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Dose-dependent association of smoking and bronchial hyperresponsiveness

Juusela M [1], Pallasaho P [2], Rönmark E [3,4], Sarna S [5], Sovijärvi A [1], Lundbäck B [3,6].

[1]Dept of Clinical Physiology and Nuclear Medicine, HUS Medical Imaging Center, Helsinki University Central Hospital, Helsinki, Finland; [2]Control of Hypersensitivity diseases, Finnish Institute of Occupational Health, Helsinki, Finland; [3]The OLIN studies, Sunderby Central Hospital of Norrbotten, Luleå, Sweden; [4]Dept of Clinical Medicine and Public Health, Div of Environment and Occupation, Umeå University, Umeå, Sweden; [5]Dept of Public Health, Helsinki University, Helsinki, Finland; [6] Krefting Research Centre, Institute of Medicine, University of Gothenburg, Gothenburg, Sweden.

Correspondence to:

Juusela Maria, MD

Helsinki University Central Hospital, Meilahti Hospitals

HUSLAB, Department of Clinical Physiology and Nuclear Medicine

BP 340

00029 HUS, Finland

Fax: +358-9-310 67111

GMS +358 50 4270969; +358 40 7461526

maria.juusela@helsinki.fi

Short title: BHR, smoking and ETS

Abstract

Our aim was to study the association of smoking habits and environmental tobacco smoke exposure (ETS) with bronchial hyperresponsiveness (BHR).

A random sample of 292 adults was examined with a structured interview, spirometry, skin prick tests, FENO (fractional exhaled nitric oxide) and bronchial histamine challenge.

A large majority of subjects with BHR were smokers or ex-smokers. Starting to smoke before 20 years of age was significantly associated with BHR, as did current smoking, the quantity of smoking, and ETS. The severity of BHR increased significantly with increasing pack years ($p<0.001$). Current smokers with decreased lung function were at a particularly high risk for BHR. Impaired FEV_1 and MEF_{50} were independent determinants for more severe BHR regardless of age. In multivariate analysis, smoking remained as an independent determinant for BHR after adjustment for impaired lung function and other co-variates: 15 or more pack years yielded an OR 3.00 (95%CI 1.33-6.76) for BHR. The association between BHR and FENO was dependent on smoking habits.

The results indicate that smoking is a significant risk factor for BHR with a dose-dependent pattern and that the severity of BHR increases with pack years. The findings strongly suggest assessment of smoking habits in subjects with BHR.

Introduction

Smoking causes chronic airway obstruction that mostly develops gradually from peripheral airways towards large airways[1,2]. Large scale international studies have shown that smoking is a risk factor for bronchial hyperresponsiveness (BHR)[3-5], but still questions exist whether the quantity of smoking exposure is associated with the severity of BHR. Dose-dependent association of smoking and of small airway obstruction as possible independent trigger factors for BHR severity has not been explored in detail.

There are only a few recent epidemiological studies that have assessed a large variety of possible determinants of BHR[6,7]. The majority of epidemiological studies on BHR are descriptive and have reported their results of BHR as a dose response slope or dose response rate[8]. Translating these measures of BHR into clinical practice is laborious, thus they have been used basically only in research[5,7]. BHR testing is a common tool in diagnosing asthma, but the effects of smoking have been unclear when interpreting causes of BHR[9].The association of BHR and smoking has been studied mostly in selected populations[10-12].

We aimed to study the effects of smoking, ETS and exhaled fractional nitric oxide (FENO) on BHR in adult subjects representing the general population in Helsinki, the capital of Finland. Bronchial responsiveness was assessed by a dosimetric method with histamine[13], which has been in clinical use for over three decades in Finland. The effect of the quantity of smoking exposure on BHR severity, defined by a provocative dose of histamine inducing a 15% decrement in FEV_1 in two clinically validated cut off levels ($PD_{15}FEV_1$ 1.6 mg and 0.4 mg[13]), was calculated with multiple regression analysis. When calculating the effects of current smoking status, pack years and ETS on BHR, variables of ventilatory function and of small airway obstruction were used as co-variates.

Subjects and methods

Study cohort

The study sample consisted of 292 randomly selected subjects, who had taken part in a postal questionnaire survey in Helsinki in 1996[14]. The population of the FinEsS I postal survey (n=8000) was randomly selected from the Finnish population register and designed to correspond to the general population with respect to age and gender. The participation rate of the FinEsS I study was 76% (n=6062). Of the participants, 1200 were randomly invited to the FinEsS II clinical study, and half of those (n=600) were randomly selected to take part in the BHR study. The participation rate for the FinEsS II clinical study was 54% (n=643) [15], and for the BHR study 45.4% (n=292).

