

Environmental Tobacco Smoke Affects Lung Function of Preschoolers with Asthma Even after a Decade

To the Editor:

The hazardous effects of environmental tobacco smoke (ETS) on the lung health of small children are generally recognized. Governmental actions need improvement in decreasing worldwide smoking prevalence (1). The global average of young men starting smoking is 40%; it is 9% for women (1). As children are born to young families, they are often exposed to ETS. We have previously shown that maternal smoking in particular adversely affects the lung function of preschoolers with asthma (2), and now we wanted to objectively examine whether early exposure to ETS has a long-term effect on lung function.

We enrolled 105 children (median age, 5 yr) referred to Helsinki University Hospital with multitrigger wheeze and evidence of bronchodilator response ($\geq 35\%$ decrease in respiratory resistance at 5 Hz [R5]) or exercise-induced bronchoconstriction ($\geq 35\%$ increase in R5), or both (3). Exclusion criteria included the use of corticosteroids in the previous 6 months and respiratory tract infection in the preceding 2 weeks. After 10 years, 64 children participated in a follow-up visit.

We assessed the child's exposure to ETS at preschool age with urine cotinine levels, using gas chromatography (2), as well as with a questionnaire. Cotinine is a stable metabolite of nicotine and can be used to objectively measure exposure to ETS. It is found in saliva, urine, hair, and blood a few days after exposure to ETS. We have previously presented the gas chromatographic method and the correlation between urinary cotinine levels and ETS in this particular population (2).

Fractional exhaled nitric oxide (F_{ENO}) and impulse oscillometry were performed following current guidelines both at enrollment and at the 10-year follow-up visit (2–5). Impulse oscillometry indices of interest included the total respiratory system resistance (R5) and reactance (X5) at 5 Hz, and area under reactance (AX), reflecting the capacitive component of reactance that is affected by small airway function. At the follow-up visit, lung function was also assessed by spirometry (6), and the children underwent dosimetric bronchial provocation with methacholine including five cumulative dose steps (7). Increased airway hyperresponsiveness was considered to be present if a ≤ 400 - μ g dose provoked at least a 20% decrease in the FEV_1 ($PD_{20}FEV_1$).

A blood eosinophil percentage of $\geq 4\%$ was considered eosinophilia. A wheal diameter of ≥ 3 mm was considered positive for skin prick tests to local aeroallergens (birch, timothy grass,

meadow fescue, mugwort, *Cladosporium herbarum*, dog, cat, horse, cow, and *Dermatophagoides pteronyssinus*).

Nonnormally distributed data were analyzed using the Mann-Whitney *U* test (dichotomous comparisons), and normally distributed data with Pearson's correlation test. We analyzed data with four categories, using one-way ANOVA. Because of the lack of impulse oscillometry reference values for adolescents, the upper quartile of R5 and lower quartile of X5 were considered suggestive of obstruction. A *P* value ≤ 0.05 was considered statistically significant. Data were analyzed using IBM SPSS 25.0.

Parents provided written informed consent at both study visits; children provided written informed consent only at the follow-up visit. The Research Ethics Committee of the regional university hospital approved the research protocol. Statistical approaches were verified by the biostatistics department of the university. Some of the results of this study have been previously reported in the form of an abstract (8).

In this population, current wheezing decreased during the follow-up time, whereas atopy increased (Table 1). A general decrease was observed in blood eosinophilia, F_{ENO} , and R5 levels. A significant proportion of parents ceased smoking during the follow-up time (43%). Only one parent started smoking after enrollment. Recent asthma symptoms (during the last 2 months) were reported by 22 (34%) teenagers, and a recent need for any asthma medication (during the last 2 months) by 23 (36%). None of the teenagers reported smoking.

