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Relationship between lung function and exhaled volatile organic compounds in healthy infants

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Abstract

Objective: The aim of this study is to assess, for the first time, the relationship between the volatilome and lung function in healthy infants, which may be of help for the early detection of certain respiratory diseases. Lung function tests are crucial in chronic respiratory diseases diagnosis. Moreover, volatile organic compounds (VOCs) analysis in exhaled breath is a noninvasive technique that enables the monitorization of oxidative stress, typical of some forms of airway inflammation.

Methods: Lung function was studied in 50 healthy infants of 3–8 months of age and the following parameters were obtained: forced vital capacity (FVC), forced expiratory volume at 0.5 s ($FEV_{0.5}$), forced expiratory flow at 75% of FVC (FEF_{75}), forced expiratory flow at 25%–75% of FVC (FEF_{25-75}), and $FEV_{0.5}$/FVC. Lung function was measured according to the raised volume rapid thoracoabdominal compression technique. In addition, a targeted analysis of six endogenous VOCs (acetone, isoprene, decane, undecane, tetradecane, and pentadecane) in the exhaled breath of the children was carried out by means of thermal desorption coupled gas chromatography-single quadrupole mass spectrometry system.

Results: A negatively significant relationship has been observed between levels of acetone, tetradecane, and pentadecane in exhaled breath and several of the lung function parameters. Levels of acetone (feature $m/z = 58$) were significantly negatively associated with FVC and $FVE_{0.5}$, levels of tetradecane (feature $m/z = 71$) with $FEV_{0.5}$, and levels of pentadecane (feature $m/z = 71$) with $FEV_{0.5}$ and FEF_{25-75}.

Conclusion: The findings of this study highlight a significant association between VOCs related to oxidative stress and lung function in healthy infants.

KEYWORDS

early respiratory diseases detection, exhaled breath analysis, infant lung function, monitorization of oxidative stress, volatile organic compounds

1 | INTRODUCTION

The incidence of chronic respiratory diseases such as asthma, chronic obstructive pulmonary disease, or pulmonary fibrosis has increased alarmingly over the last 20 years, with high economic direct and indirect costs for health services and for the society in developed countries.[1] Currently, lung function testing and clinical history play an essential role in the diagnosis of respiratory diseases.[2] Parameters measured in lung function tests in adults and older children such as forced expiratory volume at 1 s (FEV_1) and FEV_1/forced vital capacity (FVC) ratio are difficult to obtain from infants and thus the $FEV_{0.5}$, and the $FEV_{0.5}$/FVC ratio are preferable since the time needed for full lung emptying is shorter at an earlier age.[3] In addition, the function screening usually includes also the measurement of other parameters such as FVC, forced expiratory flow at 75% of FVC (FEF_{75}), forced expiratory flow at 25%–75% of FVC (FEF_{25-75}).[4] However, lung function parameters cannot offer any information on the severity or type of inflammation that might be underlying a low function, apart from the degree of obstruction that the inflammation may cause. Thus, having complementary information on the inflammation itself and on the lung function parameters might be of great interest.[5–7]

In this regard, although there are several techniques for oxidative stress and airway inflammation surveillance (e.g., bronchoscopy with bronchoalveolar lavage, biopsies, etc.), these tests are difficult to apply since their high degree of invasiveness. In the last decade, there has been a growing interest in the development of noninvasive methods. Volatile organic compounds (VOCs) analysis in exhaled breath is of great interest as it is a noninvasive and patient-friendly procedure.[7] In exhaled human breath, VOCs from exogenous sources, such as environmental pollutants or the product of microbiome metabolism, and endogenous ones, such as those produced by human cell metabolism, can be determined. Furthermore, their relative concentrations may change in response to pathological processes. Acetone and isoprene are the two most abundant endogenous VOCs in human exhaled air.[8,9] In recent years, this technique has been identified as potentially valuable for diagnosing and monitoring respiratory diseases, and as a useful tool for the detection of metabolic changes which involve oxidative stress.[9–11] For example, elevated levels of alkanes, such as pentane and ethane in the exhaled breath have previously been associated with asthma since these compounds are generated by lipid peroxidation due to an oxidative imbalance.[12,13] Therefore, it is of great importance to determine the relationship between VOC profile in human exhaled breath and lung function parameters so as to assess the connection between oxidative stress and lung disorders in very early childhood. This strategy could be able to diagnose preclinical asthma in children as underlying inflammatory changes might be present in the lung much earlier than alterations in lung function. In recent years, several studies have found an association between lung function and inflammation using less specific techniques than VOC analysis in exhaled breath, such as the determination of exhaled fractional nitric oxide (FeNO) and blood eosinophil counts.[14] Fuglsbjerg et al.[15] analyzed VOCs in exhaled breath and measured FEV_1 of asthmatic children older than 2 years but, did not obtain conclusive results, and they also did not analyze the lung function in healthy children to exclude latent asthma.