This BHR-study sample represents well the original study cohort from 1996 in terms of age, gender, and prevalence of asthma, respiratory symptoms and smoking habits[15]. The age-range was 26-66 years (mean 47 years), and 58% were women. The baseline FEV_1 of the studied subjects ranged from 60% to 136% of predicted Finnish reference values[16]. Helsinki University Central Hospital ethics committee approved the study, and all subjects signed an informed consent. Demographic data are presented in Tables 1 and 2.

Clinical examinations

BHR challenge tests were carried out within two weeks after an initial clinical visit including structured interview, spirometry with bronchodilation test and skin prick tests (SPTs)[17]. The interview was performed by a physician, and a trained nurse performed the spirometry and the SPTs. SPTs were performed in subjects < 61 years with two controls (positive control: histamine 10 mg/ml; negative control: glycerin solvent) and 15 allergens[15]. The interview consisted of questions about respiratory symptoms, family history of asthma and allergy, living conditions, occupation, smoking habits and exposure from environmental tobacco smoke.

BHR test

Inclusion criteria for the BHR test were: a pre-test value of $FEV_1 \geq 60\%$ of predicted or ≥ 1.5 L, no respiratory infection within four weeks prior to testing, no marked heart diseases (myocardial infarction within 3 months, instable coronary disease, dysfunction, arrhythmia) and no stroke. Subjects were allowed to use their regular medication, except β2-agonists and antihistamines (no short-acting beta agonist [SABA] for 12 hours, long-acting beta agonists [LABA] for 48 hours and antihistamines for 5 days). 18 subjects were excluded because of low baseline FEV_1.

The bronchial challenge was conducted with histamine by a dosimetric method with controlled tidal breathing by using the Spira Electro 2 jet nebulizer (Respiration Care Center Ltd., Hämeenlinna, Finland)[13]. Subjects inhaled buffered histamine diphosphate aerosol in four-folded increasing doses. The end point was a fall of $\geq$ 15% in FEV_1 or the used maximum non-cumulative dose of histamine of 1.6 mg. After the histamine challenge, post-bronchodilatation (0.4 mg salbutamol [Ventoline®, GlaxoSmithKline, Brentford, UK) via Volumatic®, GlaxoSmithKline, London, UK] FEV_1 was measured. The provocative dose of histamine inducing a fall of FEV1 by 15% ($PD_{15}FEV_1$ value) was calculated by interpolation[18].

Within two weeks prior to the bronchial challenge tests a flow-volume spirometry with a Vmax22 Spirometer (SensoerMedics, Yorba Linda, USA) was performed according to the 1994 criteria of American Thoracic Society (ATS)[19]. We recorded the largest FEV_1 and FVC from at least three acceptable curves, and the flow parameters such as the MEF_{50} were obtained from the curve with the biggest sum of FEV_1 and FVC. Bronchodilatation response was measured after the histamine test. A nose clip was used at all spirometric examinations. In 95 % of the subjects the nitric oxide of the expired air (FENO) was measured at the 50 ml/s flow rate by the 1999 ATS criteria[20]. The FENO measurements were performed before the BHR testing.

Definitions

BHR: histamine $PD_{15}FEV_1 \leq 1.6$ mg.

Marked BHR: histamine $PD_{15}FEV_1 \leq 0.4$ mg.

Severity staging of BHR[13]: severe $PD_{15}FEV_1 \leq 0.100$ mg, moderate $0.101 \leq PD_{15}FEV_1 \leq 0.400$ mg, mild $0.401 \leq PD_{15}FEV_1 \leq 1.600$ mg, and no BHR $PD_{15}FEV_1 \geq 1.601$ mg.

Normal FEV_1 [L]: forced expired volume in 1 second ≥80% of predicted.

Normal *FVC* [L]: forced vital capacity ≥80% of predicted.

Normal FEV_1/ FVC [L]: the ratio between FEV_1 to FVC ≥88% of predicted.

Normal MEF_{50} [L/s]: maximal expiratory flow at 50% of the FVC[21] ≥63% of predicted.

Physician-diagnosed asthma: subjects who answered "yes" to the question: "Have you been diagnosed as having asthma by a physician?"

Childhood wheeze: "yes" to the question: "Have you been diagnosed as having asthma or have you had wheeze in childhood?"

