

RESEARCH LETTER

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Cluster analysis of exhaled volatile organic compounds (VOCs)—link between environmental exposure and asthma in preschool children

To the Editor,

Atopic diseases and asthma arise as a result of interrelationship of environmental factors and genetic predisposition during pregnancy, and in the first years of life.^{1,2} Urban children are exposed disproportionately to air pollution, and other environmental exposures that trigger asthma symptoms.³ Particulate matter PM_{2.5} and PM₁₀ produced during combustion, by traffic and industrial activities, are supposed to be responsible for harmful health effects.⁴ Meta-analysis based on birth cohorts data including almost 15,000 children from three European countries clearly showed that living since birth in the environment with increased PM_{2.5} was connected with increased asthma prevalence through adolescence.⁵ In children under the age of 5, diagnosis of asthma is significantly constrained,⁶ mainly because of difficulties in performing spirometry in this age group. Profiling of volatile organic compounds (VOCs) in exhaled breath adds information about underlying pathophysiology, for example inflammation, and appears as new, promising method in asthma diagnosis, monitoring and treatment.^{7–9} Endogenous VOCs are produced during metabolic processes of the human's body and their settled microorganisms and can be used as biomarkers.¹⁰ Therefore, it is of interest to evaluate the VOCs in exhaled breath in children with regard to the presence of asthma and the exposure to air pollution around their residence. The aim of the present study was to determine whether there is a link between asthma and exposure to environmental pollution in the context of exhaled VOCs patterns in preschool children.

The present study was conducted in a group of 5-year-old children attending randomly chosen kindergartens in the urban and rural areas of living, taking into account areas with low (green zone), medium (yellow zone) and high levels (red zone) of air pollution based on data obtained from reports of the Polish State Environmental Monitoring.¹¹ Participants recruitment was conducted until the total number of 138 children attending kindergartens in urban areas and 138 in rural areas was reached in each of the groups. After receiving permission from headmasters of the kindergartens, the research team organized meetings with parents/caregiver to explain the aim and procedures of the study,

to collect parental/caregivers consent for children's participation in the study and to collect the questionnaires filled by the parents/caregivers. For each child, first visit covered skin prick testing (SPT), the measurement of VOCs in exhaled breath and the distribution of a backpack containing GilAir Plus Basic personal aspirators for 24h measurement of air pollution (PM_{2.5} and PM₁₀). The study was approved by the Ethics Committee of the Medical University of Lodz (decision No. RNN/390/17/KE). The diagnosis of atopic disease (food allergy, asthma, allergic rhinitis or atopic dermatitis) was made by the allergist based on the skin prick testing and current guidelines; asthma was diagnosed based on GINA.¹² The questionnaire addressed to the caregivers of the child included data on the child's state of health, exposure to air pollution, living conditions, socio-economic data, information about doctor-diagnosed allergy and asthma, the severity and frequency of diseases, medications taken and hospitalizations. The collected dust samples from personal aspirators were analysed in the Aerosol Laboratory of the Noffer Institute of Occupational Medicine in Łódź. The measurement of VOCs in exhaled breath in each child was performed, using the gas chromatography–mass spectrometry (GC–MS) method in a laboratory in the Łódź Regional Park of Science and Technology. Breath samples were collected in Tedlar® bags and then transferred to the Tenax TA-filled tubes (GERSTEL GmbH & Co. KG, Mülheim an der Ruhr, Germany) using a peristaltic pump in maximum 3 h after material sampling. Mass spectra were collected using a Pegasus 4D time-of-flight mass spectrometer (Leco Corp., St. Joseph, MI, USA). The obtained mass spectra were compared with NIST/EPA/NIH and the Wiley Registry of Mass Spectral Data mass spectral libraries. The obtained data were processed using the Statistical Compare feature implemented in the ChromaTof software (Leco Corp., St. Joseph, MI, USA). The complete description of the methodological assumption has been published elsewhere.¹³

To determine the possible link between environmental exposure and asthma in pre-schoolers we have planned multistep analysis: (i) clustering of exhaled VOCs of children living in areas with low (green zone), medium (yellow zone) and high levels (red zone)

of air pollution, (ii) between-cluster comparisons of clinical data, (iii) between-cluster comparisons of individual exposure to PM_{2.5} and PM₁₀. We performed a non-supervised statistical cluster analysis based on the VOCs concentration in exhaled breath that enabled us to stratify the data set without any previously defined hypothesis.

