF (>70 mL/year for the PB FEV_1). Using the outcome measures based on LLN (ND COPD and DP) or SR (LoLF), 5 patients had an ND COPD, 9 had DP and 14 had a loss of FEV_1 > 1 SR. Comparisons of three EBT markers (EBTb, EBTc and ΔEBT) between the subgroups according to the progression of the disease after 2-year follow-up (based on three predefined study outcome measures) and the ROC curve analyses are shown in Tables 3 and 4.

The results for the primary outcome showed that ΔEBT measured at initial visit had a marginal discriminative power for ND COPD according to the GOLD criteria ($p = 0.148$) with the increase in the signal when ATS/ERS criteria (LLN) was used ($p = 0.036$) giving an AUC of 0.859 (95% CI, 0.781–0.917;

Table 3. EBT (EBTb, EBTc and ΔEBT) according to the outcomes of disease progression after 2-year follow-up (N = 140).

Disease progression outcome			EBTb (°C)	EBTc (°C)	ΔEBT (°C)
ND COPD	GOLD	No (n = 111); Yes (n = 7); Statistics†	33.41 ± 2.17; 31.02 ± 4.14; Z = 1.419, $p = 0.156$	33.18 ± 2.21; 32.56 ± 2.80; Z = 0.601, $p = 0.548$	−0.20 ± 1.23; 1.54 ± 2.85; Z = 1.446, $p = 0.148$
	LLN	No (n = 113); Yes (n = 5); Statistics†	33.43 ± 2.23; 30.17 ± 3.03; **Z = 2.348, $p = 0.019$**	33.25 ± 2.10; 30.98 ± 3.87; Z = 1.518, $p = 0.129$	−0.28 ± 1.05; 2.72 ± 2.43; **Z = 2.099, $p = 0.036$**
DP	GOLD	No (n = 129); Yes (n = 11); Statistics†	33.51 ± 2.05; 31.29 ± 3.85; Z = 1.758, $p = 0.079$	33.27 ± 2.05; 32.71 ± 2.63; Z = 0.320, $p = 0.749$	−0.21 ± 1.21; 1.46 ± 2.51; **Z = 2.170, $p = 0.030$**
	LLN	No (n = 131); Yes (n = 9); Statistics†	33.51 ± 2.11; 31.01 ± 3.39; **Z = 2.249, $p = 0.024$**	33.35 ± 1.96; 31.45 ± 3.19; **Z = 2.175, $p = 0.030$**	−0.16 ± 1.30; 1.12 ± 2.42; Z = 1.827, $p = 0.067$
Increased LoLF	Loss of FEV_1>70 ml/year	No (n = 80); Yes (n = 60); Statistics†	33.49 ± 2.05; 33.23 ± 2.56; Z = 0.543, $p = 0.587$	33.20 ± 2.18; 33.29 ± 2.02; Z = 0.020, $p = 0.984$	−0.20 ± 1.34; 0.13 ± 1.57; Z = 0.104, $p = 0.917$
	Loss of FEV_1>1 SR	No (n = 126); Yes (n = 14); Statistics†	33.35 ± 2.32; 33.18 ± 2.18; Z = 0.802, $p = 0.422$	33.25 ± 2.18; 33.02 ± 2.49; Z = 0.159, $p = 0.874$	−0.07 ± 1.47; −0.10 ± 0.96; Z = 0.059, $p = 0.953$

Legend: ND COPD – newly diagnosed COPD after 2 years of follow-up; DP – disease progression – ND COPD + progression of a severity of COPD from GOLD 1 stage during the 2-year follow-up; LoLF – loss of lung function; GOLD: Global Initiative for Chronic Obstructive Lung Disease; LLN: lower level of normal; FEV_1: Forced expiratory volume in 1 second; SR: standardized residuals; EBT – exhaled breath temperature; EBTb – baseline EBT; EBTc – EBT after smoking cigarette; ΔEBT – change in EBT after smoking cigarette; ∗values are presented as mean ± SD; †statistical significance was tested using Mann-Whitney U-test.

Table 4. EBT (EBTb, EBTc and ΔEBT) ROC curve analysis data according to the outcomes of disease progression after 2-year follow-up (N = 140).