Atopy: at least one positive skin prick test (SPT) reaction to any of the tested allergens or reported symptoms of allergic rhinoconjunctivitis (ARC).

Non-smoker: never smoker or smoking less than 4 cigarettes per month.

Ex-smoker: those who had quitted smoking at least 12 months prior to the study.

Exposure to environmental tobacco smoke (ETS): the subjects answered on three separate questions about ETS: "Have you ever been exposed to environmental tobacco smoke at home/ at work/ generally in the surroundings?" The answer alternatives of all three questions were: "never", "yes previously, not any more", and "yes, currently".

ETS ever: ETS at home or at work, or both, currently or previously.

ETS present: ETS currently at the time of the study at home, at work, or both.

Statistical analyses

BHR severity, risk factors and symptoms associated to BHR were determined at two different cut of levels of $PD_{15}FEV_1$. Risk factors for BHR were calculated by multiple logistic regression analysis and included as independent variables age, gender, family history of asthma and determinants that were significant in the univariate analysis.For

the analysis, the mean values of age (47 years) and pack years (8.5 were used. The results are expressed as odds ratios (OR) with 95% confidence intervals (CI). Chi-square test and Fisher's exact tests were used to assess differences between groups. Further, p-values < 0.05 was considered statistically significant. The analyses were repeated for individuals <45 and ≥45 years of age to define the effects of smoking exposure as a potential inception for BHR measured in the two age groups.

The programmes of Statistical Package for Social Sciences (SPSS version 15.0 for Windows, Chicago, IL, USA) and StatXact 8_2007(Cytel Inc., Cambridge, MA, USA) were used for the statistical analysis.

Results

Smoking

Smoking increased the risk for BHR (Table 2). BHR severity increased parallel to increasing number of pack years (p<0.001) (Figure 1). Of the subjects with marked BHR, 56% were smokers and 28% ex-smokers *versus* 28% smokers and 27% ex-smokers among the subjects without BHR. Start of smoking before the age of 20 years (n=129) yielded an OR of 4.03 *(95% CI 1.11-14.67*) for marked BHR, and the corresponding OR of start of smoking before age 15 was 5.38 *(95%CI 1.14-25.37)* with non-smokers as reference. No one who had started smoking after the age of 26 years had marked BHR.

The association of pack years with BHR and marked BHR, respectively, became significant already with one pack year, OR 1.91 *(95%CI 1.05-3.49)* and OR 4.07 *(95%CI 1.15-14.39*). A smoking history of at least 8.5 pack years yielded an OR of 2.65 *(95%CI 1.40-5.00)* for BHR, and an OR of 5.99 *(95%CI 1.67-21.45)* for marked BHR. Having a smoking history of >15 pack years resulted in an OR of 8.00 *(95% CI 2.17-29.45)* for marked BHR, and combined with obstruction in an OR of 12.85 (*95% CI 3.36-49.09*). Current smokers with impaired ventilatory function defined as FEV_1 <80% of predicted, FEV_1/FVC <0.7 and MEF_{50} <63% of predicted, were all at a high risk for BHR (OR 10.17, OR 8.37 and OR 6.85) (Table 3a).

In the multivariate analysis, smoking remained as an independent determinant for BHR and marked BHR when co-variates including impaired lung function and other determinants of BHR that were significant in the univariate analysis were taken into account (Table 4). Smoking >15 pack years remained significantly associated with both BHR and marked BHR after adjustment for age, female gender, wheezing or asthma in childhood, FEV_1 <80% of predicted and MEF_{50} <63% of predicted (Table 3b). Besides ventilatory function variables, also asthma or wheeze during childhood remained as significant risk factors for BHR in the multivariate analysis.

ETS

Of the subjects with marked BHR 33% reported ETS exposure at the time of the study *versus* 17% among those not having BHR. ETS at home and at work, respectively, associated with marked BHR, OR 3.73 *(95%CI 1.05-13.17)* and 4.65 *(95%CI 1.32-16.42)*. However, exposure to tobacco smoke in non-smokers only was not significantly associated with BHR.

Ventilatory function

Low baseline FEV_1 [L] values correlated with low $PD_{15}FEV_1$ [mg] values, $p < 0.001$.

