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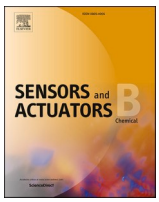

Use of the electronic nose to screen for small airway dysfunction in schoolchildren

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ABSTRACT

Accurate methods that can be used to detect asymptomatic children with asthma in schools are lacking. Small airway dysfunction is a precursor of asthma. The objective of this study was to use the electronic nose (E-nose) to detect small airway dysfunction in elementary schoolchildren at risk of asthma and evaluate the acute effects of air pollution on respiration. We conducted this study in an elementary school. The study recruited 40 asymptomatic students with a history of asthma and 40 age- and sex-matched children without a history of asthma to take the breath test. We used an E-nose to analyze volatile metabolites and gas chromatography-mass spectrometry to analyze common air pollutants in exhaled breath. After excluding eight subjects in pilot tests, we included 72 subjects in the final analysis of the breath test. The sensitivity of detecting small airway dysfunction with the E-nose was 0.92, the specificity was 0.95, the positive predictive value was 0.79, the negative predictive value was 0.98, the overall accuracy was 0.94, and the leave-one-out cross-validation accuracy was 0.74. The area under the curve was 0.98 (95 % confidence interval: 0.96–1.00). Methyl tert-butyl ether was the only ambient air pollutant that had a significant negative correlation with the maximum mid-expiratory flow (r = -0.33, $P < 0.05$). The E-nose is highly accurate at detecting small airway dysfunction in children at high risk for asthma. An analysis of exhaled breath can also be used as a personal monitoring method to assess the acute effects of air pollution on respiration.

1. Introduction

Asthma is an important chronic respiratory disease worldwide. Approximately 358 million people worldwide were estimated to have had asthma in 2015, including approximately 14 % of the world's children [1]. Asthma increases the absenteeism rate of schoolchildren and reduces test performance [2]. Asthma is even associated with increased mortality in children [3]. However, the diagnosis of asthma relies primarily on self-reports of wheezing in the past 12 months and self-reports of a physician diagnosis [4]. Accurate testing methods that

Abbreviations: E-nose, electronic nose; GC–MS, gas chromatography-mass spectrometry; VOCs, volatile organic compounds; AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; ISAAC, International Study of Asthma and Allergies in Childhood; ACT, Asthma Control Test; ATS/ERS, American Thoracic Society/European Respiratory Society; FVC, forced vital capacity; FEV1, forced expiratory volume in the first second; FEV1/FVC, the ratio of FEV1 to FVC; MMEF, the maximum mid-expiratory flow; FEF_{25-75} %, forced expiratory flow between 25 and 75 % of the total lung volume; FeNO, fractional exhaled nitric oxide; NIST, National Institute of Standards and Technology; CI, confidence interval; MTBE, methyl tert-butyl ether.

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can be used to detect asymptomatic children with asthma in school or the community are lacking.

Small airway dysfunction is considered a precursor of asthma [5]. In asthma, the small airways are thickened by chronic inflammation in the epithelium, submucosa, and muscle [6], which correlates with the frequency and severity of dyspnea and asthma exacerbations [7]. Obstruction of the small airways results in very little change in measurable airway resistance, which is why the small airways are referred to as the silent zone for lung disease. A prospective longitudinal study suggested that small airway dysfunction can precede the development of asthma [8].

Air pollution is a significant environmental trigger of asthma attacks [9]. Children are uniquely vulnerable to the effects of air pollution because the airway epithelium of growing children is more permeable to air pollutants, and their pulmonary defenses against particulate pollution and gaseous pollution have not yet fully evolved [10]. The objective of this study was to use the electronic nose (E-nose) to detect small airway dysfunction in elementary schoolchildren at risk of asthma and evaluate the acute effects of air pollution on respiration.

2. Methods

2.1. Study design and participants

We conducted this study at an elementary school in New Taipei City, Taiwan. There were two steps of enrollment. First, we identified students with a past history of asthma in the school's student health information system. We invited these high-risk children with a history of asthma to participate in a health promotion program. The exclusion criteria for the breath test were that the children did not provide a medical certificate from their physicians or informed consent from their legally authorized representative. After the enrollment of these high-risk children with a history of asthma, we recruited age- and sex-matched children without a history of asthma in the second step of enrollment. We used an E-nose to analyze volatile metabolites and gas chromatography-mass spectrometry (GC–MS) to analyze common air pollutants in exhaled breath. This study was approved by the Institutional Review Board of National Taiwan University Hospital (No. 201902044RIND).