Two hundred thirty-five children completed the study. All participants were reported being Caucasians with a mean age of 5 years (SD 2.8 months). Study participants included 53.2% females. The majority of the subjects (69.8%) resided in non-smoking homes, and 41.7% in homes that had at least one pet. About 23% of the subjects were diagnosed with asthma, 13.6% with allergic rhinitis, 8.9% with food allergy, 11.5% with atopic dermatitis within the last 12 months. The mean concentrations of PM_{2.5} and PM₁₀, measured by personal meters, were 19.8 and 47.6 $\mu\text{g}/\text{m}^3$, respectively, without any significant difference between Łódź region and province region. The mean concentration of PM_{2.5} and PM₁₀ were statistically higher in red zone of Łódź than in red zone in the province. There were no differences in concentrations of PM_{2.5} and PM₁₀ between the green zones and of PM₁₀ concentration between the yellow zones. Mean concentration of PM_{2.5} was significantly higher in the yellow zone of province comparing with the yellow zone of Łódź. Type of building of living was significantly differentiating PM_{2.5} ($p < 0.001$) and PM₁₀ ($p = 0.004$) as measured by personal meters. Based on post hoc test, PM_{2.5} level was significantly higher in group living in tenement house vs. all other types of buildings, and PM₁₀ was significantly higher in tenement house group vs. all other groups but tower blocks. Children living at coal-fired homes or heated with tiled-stove had significantly different level of PM_{2.5}, MD = 4.00 CI95 [0.01; 8.00]; $p = 0.016$ as well as PM₁₀, MD = 11.90 CI95 [0.01; 19.80]; $p = 0.021$ than children living in homes heated with more ecological methods (central heating/gas/oil). Children with smoking parents had significantly different level of PM_{2.5}, MD = 4.00 CI95 [0.01; 11.90]; $p = 0.018$ as well as PM₁₀, MD = 15.90 CI95 [4.00; 23.80]; $p = 0.008$ than children with non-smoking parents. PM_{2.5} and PM₁₀ from personal meters were not significantly different in regard to: presence of carpets in house, frequency of cleaning, presence of pets at home, contact with animals outside of home, presence of industrial plant in the neighbourhood. There was no relationship between PM and occurrence of asthma and atopy. The lack of a link between the current exposure to PM and the diagnosis of asthma (and atopy) prompted us to look for the missing element—VOCs. We identified

Key Messages

- The clustering results of exhaled volatile organic compounds (VOCs) in exhaled breath revealed that children diagnosed with asthma have specific gaseous profile.
- Our observation suggests that VOCs may be used as potential asthma markers
- VOC collection may be influenced by different factors, for example environmental

37 VOCs compounds that could accurately differentiate between children with asthma and children with no asthma exposed to environmental pollution. Clustering of exhaled VOCs revealed two clinically distinct groups of children (Table 1). Cluster 1, defined as *non-asthmatic*, included 72.8% ($n = 171$) of our cohort. Children from Cluster 1 were recruited from the green zone predominantly ($p < 0.0001$). Among children from Cluster 1, we observed atopic dermatitis more frequently (Table 1). Cluster 2, defined as *asthmatic*, included 27.2% ($n = 64$) of our cohort. Children from this cluster were living in the red zone predominantly ($p < 0.0001$) and more frequently suffered from asthma (Table 1). Between-cluster comparison showed significantly higher individual exposure to PM_{2.5} (measured with personal meters) in children from Cluster 2 compared with children from Cluster 1 ($p = 0.0144$). There was no such difference for PM₁₀ individual exposure ($p = 0.2959$) (Figure 1).

The clustering results of volatile organic compounds in exhaled breath (VOCs) analysis revealed that the group of children diagnosed with asthma has its specific VOCs profile. Our observation suggests that extracted compounds may be used as potential asthma markers and link with environmental exposure. In our study, living in the red zone with the highest level of dust pollution defined on account of distribution of annual mean concentration values of PM_{2.5} (but not PM₁₀) of the Polish State Environmental Monitoring was significantly more often associated with asthma diagnosis as measured by VOCs profile.

Our observation also suggests that air pollutions are likely to have stronger influence on triggering asthma in children than sensitization, perhaps due to the induction of non-allergic inflammation. The value of the study is represented by the identification of particular VOCs in relation to asthma in children and additional confirmation that worse air quality indeed plays crucial role in the