Baseline FEV_1 < 80% of predicted together with obstruction (FEV_1/ FVC <0.7) increased the risk for BHR yielding an OR of 5.73 *(95%CI 1.75-18.73)* (Table 3a). In univariate analysis of lung function variables, MEF_{50} below lower limit of normal (LLN) appeared as a strong determinant for BHR and marked BHR. When MEF_{50} < LLN was the only sign of decreased ventilatory function, it was significantly associated with BHR, OR 2.65 *(95% CI 1.21-5.82).*

FENO in relation to BHR

The association between FENO and BHR was strongly dependent on smoking habits (Figure 2). Only in non-smokers with BHR, FENO was >25 ppb, and significantly higher compared to the remaining subjects (p=0.008). Current exposure to ETS was associated with a lower FENO (13.2 ppb) compared to non-exposed (19.3 ppb) (p=0.002).

Influence of age

The association of smoking with BHR was examined in two age groups: those <45 years of age (group 1,mean age 36 years; n=126) and those ≥45 years of age (group 2, mean age 54 years; n=166). The prevalence of BHR did not differ between the groups 1 and 2 (19.8% *versus* 22.3%), whereas marked BHR was more common in the group 2 (4.8% *versus* 7.2%). The group 1 included more non-smokers (46.8% *versus* 37.3%), and the number of pack years was lower than in group 2 (mean 5.6 *versus* mean 10.8), respectively. The proportion of subjects having obstruction defined as FEV1/ FVC < 88% of predicted was the same in both the groups 1 and 2 (30.4% *versus* 30.1%), but obstruction defined as $FEV_1/FVC < 0.7$ was more common in group 2 (1.6% *versus* 12.0%, respectively).

In group 1, smoking and LLN of FEV_1 were not significantly associated with BHR (OR 1.78, 95%CI 0.68-4.46, and OR 1.93, 95%CI 0.54-6.86, respectively), but both these factors increased the risk for BHR in group 2 (OR 3.55, 95%CI 1.42-8.91, and OR 11.10, 95%CI 3.84-32.10, respectively). In the multivariate analyses, age adjusted determinants for BHR did not differ from analyses performed without age in the models (Tables 3b and 3c, Table 4). Of the lung function parameters, MEF_{50} < 63% of predicted increased the risk regardless of age: in group 1, when sex, wheezing or asthma in the childhood, and smoking (pack years) were included in the multivariate model, ORs for BHR and marked BHR were 3.39 (95%CI 1.20-9.55) and 13.60 (95%CI 1.88-98.23), respectively.

Discussion

In this study, we found a dose-dependent association of smoking and the severity of BHR in an adult sample of the general population. The present study indicates that increasing smoking exposure, defined by pack years is associated with more severe BHR. The association remained significant even after adjustment of effects on BHR of decreased lung function (FEV_1), airway obstruction and peripheral airflow

limitation at baseline. In the multivariate model, a history of asthma or wheezing in childhood and female gender were also independent determinants of BHR.
Generally the risk factors were most strongly associated among subjects ≥45 years, whereas in subjects <45 years significant associations with BHR were diluted, except for MEF_{50} < 63% of predicted.

We found that the majority of the current smokers had started to smoke at the age of 15-19 years. Starting to smoke at age 7-20 years doubled the risk of having BHR and increased the risk for marked BHR fourfold. Starting to smoke very early in life, at age 7-14 years, increased the risk for marked BHR in adulthood more than five-fold. Categorization of the smoking exposure by the pack years revealed more significant associations than the use of general terms of current smoking status, i.e. non-, ex- or current smokers. Acute effects of exposure to tobacco smoke were not studied.

Our results indicate that ETS and smoking interfere with FENO values in detecting airway inflammation in a general population, similar to the results recently found by Nadif et al.[22]. Smoking exposure plays an inestimable role in evaluating FENO levels of an individual, thus probably explaining some of the contradictory results found in former studies of the associations of BHR with other measurements of airway regulation and inflammation[23,24]. Biological measurements of exposure to tobacco smoke were not performed, which results in a somewhat incomplete quantification of the ETS exposure.

The inclusion criteria for participation in a study of BHR have an impact on the final outcomes. In this general population cohort the prevalence of BHR was 21%, and severe or moderate BHR constituted in 6% of the studied[17], the latter result fairly consistent with current data on prevalence of asthma among adults in Finland[14,15].

However, in our study as in all BHR studies, several of the most severe patients were excluded because of their low baseline FEV_1 value. Thus their severely decreased ventilatory function cannot be taken into account when calculating the risk factors or determinants of increased BHR. The hypothesis of the effect of the size of the airway calibre, and gender differences, are both important determinants of the BHR[25], as we could see in the presented multivariate model. The methodological considerations of BHR testing and comparison of the results in epidemiological studies lack this part of

critical evaluation[26]. In a majority of the BHR studies, only lung function values of predicted are used in the evaluation of risk factors. This might exclude the eventual effect of decreased ventilatory reserves on BHR particularly among elderly subjects.