Disease progression outcome			EBTb	EBTc	ΔEBT
ND COPD	GOLD	AUC, 95% CI, Statistics	0.664, 0.571–0.748, Z = 1.199, p = 0.231	0.568, 0.466–0.667, Z = 0.547, p = 0.584	0.669, 0.568–0.759, Z = 1.409, p = 0.159
	LLN	AUC, 95% CI, Statistics	0.788, 0.703–0.858, **Z = 2.330, p = 0.020**	0.704, 0.605–0.791, Z = 1.124, p = 0.261	0.859, 0.781–0.917, **Z = 2.533, p = 0.011**
DP	GOLD	AUC, 95% CI, Statistics	0.663, 0.579–0.740, Z = 1.595, p = 0.111	0.530, 0.438–0.621, Z = 0.274, p = 0.784	0.711, 0.622–0.789, **Z = 2.404, p = 0.016**
	LLN	AUC, 95% CI, Statistics	0.716, 0.634–0.789, **Z = 2.246, p = 0.025**	0.731, 0.644–0.808, **Z = 1.993, p = 0.046**	0.614, 0.520–0.702, Z = 0.783, p = 0.433

Legend: ND COPD – newly diagnosed COPD after 2 years of follow-up; DP - disease progression – ND COPD + progression of a severity of COPD from GOLD 1 stage during the 2-year follow-up; GOLD: Global Initiative for Chronic Obstructive Lung Disease; LLN: lower level of normal; AUC: area under the curve; CI: confidence interval; EBT – exhaled breath temperature; EBTb – baseline EBT; EBTc – EBT after smoking cigarette; ΔEBT – change in EBT after smoking cigarette; statistical significance was tested using z statistics.

z statistic = 2.533, p = 0.011). A cut-off value of 1°C had a sensitivity of 66.7%, specificity of 98.1%, PPV of 75% and NPV of 97.3%. We found a significant discriminative power for DP according to the GOLD criteria (p = 0.030) with a comparative signal when ATS/ERS criteria (LLN) was used (p = 0.067) giving an AUC (GOLD criteria) of 0.711 (95% CI, 0.622–0.789; z statistic = 2.404, p = 0.016). A cut-off value of 0.17°C had a sensitivity of 60.0%, specificity of 75.0%, PPV of 17.7% and NPV of 95.5%. No significant difference was found for ΔEBT for the third outcome measure LoLF (p > 0.9 for both; Table 3).

Results for the secondary outcomes showed that EBTb was significantly discriminative for patients with two (out of three) outcome measures (ND COPD and DP) but only if ATS/ERS criteria were used (LLN) for the definition of airway limitation (p = 0.019 and p = 0.024, respectively; Table 3) giving moderately good results using ROC curve analysis (AUC = 0.788 and AUC = 0.716, respectively; Table 4) with EBTb at initial visit significantly lower in patients with the progression of disease. Results for the EBTc showed the same pattern but were significantly discriminative only for DP when ATS/ERS criteria were used (LLN) for the definition of airway limitation (p = 0.030; Table 3) giving a moderately good result using ROC curve analysis (AUC = 0.731; Table 4). Both EBTb and EBTc have not showed a significant difference for LoLF (p > 0.87 for all; Table 3).

Logistic regression analysis for ND COPD and DP using ATS/ERS criteria (LLN) taking into account sex, age, smoking exposure, lung function, 6MWT and EBT was carried out to assess markers of susceptibility to cigarette smoke for the progression of disease. For ND COPD, only ΔEBT was significantly associated with this outcome and had an OR equal to 1.74 (95% CI, 1.09–2.78; p = 0.021). For DP EBTb, PB FVC and PB FEV_1 (expressed as SR) were significantly associated with this outcome (EBTb OR = 0.71, 95% CI 0.54–0.92, p = 0.010; PB FVC OR = 13.34, 95% CI 1.79–99.38, p = 0.012; PB FEV_1 OR = 0.06, 95% CI 0.01–0.50, p = 0.010).

Discussion

The main result of this preliminary analysis is the potential of an average equilibrium exhaled breath temperature (EBTb and ΔEBT) measured at a single time point (at initial visit) to predict future (after 2-year follow-up) development of COPD or progression of disease with the increase in signal and more consistent results when using a more stringent ATS/ERS criteria for airway limitation (LLN). A much clearer signal when using LLN for diagnosing COPD is especially important in determining whether GOLD is a meaningful method for diagnosing COPD. These results were further corroborated by the results of logistic regression analysis (taking into account also sex, age, smoking, lung function, 6MWT) showing that ΔEBT was the only significant factor predicting ND COPD (using LLN) with 74% more odds for ND COPD with an increase in EBT per°C after smoking cigarette. Logistic regression analysis revealed that EBTb together with PB FVC and PB FEV_1 (both expressed as SR) were significant independent predictors for DP, but confidence intervals for lung function indexes were wide. On the other hand, EBT was not predictive for the LoLF either as absolute value or SR values (recommended for the lung function follow-up studies by ATS/ERS to exclude age-related bias especially).