Table 1. Demographics and Test Results of Study Patients (*n* = 64)

Patient Characteristics and Measurements	Preschool	Teenage
Age, yr, mean (range)	5.62 (3–7)	14.22 (12–16)
ISO-BMI, kg/m ² , mean (SD)	22.92 (4.16)	23.1 (3.8)
ISO-BMI > 25 kg/m ² , <i>n</i> (%)	16 (25)	15 (23)
Wheezing during previous yr, <i>n</i> (%)*	53 (83)	7 (11)
Parental smoking, <i>n</i> (%)*	21 (33)	13 (20)
Parental asthma, <i>n</i> (%)*	15 (24)	18 (28)
Positive SPT, <i>n</i> (%)	45 (70)	59 (92)
Urine cotinine, μ g/L, mean (SD)	2.06 (3.56)	—
F_{ENO} z-score, mean (SD)	2.54 (1.57)	2.08 (2.61)
Abnormal F_{ENO} (z-score ≥ 2 SD), <i>n</i> (%)	36 (60)	31 (49)
Blood eosinophils, %, mean (SD)	6.92 (4.76)	5.16 (4.19)
Blood eosinophilia ($\geq 4\%$), <i>n</i> (%)	53 (83)	34 (54)
FEV_1 z-score, mean (SD)	—	−0.45 (1.16)
Abnormal FEV_1 (z-score ≤ -1.645 SD), <i>n</i> (%)	—	9 (14)
R5, kPa/L/s, mean (SD)	0.89 (0.22)	0.36 (0.09)
Upper quartile R5 (R5 < 0.451 kPa/L/s), <i>n</i> (%)	—	12 (8)
X5, kPa/L/s, mean (SD)	−0.27 (0.10)	−0.09 (0.03)
Lower quartile X5 (X5 > −0.121 kPa/L/s), <i>n</i> (%)	—	7 (5)
Methacholine challenge, μ g, median (IQR)	—	1,709 (283–2,600)
AHR to methacholine ($PD_{20}FEV_1 \leq 400$ μ g), <i>n</i> (%)	—	17 (27)

Definition of abbreviations: AHR = airway hyperresponsiveness; F_{ENO} = fractional exhaled nitric oxide; ISO-BMI = sex- and age-adjusted body mass index for children; IQR = interquartile range; $PD_{20}FEV_1$ = provocative dose causing 20% decrease in FEV_1 ; R5 = respiratory resistance at 5 Hz; SPT = skin prick test; X5 = respiratory reactance at 5 Hz.

*Self-reported.

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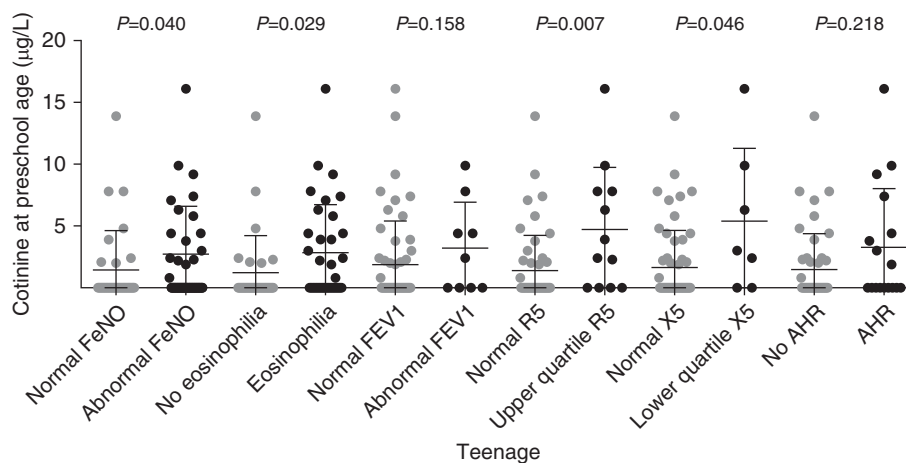


Figure 1. Preschool urinary cotinine levels compared with multiple pulmonary function tests and blood eosinophilia in teenage years. AHR = airway hyperresponsiveness measured with methacholine challenge; FE_{NO} = fractional exhaled nitric oxide; R5 = respiratory resistance at 5 Hz; X5 = respiratory reactance at 5 Hz.