As a surrogate variable of peripheral airway obstruction, we used the MEF_{50} flow of the baseline spirometry to investigate the role of flow limitation typical for a history of smoking. The repeatability and reliability of the measure is known to be lower and less precise than that of FEV_1 [27]. However in our study cohort, the quality and the representativeness of the spirometric measurements have been evaluated [28], and the mean FEV_1 and FVC of predicted values in the present study sample conformed well to current Finnish reference values.

We found that impaired MEF_{50} was strongly associated with BHR. Smoking more than 15 pack years as an independent risk factor for BHR remained stable also after adjustment for both MEF_{50} and FEV_1 below LLN in the multivariate model. As a sign for early airway closure, the MEF_{50} below LLN independently associated with an increased risk for BHR and marked BHR of the same magnitude as a decreased FEV_1 value. Results from others, also assessed in general adult population samples, report a close association of decreased FEV_1 and increased BHR[3,29,30],but the associations between MEF_{50} and BHR to histamine in adult general populations have not previously been published to our knowledge.

Results of the analysis in individuals <45 and ≥45 years of age suggested that exposure to tobacco smoke is a potential inception for BHR after mid-ages. Pathologically defined BHR appears after a life along exposures, such as tobacco smoke. This is in line with the results gained from larger epidemiological BHR studies, in which remodelling changes caused by tobacco have been suggested to cause the increased BHR in a longitudinal setting[7]. As published by van den Berge and colleges (2012) the critical role of inflammatory cells, such as neutrophils, macrophages and lymphocytes, and air trapping in relation to BHR serves as a characterization tool in distinction of phenotypes of chronic airway diseases. Prospective studies have shown significant reduction in BHR in asthmatic smokers after quitting, thus smokers should be assisted in quitting[10,11].

Limitations of the present general population study are obvious due to the small sample size of slightly less than 300 subjects. However, we could show similar associations of BHR and smoking as presented in studies among selected patient populations, such as asthmatics, subjects with allergy and with COPD[10-12,31].

Our present study was performed before 2006, when smoking became forbidden in public places and restaurants in Finland. Along with the public ban of smoking, smoking habits have started to decrease also in Finland[32]. A decrease in the prevalence of respiratory symptoms and lung function disturbances may become a consequence of the decrease in smoking, as found in prospective studies in asthmatic smokers after quitting[10,11,31,33].

In conclusion, smoking and BHR were dose-dependently associated even after correction of effects of impaired lung function, female gender and a history of asthma or wheezing during childhood. The severity of BHR increased by increasing number of pack years and starting to smoke before age 20 yielded over four-folded risk for marked BHR, thus indicating that smoking exposure is a trigger factor for BHR in mid-ages and older. Low MEF_{50} as a single spirometric measure presented the highest OR for BHR, indicating a significant association of impaired air flow limitation with BHR. Smoking and ETS confounded the association of FENO and BHR. Our results support anti-smoking actions and legislative restrictions of ETS both at work and at home. Assessment of smoking habits in subjects with BHR is important..

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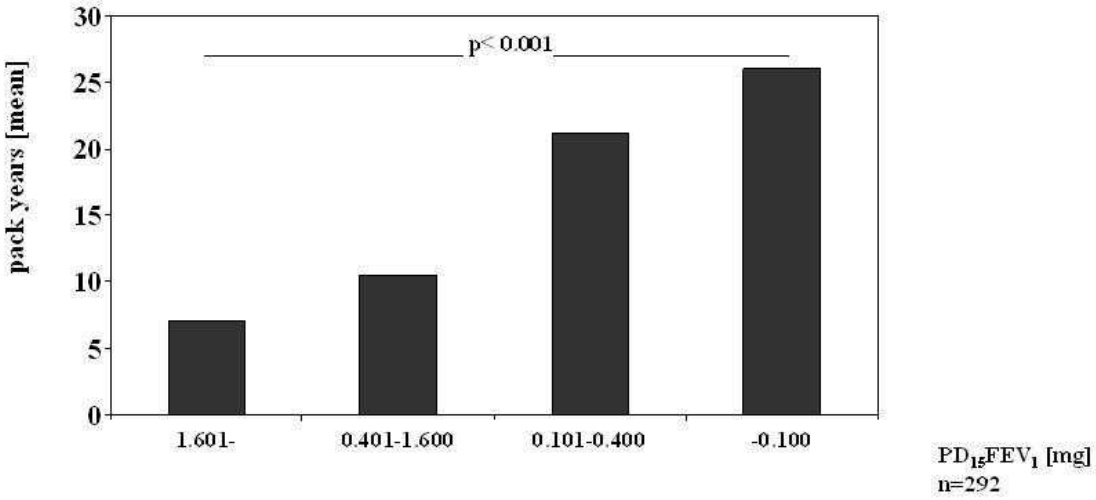
30
25
20
15
10
5
0
pack years [mean]
p< 0.001
1.601-
0.401-1.600
0.101-0.400
-0.100
PD15FEV1 [mg]
n=292