2.2. Medical history

We obtained each child's medical history with the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire and the Asthma Control Test (ACT). The school nurse assisted the child's parents with completing the questionnaire. The ISAAC questionnaire was designed for an epidemiological study involving a survey of 2 million children worldwide to evaluate the prevalence of atopic disease in individuals with various ethnic backgrounds. We used the ISAAC questionnaire to collect the children's asthma symptoms [11]. We also collected the allergy history of the children and their parents: (1) Has your child used any asthma medication in the past two weeks? (2) Has your child been diagnosed with "allergic rhinitis" by a doctor in the past? (3) Has your child had a fever or cold symptoms in the past week? (4) Has your child had a fever and cold symptoms in the past week? (5) Has the doctor or nursing staff ever said that your child has had an allergic reaction? (6) In the past 12 months, have you kept a cat? (7) Have you kept a dog in the past 12 months? (8) Do family members living with you now smoke? (9) Does the child's father have asthma, allergic rhinitis, or atopic dermatitis? (10) Does the child's mother have asthma, allergic rhinitis, or atopic dermatitis?

2.3. Environmental exposure

We obtained information on indoor air pollution, outdoor air pollution, and lifestyle factors in the questionnaire. The questions about outdoor air pollution included the following: (1) Is your current residence near the main road (the main road refers to a secondary road)? (2) How many trucks (cars) pass along your residential street every day? (3) Do you smell weird odors around your environment (home, school, cram school, etc.)? (4) Are there factories near the home(s) in which you lived after your child turned two years old (within a straight-line distance of 3 km, approximately 8 min by train)? (5) Can the following be found near your child's home: factories, chimneys, farmland, or sites for open burning? (6) What kind of transportation does your child use to get to school (walk, bicycle, motorcycle, car, bus)? The questions about indoor air pollution included the following: (1) Does your family have the habit of burning incense to worship? (2) Do you have the habit of using fragrant incense in your home (including sandalwood, aromatic incense, etc.)? (3) Do you have the habit of using mosquito repellent incense in your home (including electric mosquito repellent)? (4) Is there any carpet in your home? (5) Do you have the habit of using insecticides in your home? (6) Are there mold spots (dark or green stains) on the walls of your house? (7) Are there mold spots (dark or green stains) in the bathrooms in your house? (8) Does your house smell musty? (9) Have you ever found stains caused by moisture on the ceiling, floor, or wall of your house? (10) In general, how much water accumulates in your house every year? (11) In the last month, how many cockroaches did you see in your house (each time you saw one, it counts as one)? The questions about lifestyle factors included the following: (1) Have you ever kept a cat in the past 12 months? (2) Have you ever kept a dog in the past 12 months? (3) Do you have the habit of using fragrant incense in your home (including sandalwood, aromatic incense, etc.)? (4) Do you use a dehumidifier in your child's room every day? (5) Do you use air conditioning in your child's room every day? (6) Do you have the habit of using an air purifier in your home? We also obtained the average daily concentration of seven air pollutants (CO, NO, NO_2, NO_x, O_3, PM_{10}, $PM_{2.5}$) on the day of the breath test from the air monitoring station near the school.

2.4. Pulmonary function test

A spirometric pulmonary function test was performed with a Spirolab III device (Medical International Research, Roma, Italy). We performed a standard pulmonary function test according to the recommendations of the American Thoracic Society (ATS)/European Respiratory Society (ERS) [12]. We obtained the forced vital capacity (FVC), forced expiratory volume in the first second (FEV_1), the ratio of FEV_1 to FVC (FEV_1/FVC), and the maximum mid-expiratory flow (MMEF). The MMEF is the forced expiratory flow between 25 and 75 % of the total lung volume ($FEF_{25–75}$ %). For screening, we defined small airway dysfunction as $FEF_{25–75}$ % <80 % of the predicted value [13].

2.5. Fractional exhaled nitric oxide (FeNO)

Fractional exhaled nitric oxide (FeNO) is a noninvasive biomarker of eosinophilic airway inflammation [14]. We measured FeNO according to the Guidelines of the ATS. We used the NObreath system (Bedfont Scientific Ltd, ME17 1JA, UK) and followed the ATS/ERS recommendations [15].