Preschool cotinine levels were not associated with recent symptoms or need for medication in adolescence. Children with high urinary cotinine levels had parents with asthma ($P = 0.046$) more often than those with low levels. However, we observed that parental asthma was not connected with symptoms, need for medication, or lung function results.

Overweight (body mass index $> 25 \text{ kg/m}^2$) in adolescence correlated with baseline FEV_1/FVC ($P = 0.025$), R5–20 ($P = 0.007$), resonant frequency ($P = 0.002$), and AX ($P = 0.014$) at the same age, but not with preschool cotinine levels. Preschool urinary cotinine results correlated with FE_{NO} z-scores ($R = 0.227$; $P = 0.009$), blood eosinophils (%) ($R = 0.496$; $P < 0.001$), baseline FEV_1 z-scores ($R = -0.219$; $P = 0.041$), baseline R5 (kPa/L/s; $R = 0.272$; $P = 0.015$), baseline X5 (kPa/L/s; $R = -0.297$; $P = 0.009$), and baseline AX (kPa/L; $R = 0.316$; $P = 0.012$) in adolescence. Nevertheless, no correlation with methacholine responsiveness ($PD_{20}FEV_1$) or baseline FEV_1/FVC was observed.

In the dichotomous analysis of the data, a significant association with cotinine remained with FE_{NO} , blood eosinophilia, increased baseline R5, and decreased baseline X5. A significant association was not observed with decreased baseline FEV_1 or decreased $PD_{20}FEV_1$ (Figure 1). Only the children's baseline X5 remained low ($P = 0.020$) if the parents had quit smoking before the follow-up visit compared with nonsmoking families. The adverse effects of maternal smoking only could be detected with impulse oscillometry indices, X5 ($P = 0.003$) and AX ($P = 0.048$), as well as with blood eosinophilia ($P = 0.045$). Other measures showed a similar pattern.

The influence of overweight and preschool ETS on lung function and inflammation markers were significant but essentially different. In our study, children exposed to ETS were generally not overweight, and thus the mechanisms of lung function deficits are probably distinct. Both factors are known to be associated with lower economic status. As a result of questionnaire limitations, the confounding effect cannot be excluded.

We quantified the child's exposure to ETS at preschool age and compared it with objective measures of lung function in adolescence. Although the small sample size restricted the power of the analyses, we found long-lasting multifactorial effects of ETS on children with asthma. It is notable that the defects measurable with

impulse oscillometry suggest small airway dysfunction similar to chronic obstructive pulmonary disease (9) and persisted even when parents quit smoking during the follow-up. It is possible that the actual airway remodeling might occur in the child's epigenetic environment (10).

In conclusion, early-life exposure to tobacco smoke causes chronic airway inflammation and defects in lung function in children with asthma. These defects are measurable even a decade later as changes in FE_{NO} , blood eosinophil levels, impulse oscillometry, and spirometry. Smoking cessation is good; prevention is even better. ■

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Persistent Reduction of Mucin Production after Bronchial Thermoplasty in Severe Asthma

To the Editor:

Bronchial thermoplasty (BT) has been shown to reduce airway mucosa smooth muscle mass, reticular basement membrane thickness (1–3), and epithelium nerve fibers (4). Because an improvement of bronchial epithelial integrity was reported after BT, suggesting that it induces an epithelium injury–repair cycle (5), we evaluated the effects of BT on airway cell regeneration and proliferation, and mucin MUC5AC and IL-13 expression.