Figure 1

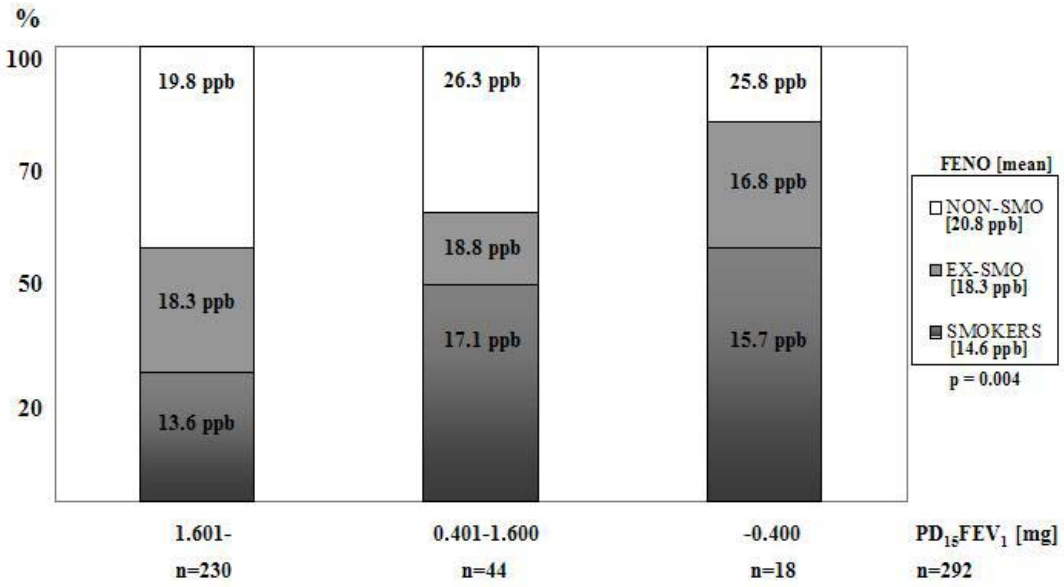
%
100
70
50
20
19.8 ppb
18.3 ppb
13.6 ppb
26.3 ppb
18.8 ppb
17.1 ppb
25.8 ppb
16.8 ppb
15.7 ppb
FENO [mean]
NON-SMO [20.8 ppb]
EX-SMO [18.3 ppb]
SMOKERS [14.6 ppb]
p = 0.004
1.601-
n=230
0.401-1.600
n=44
-0.400
n=18
PD15FEV1 [mg]
n=292

Figure 2

Table 1. Demographic data of the subjects studied, n=292.

		Men (n=123)	**Women (n=169)**	**Total (n=292)**
		mean±SD (range)	mean±SD (range)	mean±SD (range)
Age (years)		45.2±9.5 (28-65)	47.3±10.6 (26-66)	46.4±10.2 (26-66)
Height (m)		1.74±0.06 (1.61-1.86)	1.63±0.07 (1.46-1.74)	1.69±0.08 (1.46-1.86)
Weight (kg)		80.0±12.6 (43-110)	70.6±13.8 (48-105)	75.6±14.0 (43-110)
Spirometry	FEV_1 [L]	4.06±0.70 (2.35-5.90)	2.87±0.51 (1.71-4.50)	3.37±0.84 (1.71-5.90)
	FEV_1 of predicted [%] #	94±12 (62-127)	94±12 (71-129)	94±12 (62-129)
	FVC [L]	5.28±0.82 (3.09-8.03)	3.65±0.61 (2.15-5.39)	4.34±1.07 (2.15-8.03)
	FVC of predicted [%] #	99±11 (67-127)	99±12 (72-145)	99±12 (67-145)
	FEV_1/FVC [%]	77±6	78±6	78±6
	FEV_1/FVC of predicted [%] #	95±7 (71-113)	95±6 (80-115)	95±7 (71-115)
	MEF_{50} [L/ s]	4.43±1.33 (1.40-8.11)	3.37±0.98 (1.41-6.33)	3.82±1.26 (1.40-8.11)
	MEF_{50} of predicted [%] #	82±24 (30-147)	77±20 (39-137)	79±22 (30-147)
Smoking	pack years	10.30±12.57 (0-47)	7.21±10.18 (0-39)	8.51±11.33 (0-47)
		n (%)	n (%)	n (%)
Smoking history	non-smokers n(%)	47 (38.2%)	74 (43.8%)	121 (41.4%)
	ex-smokers n(%)	30 (24.4%)	45 (26.6%)	75 (25.7 %)
	smokers n(%)	46 (37.4%)	50 (29.6%)	96 (32.9%)