2.6. Collection of breath

Our laboratory has designed a device for collecting breath [16]. The breath collection device is equipped with an active carbon gas filter (Spacciani Spa, Origgio, Italy) to reduce contamination with environmental volatile organic compounds (VOCs) when the subjects inhale air through the device and a silica reservoir to reduce the influence of humidity on the sensor. The device contains a VOC filter (Spacciani Spa, Origgio, Italy) to reduce contamination with environmental VOCs and a flow resistance standard (Model 7100R-R200, Hans Rudolph, Shawnee, KS, USA) to maintain a constant flow rate of 6 L/min. Using a

mainstream capnometer (EMMA Emergency Capnograph, Masimo, CA, USA), we monitored the concentration of CO_2 in the exhaled breath and only collected alveolar air when the concentration of CO_2 reached its peak. The air was stored in a FlexFoil Plus gas sampling bag (SKC Inc., PA, USA), which is specially designed for breath-gas analysis and has good storage reliability for low-molecular-weight VOCs at the ppb level. The participants were asked to refrain from eating before the test for eight hours. The subjects gargled with water, wore a nose clip, and then exhaled into a disposable mouthpiece connected to the device (Fig. 1).

2.7. E-nose analysis

One liter of exhaled air was analyzed with the E-nose within 30 min by trained personnel. We used the Cyranose 320 E-nose (Sensigent, CA) to analyze the breath samples. The E-nose has 32 thin-film nanocomposite sensors. Because the expiratory flow rate significantly affects the measurement [17], a constant flow rate of 120 cc/min was used for all measurements. The room air pumped into the E-nose was analyzed to provide the baseline sensor response (R_0). Since the sensor is sensitive to humidity, the purge inlet of the E-nose was connected to a silica reservoir to absorb moisture from the breath. The raw data were normalized and autoscaled to eliminate background noise and exclude outliers, and then the prediction model was constructed [18,19]:

$$\text{Sensor response}: \frac{\Delta R}{R_o} = \frac{(R_{max} - R_0)}{R_0} \tag{1}$$

The raw data were normalized using the equation:

$$\sum_{k=1}^{NV} x_{ik}^2 = ci \tag{2}$$

where k designates the sensor, i designates the gas, and NV is the total number of sensors. Then, the data were autoscaled to the unit variance, which refers to mean centering, and then divided by the standard deviation:

$$x_{ik}^{'} = \frac{x_{ik} - \bar{x}_k}{s_k} \tag{3}$$

where $x_{ik}^{'}$ is the autoscaled response, x_{ik} is the relative sensor response, $\bar{x}_k$ is the mean value of the normalized response for the specific sensor, and s_k is the standard deviation:

$$S_k = \left[\frac{1}{\text{NP-1}} \sum_{i=1}^{NP} (x_{ik} - \bar{x}_k)^2\right]^{1/2} \tag{4}$$

Autoscaling removes any inadvertent weighting that arises due to arbitrary units. After autoscaling, the value distribution of each sensor across the entire database was set to a mean value of zero and unit standard deviation [18]. Each sample was analyzed ten times. Then, we deleted the first measurement and obtained a mean value of each sensor's responses, as suggested by the manufacturer [20].

2.8. GC–MS analysis

One liter of exhaled air was stored in a Bottle-Vac canister (Entech Instruments Inc., Simi Valley, CA) and analyzed within 48 h in the Green Energy and Environmental Research Laboratories of the Industrial Technology Research Institute. The gas samples were analyzed using an Entech 7500A Robotic Headspace Autosampler attached to an Entech 7150 Air/Headspace Preconcentrator (Entech Instruments Inc., Simi Valley, CA, USA) coupled with an Agilent 6890 N GC/5975C MS (Agilent Technologies, Santa Clara, CA, USA). An Agilent J&W DB-1 nonpolar column was used. The analysis was performed in accordance with the U.S. Environmental Protection Agency Method TO-15 for the analysis of VOCs in air samples. An internal standard spiking mixture containing bromochloromethane, 1,4-difluorobenzene, chlorobenzene-d5, and 1-bromo-3-fluorobenzene was added to the sample as a calibration standard. The internal standards were introduced into the trap during the collection time for all calibration, blank, and sample analyses. A total of 63 VOC (63-VOC) standards, which are included in Method TO-15, were quantitated and included in the 63-VOC analysis (Supplementary Table 1).

2.9. Data preprocessing

Raw data preprocessing for GC–MS was first performed by MSD ChemStation Data Analysis software (Agilent Technologies, Santa Clara, CA, USA) in the quantitative analysis. The compounds were then identified using the National Institute of Standards and Technology (NIST) library of the NIST11 database (NIST/EPA/NIH Mass Spectral Library, 2011 version). A missing value for a concentration that was under the detection limit or for which the limit of detection was less than 60 was replaced by the minimum value in the entire dataset divided by the square root of 2 [21].