As cigarette smoking represents the most significant single cause of death and disability in developed countries, as in the pathogenesis of COPD, the results of our study show a potential for the future research in this area – both for new markers of an early diagnosis and progression of COPD as well as for the possible need to revise the current GOLD criteria for diagnosing COPD. Need for further research is emphasized by COPD disease characteristics: COPD developed in less than one-third of smokers during their lifetime, preventive measures being difficult to implement with a low rate of success, progressive disease with an unsatisfactory treatment success, high public health impact with high morbidity, disability and early mortality rates. Thus, identification of individuals with a significant susceptibility to cigarette smoke and future development of COPD is still an important (major) research goal (2). Data analysis for EBTb and ΔEBT showed a moderate sensitivity and specificity for two of three outcomes ('newly diagnosed COPD' and 'newly diagnosed COPD + progression of severity of COPD'). This allows moderately differentiating patients at risk as either 'healthy' smokers or as patients with a significant potential to develop COPD in future or patients that will have a DP, with the potential for an early intervention.

EBT is a biological marker for which research was started during last decades especially as a noninvasive measure of airways inflammation in children and adults (30,31). Higher EBT found in asthmatics compared to healthy individuals was understood to be an indicator of airways inflammation, while at the same time axillary and ear temperature were comparable between groups supporting the fact that EBT was representing a local inflammatory process (25). Measurement methods used

in early research (instantaneous EBT measurement) produced EBT results that were closely associated with age, lung volume, type of breathing and environmental factors (room temperature and humidity), and so the results had to be adjusted for these factors (32). In our study we used the newer method developed by Popov et al. (X-halo® device), measuring EBT at the maximal plateau of stabilization (average equilibrium EBT) with spontaneous breathing pattern because measurements done using this method were not influenced by the pattern of breathing, lung volume and environmental factors, while the discriminating power of the method was preserved (15, 33, 34). The EBT results in our study show a wider range of results than other studies but patients were closely monitored during the measurements; we used a one-way valve, and when in doubt regarding the measured value, the measurement was repeated. To exclude further artifacts, patients (n = 6) with EBTb < 25°C were excluded. Also, the change in a breathing technique of the patient (breathing in through mouth instead of through nose or rebreathing through the device) can change the final result by less than 0.5°C which could not explain this wide range (unpublished results). As we had several devices, subjects did not use the same device one after another, excluding the possibility that the measurement from the previous subject could influence the results of the other. So the difference in the scatter of EBT values was most probably the consequence of the studied population which was different from other studies.

Previous research showed that the change in EBT during expiration (change from environmental temperature to the plateau) was lesser and slower in patients with COPD when compared to healthy controls, possibly as a consequence of mucus hypersecretion, remodeling and/or reduction in blood vasculature (35). This data, together with the fact that this change was not significantly increased after the inhalation of vasodilator drug (e.g. salbutamol), supports the concept of decreased vascularity of airways in COPD compared to patients with asthma (30, 36). Mean (±SD) EBT in our study was 33.33 ± 2.30°C, which was comparable to similar studies (37, 38). When we compared the subgroups of smokers according to symptomatic status and diagnosis of COPD, although we found the highest temperature in the subgroup of symptomatic smokers (33.98°C) and lowest in the subgroup of asymptomatic smokers (32.56°C), the difference did not reach statistical significance because of the significant overlap. As symptoms represent an important specific facet in COPD (different from lung function), as recognized in recent GOLD document, the association of symptoms with EBT found in our study might be important and deserves further research. The other reason for the association observed between EBT and symptoms could be that these subgroups represent different mixtures of phenotypes regarding airways inflammation and vascularity changes associated with the effect of cigarette smoke on the airways.