Patients with asthma and suboptimal asthma control despite the use of inhaled corticosteroids and long-acting β_2 -agonists, optimal environmental control, and treatment of asthma comorbidities were offered BT. The 33 patients who accepted BT consented to bronchial biopsies taken in the left lower lobe at the first bronchoscopy for right lower lobe treatment and 3 weeks later in the right lower lobe at the time of left lower lobe treatment. Eighteen of these patients accepted bronchial biopsies ≥ 12 months later (median: 28 mo, 12–48 mo). BT and bronchial biopsies were performed as previously described (2). Morphometry analysis was used to measure epithelium, and

immunohistochemistry was used to evaluate expression of MUC5AC, IL-13, Ki-67 (cell proliferation), Foxj1 (ciliated epithelial cells), and p63 (epithelial basal cells). MUC5AC staining was quantified as nontangential nonmetaplastic stained epithelium area reported to epithelium thickness, and expressed as percentage of staining intensity per micrometer of epithelium length using ImageJ. The numbers of Ki-67⁺, Foxj1⁺, IL-13⁺, and p63⁺ cells were quantified using ImageJ. A total of 500–1,000 epithelial cells with positive and negative nuclei for Ki-67 or Foxj1 were counted in randomized areas of the total biopsy surface, and results were expressed as the percentage of positive cell numbers against the total cell number. IL-13⁺ cells were counted in the total biopsy area and reported as the number of positive cells/mm². Double staining for p63 and Ki-67 by immunofluorescence was performed and the ratio of Ki67⁺ cells was expressed as the percentage of total p63⁺ cells. All measurements were done by two independent observers blinded to the status of BT treatment, with a mean observer variability between 4% and 8%.

Clinical data document the severity of asthma and improvement of clinical parameters 1 year after BT (Table 1). MUC5AC expression showed a persistent decrease at 3 weeks and ≥ 12 months after BT (Figure 1). IL-13⁺ cell numbers decreased at 3 weeks and ≥ 12 months after BT (3.3 ± 0.6 cells/mm² and 4.5 ± 0.9 cells/mm²) compared with baseline (6.05 ± 1.1 cells/mm², $P = 0.01$) and correlated with MUC5AC decreases at 3 weeks ($r = 0.81$, $P = 0.002$) and ≥ 12 months after BT ($r = 0.67$, $P = 0.024$, Pearson's correlation coefficient). The percentage of ciliated cells decreased 3 weeks after BT ($21.82 \pm 1.51\%$ Foxj1⁺ cells; $P = 0.04$) compared with baseline ($27.02 \pm 2.02\%$) and returned close to baseline level at ≥ 12 months ($24.81 \pm 2.67\%$ of Foxj1⁺ cells). Epithelial cell proliferation increased 3 weeks after BT ($24.37 \pm 3.85\%$ Ki-67⁺ cells) compared with baseline ($10.47 \pm 1.38\%$, $P = 0.002$) and decreased to baseline level at ≥ 12 months ($11.64 \pm 2.28\%$). The percentage of p63⁺ basal cells increased at 3 weeks after BT ($30.7 \pm 2\%$, $P = 0.009$) compared with baseline ($21.8 \pm 2.4\%$) and remained elevated at ≥ 12 months ($38.01 \pm 1.8\%$, $P = 0.0002$). The percentage of double-stained Ki-67⁺ and p63⁺ cells increased at 3 weeks after BT ($16.6 \pm 6\%$, $P = 0.04$) compared with baseline ($5.68 \pm 0.37\%$) and decreased to baseline values ($6.56 \pm 1.12\%$; $P = 0.001$) at ≥ 12 months.

This observational study reports subacute (3 wk) and long-term (12–48 mo) effects of BT on airway epithelium in patients with severe asthma. It shows a significant decrease in MUC5AC expression that persisted for years and correlated with a decrease in expression of IL-13, a potent inducer of mucus secretion and goblet cell hyperplasia (6), suggesting that a BT-induced reduction of IL-13 is one of the mechanisms involved in the decrease of MUC5AC. It also documents a decrease in ciliated cell (Foxj1⁺) numbers, which returned to baseline levels in association with the proliferation of basal progenitor cells. The balance of progenitor differentiation into secretory versus ciliated lineages is critical to maintain airway epithelium homeostasis. This suggests that the epithelium regeneration in response to BT-induced epithelial injury decreases mucin expression, improves goblet cell metaplasia, and regenerates the ciliated cell layer, providing a durable improvement of airway epithelium integrity. This is likely clinically significant because the goblet cell hyperplasia/metaplasia observed in

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