2.10. Validation analysis to quantify the volatile compounds

Detected peaks in ion chromatograms were identified and confirmed using the NIST11 database. For the identification of each compound, this study first matched the observed peak and standard fragmentation provided by the NIST library and then matched the retention indices (RIs) of each compound. The procedure of peak accuracy validation included the following steps:

Step 1. The match factor was set at 60 % to measure the fitness between the sample spectrum and the reference spectrum.

Step 2. Isothermal Kovats RIs were further compared to identify volatile compounds [22]. The formula is shown below:

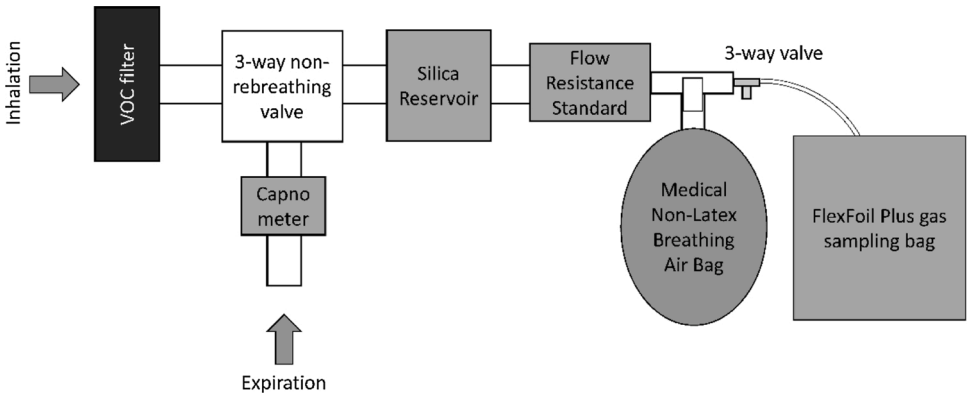

Fig. 1. Breath collection device.

$$RI_x = 100n + \frac{\log(t_x) - \log(t_n)}{\log(t_{n+1}) - \log(t_n)} \quad (5)$$

where t_n and t_{n+1} are retention times of the reference n-alkane hydrocarbons eluting immediately before and after chemical compound "X", and t_x is the retention time of compound "X."

Step 3. If the comparison between the observed peaks and standards in the NIST library showed more than 75 % conformity, the RI value for each compound was checked against the reference data.

2.11. Statistics

This study used an independent *t*-test to compare numerical variables and a chi-square test to compare categorical variables for the demographic characteristics of children. We applied Pearson's correlation analysis to show the correlation coefficients between air pollutants and pulmonary function. Using standard pulmonary function tests as the reference standard for small airway dysfunction, we assessed the performance of the ISAAC questionnaire and the E-nose based on sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, and area under the receiver operator characteristic (ROC) curve (AUC). The 95 % confidence interval (CI) was computed with 2000 stratified bootstrap replicates. AUC values of 0.7−0.8, 0.8−0.9, and 0.9–1 were regarded as good, very good, and excellent diagnostic accuracy, respectively [23]. We used a bootstrap method and calculated the accuracy of 2000 iterations to determine the parameters of the machine learning methods that yielded the highest predictive accuracy. We applied linear discriminant analysis (LDA) to construct the prediction model with the MASS package in R and used the pROC package to construct the ROC curve. To explore the effect of medication on the breath test, we conducted a metabolite set enrichment analysis (MSEA) to identify biologically meaningful patterns enriched in quantitative metabolomic data [24]. A two-tailed *P*-value less than 0.05 was considered statistically significant.

2.12. Sample size estimation

We calculated the sample size by estimating the standard error of the percentage of correctly classified patients [25]:

$$SE = \sqrt{\frac{C(1-C)}{n}} \quad (6)$$

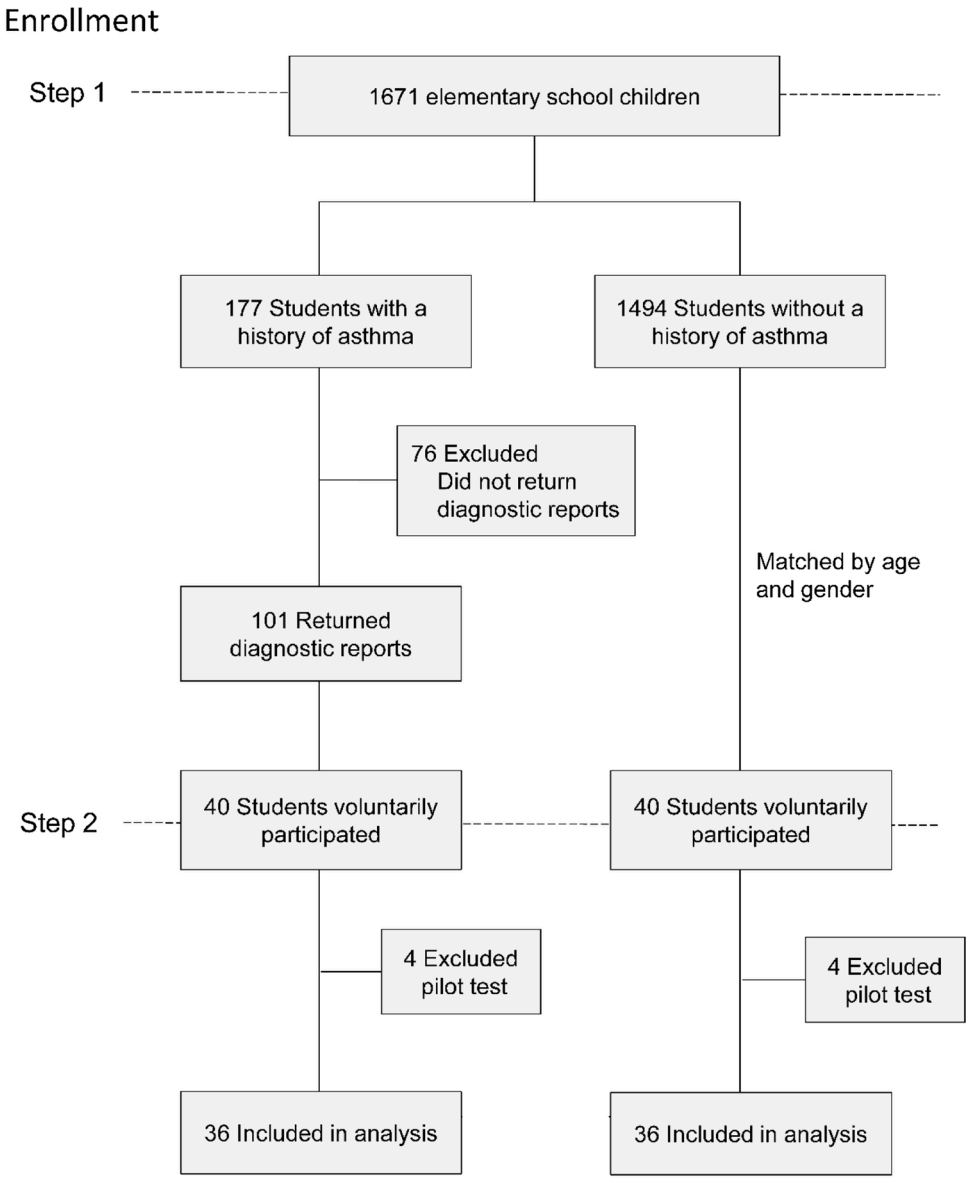

Fig. 2. Flowchart of the study protocol.

where SE is the standard error, C is the percentage of patients classified correctly, and n is the estimated sample size. Based on a previous study of diagnostic accuracy using the E-nose to diagnose asthma, the accuracy was 0.79 (95 % CI: 0.63−0.94) [26]. We used an SE of 5% and an acceptable accuracy (C) of 0.8. The required sample size was 66.

3. Results

There were 1671 students in the school, and 177 of them were high-risk children. After excluding 76 students without medical certificates and 61 students who did not provide informed consent, 40 high-risk children and 40 healthy children underwent our breath test between April 2019 and November 2019. Our pilot study used eight schoolchildren to test different methods of collecting children's breath samples. After excluding those eight subjects, a total of 72 subjects were included in the final analysis, including 36 subjects with a past history of asthma and 36 subjects without a past history of asthma (Fig. 2). Among the 72 subjects, we identified 12 subjects with small airway dysfunction and 60 subjects without small airway dysfunction by the pulmonary function test. The mean age was 9.1 years (SD 1.6), and 59.7 % were male. Subjects with small airway dysfunction had higher concentrations of $PM_{2.5}$ and PM_{10} on the day of the examination. The FEV1, FEV1/FVC, and MMEF were significantly lower in subjects with small airway dysfunction than in the control group. The FeNO concentration was elevated in subjects with small airway dysfunction. There was no difference in the asthma control scores between subjects with and without small airway dysfunction (Table 1).

Table 1
Characteristics of the schoolchildren.