ETS	ETS ever[#]	84 (68.3%)	131 (77.5%)	215 (73.6%)
	ETS both at work and at home	49 (39.8%)	65 (38.5%)	114 (39.0%)
Inhaled corticosteroids[§]		3 (2.4%)	6 (3.6%)	9 (3.1%)

All values given as mean ±SD (range).Lung function values are also presented in values of the predicted [%]# as mean ±SD and range (n=291). Predicted values according to Viljanen et al. (1982).
at work and/ or at home
§ daily use of inhaled corticosteroids ≥ 200 µg

Table 2. Smoking as a risk factor for BHR in terms of odds ratios (OR), univariate analysis.

			PD15FEV1 ≤ 1.6 mg		**PD15FEV1 ≤ 0.4 mg**	
		n=292	*	**OR (95%CI)**	*	**OR (95%CI)**
Age started						
	non smokers	121	19	1	3	1
	≥ 20 years	42	9	1.46 (0.60-3.55)	3	3.03 (0.59-15.61)
	< 20	129	34	**1.92 (1.03-3.60)**	12	**4.03 (1.11-14.67)**
Number of pack years						
	non smokers	121	19	1	3	1
	< 8.5 [¤]	65	8	0.75 (0.31-1.83)	1	0.62 (0.06-6.03)
	≥ 8.5	106	35	**2.65 (1.40-5.00)**	14	**5.99 (1.67-21.45)**
	non smokers	121	19	1	3	1,00
	< 5	45	4	0.52 (0.17-1.63)	0	:
	5 ≥ 15	55	12	1.50 (0.67-3.35)	3	2.27 (0.44-11.62)
	>15-	71	27	**3.29 (1.66-6.54)**	12	**8.00 (2.17-29.45)**

Smoking status					
non smokers	121	19	1	3	1
ex-smokers	75	12	1.02 (0.47-2.25)	5	2.81 (0.65-12.12)
current smokers	96	31	**2.56 (1.34-4.91)**	10	**4.57 (1.22-17.12)**
smoking history ≥ 1 year	160	39	1.53 (0.86-2.72)	15	**4.45 (1.26-15.72)**
Current smoking status					
non smokers and ex-smokers'	197	31	1	8	1
0-4 cigarettes per day	36	11	**2.34 (1.05-5.25)**	2	1.38 (0.28-6.80)
5-14 cigarrettes per day	29	10	**2.80 (1.19-6.60)**	2	1.74 (0.35-8.63)
≥ 15 cigarrettes per day	30	10	**2.66 (1.14-6.23)**	6	**5.88 (1.88-18.38)**

*number of those subjects having BHR
¤ the mean of pack years in the cohort, n=292
' non smokers and ex smokers, and one missing included, n=197
: number of subjects is 0.

Table 3. Lung function and smoking, risks in odds ratios (OR) with 95 % intervals for $PD_{15}FEV_1 \leq 1.6$ mg and $PD_{15}FEV_1 \leq 0.4$ mg, analysed by univariate (3a), and multivariate analysis (3b and 3c)

Independent variables			Dependent variables			
			$PD_{15}FEV_1 \leq 1.6$ mg		$PD_{15}FEV_1 \leq 0.4$ mg	
Variables	Categories		*	OR (95%CI)	*	OR (95%CI)
3a. Univariate analyse		n=292				
Lung function	FEV_1 < 80% pred	38	19	**4.91 (2.40-10.04)**	10	**10.98 (4.01-30.11)**
	FEV_1/ FVC < 0.7	23	11	**3.92 (1.64-9.38)**	8	**13.81 (4.76-40.09)**