Spirometry with a bronchodilator test with the cut-off value of FEV_1/FVC of < 0.7 still represents the 'gold standard' for the COPD diagnosis according to GOLD initiative, although this single cut-off value was challenged by ATS/ERS recommendations for criteria in support of airway limitation using LLN, especially for patients older than 70 years of age (shown very clearly by the results of GLI (19)). The unique criterion (FEV_1/FVC < 0.7) for airway limitation yields a significant overestimation of COPD in older populations. Because of that, we used both criteria and showed that using more stringent criteria, although reducing the number of subjects with outcomes (lowering the statistical power), can enhance the 'signal'. This is an important finding that challenges the globally accepted GOLD criteria for the diagnosis of COPD. On the other hand, our study evaluated EBT as a marker of susceptibility to a cigarette smoke in a population at risk having already significant cigarette smoke exposure (active smokers with a smoking history of >20 pack-years), and as a possible predictor of future COPD and DP based on the single time-point measurement. This was evaluated using baseline EBT (EBTb) measurement, the EBT measurement after smoking the cigarette (EBTc), and the change in EBT after smoking cigarette (ΔEBT) to additionally evaluate the effect of cigarette smoke on EBT. Although the data is preliminary and needs further evaluation regarding the larger patient sample and longer follow-up, it showed a potential for the predictive power of EBT measured at single time point for the future development of COPD. High negative predictive value (>95%) gives the physician assurance that individuals with a risk for the development of COPD and a negative test do not have a specific susceptibility to cigarette smoke regarding the development and progression of COPD. This was however not associated with the rate of LoLF in our study. This could be explained by a significant variability of lung function measurements during this relatively short follow-up, thus significantly influencing measured trend.

Although the predictive power of EBTb and ΔEBT was somewhat comparable for COPD DP based on the results of our study, associations found using logistic regression analysis showed a somewhat different pattern for these two EBT markers; so further research in regard to the underlying mechanisms behind these two markers is needed. The results of our study that lower EBTb represents a higher risk for DP challenge the hypothesis that inflammation is the single driver of DP under the smoke exposure (at least in this population); therefore other possible mechanisms should also be taken into account (39, 40). The results of our study corroborated by the previously published data support the potential of EBT as a possible marker of susceptibility to cigarette smoke and future development and progression of COPD. The limitations of our study were: relatively small sample size and limited follow-up time, in particular related to the low COPD progression rate found in our cohort. Further research is needed in this and other comparative populations at risk to complete the validation of this method.

Conclusions

Results of this preliminary analysis of the 2-year follow-up of a cohort of patients at risk for COPD have shown the potential of EBT in identifying patients susceptible to cigarette smoke, which would develop into clinically manifest COPD or would have a progression of disease with a better signal using LLN as criteria for COPD diagnosis. This is the first time that we have a potential biomarker measured at a single time point that can potentially predict future development of COPD. As this preliminary data analysis had a limited number of patients who were followed for 2 years with a low rate of DP, these results need additional confirmation in a larger sample with

a longer follow-up time including other comparative populations as well. If these results are confirmed in future studies, this could allow new preventive actions to be developed in patients at risk for COPD, thereby possibly lowering the burden of the disease.

List of abbreviations

ANOVA	analysis of variance
ATS	American Thoracic Society
AUC	area under the curve
BMI	body mass index
CAT	COPD Assessment Test
CI	confidence interval
COPD	chronic obstructive pulmonary disease
CRP	C-reactive protein
CVI	cerebrovascular infarction
DP	disease progression
EBT	exhaled breath temperature
EBTb	baseline EBT
EBTc	EBT after smoking cigarette
ΔEBT	change in EBT after smoking cigarette
ERS	European Respiratory Society
FEV_1	forced expiratory volume in 1. second
FVC	forced vital capacity
GCP	Good Clinical Practice
GLI	Global Lung Initiative
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GP	general practitioner
HR	health related
hs	highly sensitive
LLN	lower level of normal
LoLF	rate of loss of lung function
MI	myocardial infarction
ND	newly diagnosed
NPV	negative predictive value
PB	postbronchodilator
PEF	peak expiratory flow
PPV	positive predictive value
QoL	Quality of Life
ROC	receiver operator curve
SD	standard deviation
SGRQ	Saint George Respiratory Questionnaire
SR	standardized residuals
TIA	transient ischemic attack
WBC	white blood cell count
6MWT	6-minute walk test
%Pred	% of a predicted value.

Declaration of interests

The authors declare that they have no conflict of interests. This study was part of MARKO study. Prof. Davor Plavec and Dr. Žarko Vrbica as principal investigators and Children's Hospital Srebrnjak have initiated study MARKO (ClinicalTrials.gov Identifier NCT01550679) and received an unrestricted grant from GlaxoSmithKline. GlaxoSmithKline has not influenced the study design or study protocol, has no ownership rights over data gathered by the study and has no influence on this or further publications of data gathered throughout this study.

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