Characteristics	Small airway dysfunction (n = 12)	Control (n = 60)	P value
Gender, male/female, No.	8/4	35/25	0.75
Age, mean (SD), y	9.17 (1.47)	9.13 (1.59)	0.95
Preterm birth, No. (%) [a]	2 (16.67)	9 (15.00)	0.88
Breastfeeding, No. (%)	9 (75.00)	49 (81.67)	0.20
Low birthweight, No. (%) [b]	0 (NA)	3 (5.00)	0.57
Weight, mean (SD), kg	31.53 (8.51)	31.17 (9.35)	0.90
Height, mean (SD), cm	132.73 (8.44)	132.22 (9.42)	0.87
BMI, mean (SD), kg/m^2	17.74 (3.48)	17.49 (3.32)	0.82
Asthma control test score	23.29 (2.63)	23.12 (3.46)	0.91
Daily mean (SD) $PM_{2.5}$, μg/m^3 [c]	23.00 (12.81)	19.48 (10.73)	0.36
Daily mean (SD) PM_{10}, μg/m^3 [c]	39.16 (19.85)	37.90 (17.87)	0.84
FVC, mean (SD), %	102.77 (11.23)	105.43 (12.99)	0.51
FEV1, mean (SD), %	92.38 (7.08)	107.77 (12.06)	<0.05
FEV1/FVC, mean (SD), %	80.53 (6.65)	90.41 (5.18)	<0.05
MMEF, mean (SD), %	69.01 (8.47)	111.44 (20.50)	<0.05
FeNO, mean (SD), ppb	23.50 (24.33)	19.77 (17.93)	0.54
Medication for asthma in the past two weeks, No. (%)	4 (33.33)	12 (20.00)	0.21
Inhaled corticosteroids or bronchodilators, No. (%)	3 (25.00)	6 (10.00)	0.15
Oral drugs, No. (%)	1 (8.33)	7 (11.67)	0.60

[a] Preterm birth is defined as a birth that occurs between 20 and 37 weeks of pregnancy.
[b] Low birthweight is defined as birthweight less than 2,500 g (5 pounds, 8 ounces).
[c] Daily mean $PM_{2.5}$ and PM_{10} were obtained from nearby air monitoring stations on the examination day.

The ISAAC questionnaire had a good NPV for the detection of small airway dysfunction; however, the sensitivity, specificity, PPV, and AUC were low (Table 2). The sensitivity of the E-nose was 0.92, the specificity was 0.95, the PPV was 0.79, the NPV was 0.98, the overall accuracy was 0.94, and the leave-one-out cross-validation accuracy was 0.74. The AUC was 0.98 (95 % CI: 0.96–1.00) (Fig. 3).

Methyl tert-butyl ether (MTBE) was the only ambient air pollutant that had a significant negative correlation with the MMEF (r = -0.33, P < 0.05) (Fig. 2). The concentration of MTBE was negatively correlated with the MMEF (r = -0.31, P = 0.01), FEV1 (r = -0.24, P = 0.04) and FEV1/FVC (r = -0.30, P = 0.01) and was positively associated with the FeNO concentration (r = 0.03, P = 0.83). The concentration of $PM_{2.5}$ was negatively correlated with the MMEF (r = -0.27, P = 0.03), FEV1 (r = -0.33, P = 0.01) and FEV1/FVC (r = -0.14, P = 0.27) (Fig. 4).

4. Discussion

To the best of our knowledge, this is the first study using the E-nose to detect small airway dysfunction, a precursor of asthma. This study showed that the E-nose was highly accurate at detecting small airway dysfunction among asymptomatic schoolchildren at risk of asthma. Inhalation is the most important pathway by which humans are exposed to common air pollutants; however, methods of assessing the internal exposure dose of air pollutants are still lacking. In this study, we showed that the internal dose of air pollution could be quantified by analyzing the air pollutants in exhaled breath.

Children with asthma might not have regular follow-up in the hospital, and their parents might not be aware of asthma attack symptoms. Therefore, school is the best place to provide health management and education programs for asthmatic children. However, the methods currently available are inadequate for detecting asymptomatic small airway dysfunction in children with asthma. Asthma is characterized by small airway inflammation and airflow limitation. Small airway dysfunction plays a role in the pathobiology of asthma and is recognized as a potential target for optimal control of the disease [27]. Hederos et al. used the ISAAC questionnaire in outpatient children; the sensitivity of the ISAAC questionnaire was 77 % and the specificity was 97.5 %. The

Table 2
Accuracy of the ISAAC questionnaire and electronic nose in the screening of small airway dysfunction.