	MEF_{50} < 63% of pred	77	37	**6.99 (3.79-12.89)**	15	**17.02 (4.77-60.68)**
	FEV_1 < 80% of pred & FEV_1/FVC < 0.7	12	7	**5.73 (1.75-18.73)**	7	**34.24 (9.36-125.17)**
	FEV_1 < 80% & MEF_{50} < 63% of pred	24	15	**7.80 (3.22-18.89)**	10	**23.13 (7.90-67.69)**
Current smokers and lung function		n=96				
	smokers with FEV_1 < 80% pred	22	15	**10.17 (3.93-26.31)**	8	**14.86 (5.08-43.49)**
	smokers with FEV_1/FVC <0.7	12	8	**8.37 (2.43-28.82)**	7	**34.24 (9.36-125.17)**
	smokers with MEF_{50} < 63% of pred	37	21	**6.85 (3.30-14.23)**	10	**11.44 (4.16-31.43)**
3b. Multivariate analysis		n=292				
Age	≥ 45 years			0.56 (0.27-1.14)		0.52 (0.14-2.00)
Lung function						
	FEV_1 < 80% of pred			**2.69 (1.06-6.84)**		**5.78 (1.55-21.54)**
	MEF_{50} < 63% of pred			**5.53 (2.70-11.32)**		**8.34 (1.82-38.18)**
Sex	Women (reference: men)			**2.12 (1.04-4.34)**		0.93 (0.26-3.34)
Wheezing or asthma in childhood	Yes (reference: no)			**3.99 (1.24-12.85)**		1.05 (0.09-11.74)
Smoking in pack years	non smokers			1		1
	0 ≥ 5 pack years			0.45 (0.14-1.50)		:
	5 ≥ 15 pack years			1.30 (0.53-3.22)		1.40 (0.23-8.61)
	>15- pack years			**3.00 (1.33-6.76)**		**5.80 (1.27-26.62)**
3c. Multivariate analysis		n=292				
Age	≥ 45 years			0.61 (0.31-1.21)		0.67 (0.20-2.31)
Lung function						
	MEF_{50} < 63% of pred			**7.64 (3.92-14.88)**		**19.04 (4.77-75.97)**
Sex	Women (reference: men)			1.92 (0.97-3.80)		0.69 (0.22-2.14)
Wheezing or asthma in childhood	Yes (reference: no)			**4.15 (1.35-12.76)**		1.77 (0.17-18.13)
Smoking in pack years	non smokers			1		1

	< 8.5 pack years	0.64 (0.24-1.68)	0.45 (0.04-4.79)
	≥ 8.5 pack years	**2.58 (1.26-5.31)**	**5.00 (1.25-19.92)**

* number of those subjects having BHR
: number of subjects is 0

Table 4. Risk in odds ratios (OR) with 95 % confidence intervals (CI) for histamine $PD_{15}FEV_1 \leq 1.6$ mg and $PD_{15}FEV_1 \leq 0.4$ mg, multivariate analysis

Independent variables		Dependent variables	
		$PD_{15}FEV_1 \leq 1.6$ mg	$PD_{15}FEV_1 \leq 0.4$ mg
Variables	Categories	OR (95%CI)	OR (95%CI)
Age	>47 years	0.70 (0.36-1.38)	0.63 (0.17-2.36)
Sex	Women (reference: men)	**2.14 (1.08-4.24)**	1.05 (0.31-3.53)
FEV_1 < 80 % of pred		**4.58 (2.07-10.12)**	**10.75 (3.20-36.11)**
Family history of asthma	Yes (reference: no)	1.64 (0.75-3.62)	1.42 (0.34-5.97)
Allergy*	Yes (reference: no)	0.63 (0.33-1.21)	0.48 (0.15-1.60)
Wheezing or asthma in childhood	Yes (reference: no)	**3.66 (1.22-11.05)**	2.18 (0.23-21.11)
Smoking history #	Non smokers	1	1

<15 pack years	0.92 (0.41-2.07	1.51 (0.22-10.23)
≥ 15 pack years	**3.87 (1.77-8.43)**	**9.91 (1.83-53.53)**

* Allergy= atopy or symptoms of allergic rhinoconjunctivitis (ARC)

Smoking history in pack years, current and ex-smokers included.

BHR tested in April – June included in the model, nonsignificant: for $PD_{15}FEV_1$ 1.6 mg OR 0.95 (95%CI 0.47-1.92) and for $PD_{15}FEV_1$ 0.4mg OR 1.98 (95%CI 0.60-6.52). ETS at work included in the model (for BHR OR 2.02; 95%CI 1.00-4.10, and for marked BHR 1.98; 95% CI 0.60-6.52) did not change the significance of the factors.