In the present paper, the relationship between lung function test parameters and volatilome in healthy infants has been assessed for the first time. FVC, $FEV_{0.5}$, FEF_{75}, FEF_{25-75}, and $FEV_{0.5}$/FVC were compared with exhaled air levels of six endogenous VOCs (acetone, isoprene, decane, undecane, tetradecane, and pentadecane) in healthy infants. These compounds have been selected based on their high abundance in human exhaled breath or previous evidence in the literature of an association with respiratory/atopic diseases in children.[13]

2 | METHODS

2.1 | Study design and participants

The healthy infants (infants born at term) included in this study belong to the prospective birth cohort nutrition in early life and asthma (NELA) (www.nela.imib.es).[16–18] Recruitment of the infants was conducted over 36 months (from March 2015 to April 2018) and was carried out at the control visit of the 20th week of pregnancy at the Maternal-Fetal Unit of the Hospital Universitario Virgen de la Arrixaca (Murcia, Spain). Inclusion criteria were as follows: maternal age between 18 and 45 years; habitual residence in Health Area I, and some districts of Health Areas VI and VII of the Region of Murcia; planning to live in the same place of residence for at least 2 years; intention to deliver in the reference hospital; Spanish Caucasian origin; singleton pregnancy; unassisted conception; and normal ultrasound at 20 weeks of pregnancy (no major malformations). Exclusion criteria included: the presence of chronic disease in the mother (except asthma); complications during pregnancy (except gestational diabetes and hypertensive disorders); and the mother planning to give birth in another hospital. Of the 1350 women invited to participate, 738 were eventually included in the NELA study, and lung function tests were performed when the children were 3–8 months old, and exhaled breath samples were collected at 3 months of age in a subsample (children whose mothers who accepted to both tests). Thus, a total of 55 infants underwent both procedures. In 50 of the children, both tests were successfully performed (infants included in the present study), since it was unable to conduct the lung function test in five of the children because they did not sleep deeply enough.

The study protocol was checked and approved by the Ethics Committee of the Virgen de la Arrixaca Clinical University Hospital in accordance with the guidelines of the Declaration of Helsinki (CEIC 09/14). Written informed consent was obtained from the mothers of the participants both at the time of recruitment and before the lung function tests were performed.

2.2 | Assessment of the relationship between lung function or exhaled volatilome and demographic characteristics of the subjects

Information about the demographic characteristics of the subjects was obtained through face-to-face questionnaires administered to

the mothers of the infants during the 20th week of pregnancy. The variables included in the present study were: the age of father; the age of mother; social class of father (defined as occupation during pregnancy based on the highest social class by using a widely used Spanish adaptation of the international ISCO88 coding system: I–II, managers/technicians; III, skilled; IV–V, semiskilled/unskilled; and unemployed)[19]; social class of mother; educational level of the father (incomplete secondary or less, complete secondary, and university); educational level of the mother; asthma in the father (yes/no); asthma in the mother (yes/no); paternal smoking during pregnancy (yes/no); and maternal smoking during pregnancy (yes/no). In addition, the weight and height of the children were measured at the Pediatric Pulmonology Unit of Arrixaca Children's University Hospital (Murcia, Spain) before the pulmonary function tests were performed. The season in which exhaled air samples were obtained was also considered for data pretreatment (winter: December–February, spring: March–May, summer: June–August, and autumn: September–November).