Items	Sensitivity	Specificity	PPV	NPV	AUC (95 % CI)
Ever had wheezing or whistling in the chest	0.80	0.52	0.22	0.94	0.63 (0.43, 0.82)
Wheezing or whistling in the chest in the last 12 months	0.67	0.6	0.27	0.89	0.60 (0.39, 0.81)
Ever had asthma	0.80	0.58	0.25	0.94	0.66 (0.47, 0.85)
Chest sounded wheezy during or after exercise in the last 12 months	0.60	0.76	0.3	0.92	0.67 (0.46, 0.87)
Dry cough at night, apart from a cough associated with a cold or a chest infection in the last 12 months	0.50	0.63	0.19	0.88	0.58 (0.36, 0.79)
Mean value	0.67 (0.13)	0.62 (0.09)	0.25 (0.04)	0.91 (0.03)	0.63 (0.04)
Electronic nose	0.92	0.95	0.79	0.98	0.98 (0.96, 1.00)

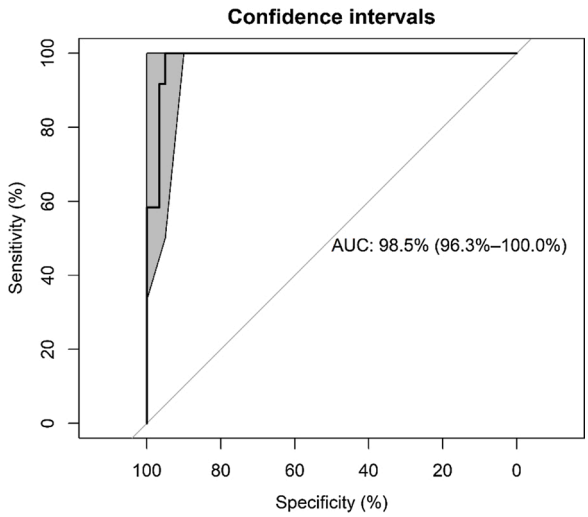

Fig. 3. ROC curves for small airway dysfunction by the electronic nose. The 95 % confidence intervals obtained using bootstrapping are shown as gray areas around the mean bootstrapped curve.

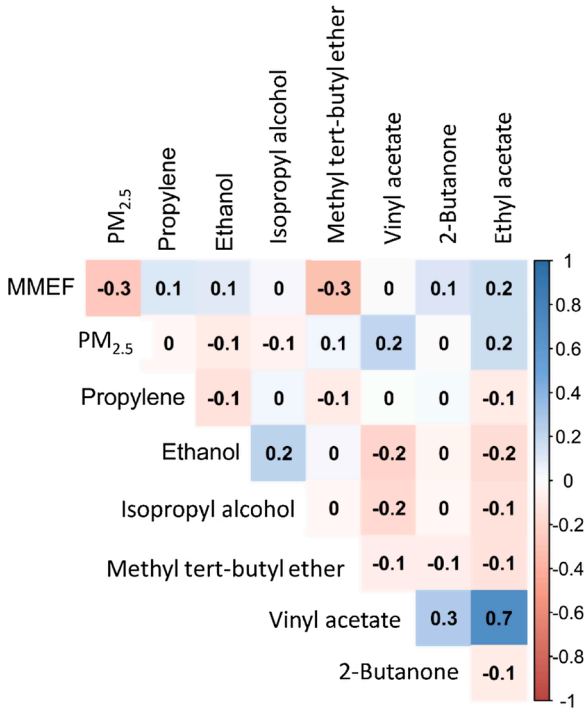

Fig. 4. The Pearson correlation matrix of the MMEF and ambient air pollutants. The correlation matrix shows that $PM_{2.5}$ and methyl tert-butyl ether in the alveolar air are negatively associated with MMEF.

high specificity may be related to the low prevalence of asthma (4.9 %) in the study population [28]. The current study found that the ISAAC questionnaire did not have sufficient sensitivity and specificity to detect small airway dysfunction. The ATS recommended that the concentration of FeNO can be used to diagnose eosinophilic airway inflammation and can be used to support a diagnosis of asthma. The FeNO concentration varies in children younger than 12 years of age. The ATS states that a FeNO concentration less than 20 ppb in children indicates that eosinophilic inflammation is less likely, a FeNO concentration greater than 35 ppb indicates that eosinophilic inflammation is likely, and FeNO values between 20 ppb and 35 ppb should be interpreted cautiously, with reference to the clinical context [29]. In this study, the mean FeNO concentration among children with small airway dysfunction was 23.5 ppb, which was not significantly different from that in healthy controls (19.8 ppb). Therefore, the high sensitivity and specificity of the E-nose for detecting small airway dysfunction are very important for the screening of asymptomatic schoolchildren.