TABLE 1 Clinical, environmental and demographic data by clustering results

	Non asthma cluster (n = 171)		Asthma cluster (n = 64)		OR ^a	95% CI		p
	N	%	N	%				
Male	80	46.8%	30	46.9%	ref			
Female	91	53.2%	34	53.1%	1.00	0.56	1.77	0.9900
Mother's education: primary school	8	4.7%	1	1.6%	ref			
Vocational school	5	2.9%	3	4.7%	4.80	0.38	59.90	0.2232
High school	55	32.2%	21	32.8%	3.05	0.36	25.93	0.3062
University	89	52.0%	39	60.9%	3.51	0.42	28.99	0.2445
Mother employed	136	79.5%	48	75.0%	0.49	0.24	1.04	0.0618
Father's education: primary school	10	5.8%	3	4.7%	ref			
Vocational school	17	9.9%	7	10.9%	1.37	0.29	6.54	0.6911
High school	56	32.7%	27	42.2%	1.61	0.41	6.32	0.4971
University	64	37.4%	26	40.6%	1.35	0.34	5.32	0.6641
Father employed	142	83.0%	61	95.3%	2.58	0.30	21.87	0.3854
Living conditions: steam on windows	82	48.0%	34	53.1%	1.06	0.60	1.90	0.8337
Signs of moisture	25	14.6%	7	10.9%	0.66	0.27	1.61	0.3590
Signs of mould	41	24.0%	8	12.5%	0.41	0.18	0.94	0.0340
Cockroaches	3	1.8%	1	1.6%	0.84	0.09	8.22	0.8799
Carpets	82	48.0%	35	54.7%	1.13	0.63	2.03	0.6736
Pets at home	73	42.7%	25	39.1%	0.76	0.42	1.36	0.3519
Parents smoking	50	29.2%	21	32.8%	1.06	0.57	1.98	0.8430
Place of living: city	102	59.6%	9	14.1%	ref			
Rural areas	69	40.4%	55	85.9%	9.03	4.19	19.47	<0.0001
Quality of the outdoor air: red zone	40	23.4%	35	54.7%	ref			
Yellow zone	56	32.7%	21	32.8%	0.43	0.22	0.84	0.0141
Green zone	75	43.9%	8	12.5%	0.12	0.05	0.29	<0.0001
Allergy diseases: atopic dermatitis	25	14.6%	2	3.1%	0.17	0.04	0.75	0.0189
Food allergy	14	8.2%	7	10.9%	1.27	0.49	3.31	0.6225
Allergic rhinitis	26	15.2%	6	9.4%	0.53	0.21	1.35	0.1844
Asthma	31	18.1%	23	35.9%	2.32	1.22	4.41	0.0106
Skin prick tests (SPT) Positive-atopy	50	29.2%	18	28.1%	0.95	0.50	1.79	0.8668
House dust mites (HDM)	19	11.1%	8	12.5%	1.19	0.49	2.88	0.6995
Trees	23	13.5%	4	6.3%	0.44	0.15	1.34	0.1502
Grasses	19	11.1%	10	15.6%	1.55	0.67	3.55	0.3027
Moulds	10	5.8%	5	7.8%	1.42	0.46	4.33	0.5397
Mugwort	4	2.3%	1	1.6%	0.69	0.08	6.27	0.7388
Cat	7	4.1%	5	7.8%	2.06	0.63	6.77	0.2315
Dog	2	1.2%	0	0.0%				0.9978

Note: Data are presented by odds ratios (OR) with 95% CI.

Bolded values indicate significant associations.

^aDependent variable: cluster 2 vs. cluster 1.

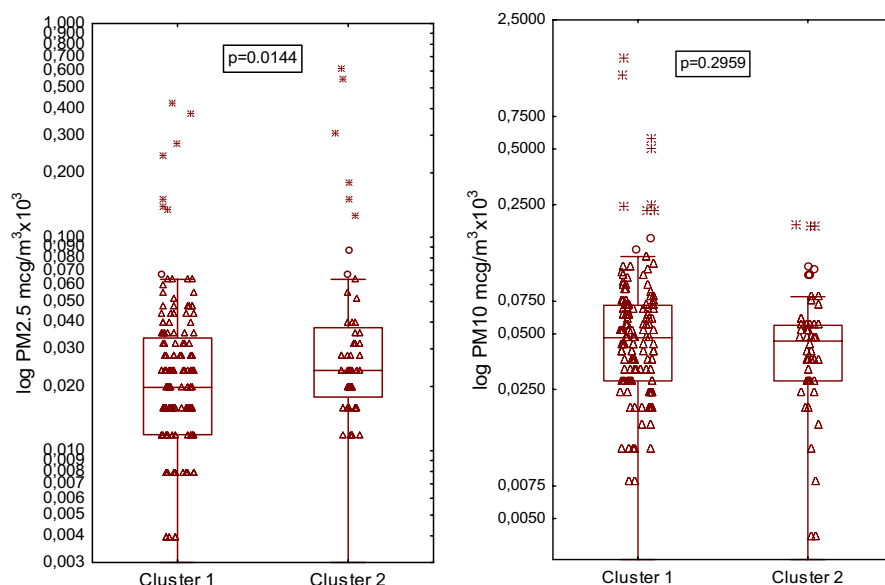


FIGURE 1 Between-cluster comparison according to individual exposure to PM_{2.5} and PM₁₀

development of respiratory diseases in paediatric population. What is more, we confirmed that the specific VOC profile may reflect the activity of the epithelium and the ecosystem of local bacteria and identifies children vulnerable to the adverse effects of the urban environment.

KEYWORDS

allergic diseases, asthma, children, environmental exposure, volatile organic compounds

AUTHOR CONTRIBUTION

MBK, AB, WS and JJ participated in the design and data collection of the study. EM and KS were responsible for the data collection and contact with the study participants. PM prepared the draft of the results upon supervision of MBK, JJ, DK and KP. All authors participated in the data analysis and interpretation. MBK, JJ, KP and DK prepared the first draft of the manuscript. All authors revised and approved the final version to be published. All authors are accountable for all aspects of the work.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this manuscript.

DATA AVAILABILITY STATEMENT

Data available on demand.

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