2.3 | Lung function measurements

FVC, $FEV_{0.5}$, FEF_{75}, FEF_{25-75}, and $FEV_{0.5}$/FVC ratio were obtained at the Pediatric Pulmonology Unit of the Arrixaca Children's University Hospital from maximal expiratory volume curves using the raised volume rapid thoracoabdominal compression technique according to the ATS/ERS specifications.[20–23] Children were sedated with chloral hydrate (80–100 mg/Kg), and oxygen saturation was monitored until recovery from sedation. Master-Screen BabyBody Plethysmograph was used for lung function measurements, and a Neopuff infant resuscitator (Fisher & Paykel Healthcare) coupled to a mask for inflating the lung at a standard pressure of 30 cm H_2O.

2.4 | Exhaled breath sampling and analysis

Further details on the protocols for exhaled breath sampling and analysis of infants can be found in Sola-Martínez et al.[17] Noninvasive collection of mixed expiratory breath from three-month-old infants was conducted. Exhaled breath samples were collected using a mask connected to 400 mL Quintron® gas sampling bags (with minimal filling resistance), and later transferred to a 1 L Tedlar® gas sampling bag due to its high airtightness. Subsequently, the exhaled air was transferred to thermal desorption tubes (Tenax TA; Markes International). Moreover, for each human exhaled breath sample, a room air content sample was obtained by means of an Easy-VOC syringe (Markes International), to assess ambient air influence. Analysis of exhaled breath and environmental samples was performed using a thermal desorption system coupled to gas chromatography-single quadrupole mass spectrometry (TD-GC/q-MS). Additionally, two chemical standards were analyzed so as to calculate the retention indices of VOCs from the samples (C7–C30 saturated alkanes standard and VOC calibration standard; Sigma-Aldrich).

2.5 | Data preprocessing and statistical pretreatment

A matrix with the normalized intensities of the features or ionic fragments (mass/charge or *m*/*z* signals) detected in the breath samples was obtained by preprocessing the raw data from exhaled breath analysis by TD-GC/q-MS using a workflow developed by our group.[17] This workflow includes functions from three R packages, *xcms*,[24] *cliqueMS*,[25] y *eRah*,[26] and allows to determine to which compound each ionic fragment belongs, as well as the identity of these compounds. The identification of the compounds was performed by matching with mass spectra and retention indices available in the National Institute of Standard and Technology library. After data preprocessing, a statistical pretreatment was carried out using analysis of variance (ANOVA)-simultaneous component analysis (*MetStaT* package)[27,28] to obtain a matrix useful for statistical analysis without the variation caused by factors like the season of breath sampling and pollution of usual residence.[18] The pollution zone of the usual residence was defined by modeling air quality using the Weather Research and Forecasting + CHIMERE modeling system, under the consideration of ozone (O_3), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), and particulate matter levels.[29,30] From the data matrix generated, the most characteristic features of the six compounds were selected: acetone (feature $m/z = 58$), isoprene (feature $m/z = 67$), decane (feature $m/z = 71$), undecane (feature $m/z = 57$), tetradecane (feature $m/z = 71$), and pentadecane (feature $m/z = 71$).

2.6 | Statistical analysis

The relationship between lung function parameters (FVC, $FEV_{0.5}$, $FEV_{0.5}$/FVC ratio, FEF_{75}, and FEF_{25-75}) and the selected exhaled breath compounds (acetone, isoprene, decane, undecane, tetradecane, and pentadecane) was assessed by three independent approaches. First, the correlation between lung function outcomes converted into *z*-scores and the features (ion fragments) of the selected compounds was checked by Pearson's correlation. *Z*-scores for FVC, $FEV_{0.5}$, $FEV_{0.5}$/FVC, FEF_{75}, and FEF_{25-75} were calculated as detailed by Lum et al.[31] It was considered a normal range of *z*-scores of lung function parameters within −1.96 (lower limit of normal [LLN]) to +1.96 (upper limit of normal [ULN]).[31,32] Afterwards, multiple linear regression models were generated with each of the lung function parameters and each of the features of the selected VOCs (lung function parameters as dependent variables and features as independent variables), as well as the following covariates: sex, height, weight, age at the time of lung function testing, and maternal smoking during pregnancy. In addition, we also constructed multiple linear regression models using the *z*-scores of the lung function parameters and the features of the selected VOCs. These models were adjusted only for maternal smoking habits during pregnancy. Finally, as an exploratory analysis, a principal component analysis (PCA) (*FactoMineR* package)[33] was performed on the lung function parameters, and the association of the first principal component with