Medication usage (i.e., inhaled corticosteroids/bronchodilators) might affect metabolism. Our MSEA suggested that the metabolism of xenobiotics by cytochrome P450 was affected by the medication (Supplementary Fig. 2). However, the results did not have statistical significance owing to the limited sample size (only 16 subjects had taken medication in the past two weeks). However, the use of asthma medications did not significantly affect the pattern of VOCs in the exhaled breath (Supplementary Fig. 3).

Breathomics is an emerging field focusing on the diagnosis of diseases based on the analysis of volatile metabolites produced by changes in metabolic processes [30]. Volatile metabolites produced during the physiological and pathological processes involved in lung diseases are released into the alveolar air [31]. Our study used the E-nose to detect the pattern of volatile metabolites from breath samples. When these volatile metabolites are presented to the sensor array of the E-nose, the chemicals interact with the sensors and change their electric resistance. The data are then processed by a machine learning model to create a pattern recognition output, allowing diseases to be diagnosed based on the compounds in exhaled breath [32]. The E-nose breath test may become a point-of-care screening method to detect children at high risk of asthma in school.

Breath concentrations may reflect pulmonary dose responses to air pollutants. In this study, we applied GC–MS to measure ambient VOCs in breath to explore the association between air pollutants and lung function. The results show that MTBE is related to small airway dysfunction. MTBE is a gasoline additive used to increase octane and reduce carbon monoxide emissions and ozone precursors. MTBE can contaminate drinking water, and exposure occurs through oral, inhalation, and dermal routes [33]. MTBE is related to asthma. Between 1992 and 1997, when the MTBE in gasoline in Philadelphia increased, the number of asthma patients increased [34]. Arif and Shah used personal exposure monitoring to measure VOC exposure and found that o-Xylene and MTBE increased the risk of asthma and adverse respiratory symptoms [35]. In a study of the kinetics of VOCs in exhaled air, VOCs could be found several hours after exposure in the third (vessel-poor tissues) or fourth (fatty tissue) compartment [36,37], reflecting not only short-term exposure but also cumulative personal exposure [36,38]. Our findings support those of a recent study in which the lung was found to act as a sink for air pollutants [39]. VOCs are important air pollutants. Outdoor VOCs are mainly emitted by petrochemical activities, fossil fuel extraction, and industrial products [40–42]. Indoor VOCs are produced by dry cleaning, paints, wood products, furnishings [43,44], and tobacco smoking [45]. Many studies have already reported that pollutant VOCs can be detected in exhaled human breath [46,47]. In the future, the analysis of VOCs in exhaled breath can be used as a new personal exposure monitoring method to assess the acute respiratory effects of air pollutants.

The method used to collect exhaled breath will affect the accuracy of the analysis. The accuracy may be affected by the expiratory flow rate, oral cavity conditions, diet, or anatomical dead space in the upper airways [48]. The dead space includes the nose, pharynx, larynx, and trachea and is not involved in gas exchange [49]. This study provided a standardized method to prevent the confounding effects of dead space air, flow rate, and humidity. Using a visual CO_2-controlled alveolar breath sampling technique [50], we sampled alveolar air to prevent contamination from the dead space. This study provides the details of these breath analysis procedures to enable future studies to reproduce the results.

There is a limitation of this study. The carbon nanotube sensor is sensitive to changes in humidity. This study used a silica reservoir in the breath collection device to prevent the influence of humidity. The use of

a silica reservoir can effectively decrease the influence of vapor. The mean humidity decreased from 95.67 % relative humidity (RH) to 27.51 % RH. (RH was measured at 24 °C) [51]. Since the collection of breath through a silica reservoir was applied throughout all our samples, we cannot estimate the effect of silica in regard to trapping and/or adding VOCs to the VOC signal/breathprint. We suggest conducting an independent study that uses a set of VOC standards and compares VOC concentrations before and after breath air passes through a silica reservoir.

5. Conclusion

This study applied the E-nose to detect small airway dysfunction in children at high risk for asthma. We provide evidence that the E-nose is highly accurate at detecting small airway dysfunction. The analyses of the volatile metabolites in the exhaled breath must be standardized to increase the accuracy. An analysis of exhaled breath can also be used as a personal monitoring method to assess the acute respiratory effects of air pollution.

CRediT authorship contribution statement

Yi-Giien Tsai: Conceptualization, Investigation. **Ruei-Hao Shie:** Methodology, Software. **Chi-Hsiang Huang:** Methodology. **Chih-Dao Chen:** Investigation. **Wei-Chi Lin:** Project administration, Data curation, Formal analysis, Writing - original draft. **Hsiao-Yu Yang:** Conceptualization, Formal analysis, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.snb.2021.130395.

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