the features of the selected VOCs was checked by Spearman's rank correlation tests. Assessment of correlation between levels of VOCs in exhaled breath and the first principal component was carried out to study the relationship of volatilome and global lung function in healthy infants, considering lung function parameters as a whole.

To avoid possible biases in the analysis of the association between volatilome and infant lung function, the relationship between the demographic characteristics of the subjects and lung function parameters or level of features of selected endogenous VOCs was assessed by Pearson's correlation or Spearman's rank correlation tests (continuous variable vs. continuous variable), Student's *t*-tests or Mann–Whitney U-tests (continuous variable vs. categorical variable with two groups), or ANOVA or Kruskal–Wallis tests (continuous variable vs. categorical variable with more than two groups). Data distribution was assessed by Shapiro–Wilk tests for the selection of the most suitable statistical tests (parametric or nonparametric tests). All statistical analyses of this study were conducted in R (version 4.0.5) and a $p < 0.05$ was considered as the significance threshold.

TABLE 1 Demographic characteristics of the infants included in this study

	Boys ($n = 23$)	Girls ($n = 37$)	p
Weight (kg), mean (SD)	7.25 (1.01)	6.93 (0.77)	0.216
Height (cm), mean (SD)	66.8 (3.5)	65.3 (2.6)	0.090
Age (weeks), mean (SD)	22.02 (3.81)	22.05 (4.87)	0.977
Gestational age at birth (weeks), mean (SD)	39.43 (1.47)	40.15 (1.26)	0.084
FVC (ml), median (IQR)	230 (51)	215 (35)	0.137
$FEV_{0.5}$ (ml), median (IQR)	178 (38)	175 (23)	0.984
$FEV_{0.5}$/FVC (ml), median (IQR)	**0.79 (0.15)**	**0.82 (0.08)**	**0.038**
FEF_{75} (ml), median (IQR)	185 (60)	193 (51)	0.104
FEF_{25-75} (ml), median (IQR)	314 (91)	316 (67)	0.298

Note: Bold characters mean statistically significant results ($p < 0.05$).
Abbreviations: FEF_{25-75}, forced expiratory flow at 25%–75% of FVC; FEF_{75}, forced expiratory flow at 75% of FVC; $FEV_{0.5}$, forced expiratory volume at 0.5 s; FVC, forced vital capacity; IQR, interquartile range.

3 | RESULTS

3.1 | Relationship between lung function or exhaled volatilome and demographic characteristics of the subjects

The demographic characteristics of the healthy infants included in this study are shown in Table 1 and of their parents in Table S1. $FEV_{0.5}$/FVC ratio is the only lung function parameter significantly influenced by infant sex. Girls showed higher $FEV_{0.5}$/FVC ratio values than boys (Table 1). Weight, height, and age of infants were significantly correlated with many of the lung function parameters (Table 2 and Figure S1). In fact, FVC and $FEV_{0.5}$ values were significantly higher in children with higher weight, height, and age. In contrast, infant weight was negatively associated with $FEV_{0.5}$/FVC ratio values. On the other hand, no significant relationship was found between any of the features of the selected VOCs and the weight, length, age, or gender of the infants (Table 2 and Figure S2). Moreover, the season of breath sampling does not have a significant influence on lung function in infants (Table S2). Figure 1 shows *z*-scores for all lung function parameters measured in the infants involved in the present study. The mean (SD) of *z* scores for all lung function parameters was close to 0 (1) (mean [SD] FVC (*z*-score): −0.02 [0.89]; $FEV_{0.5}$ (*z*-score): −0.21 (0.76); $FEV_{0.5}$/FVC (*z*-score): 0.66 (1.31); FEF_{75} (*z*-score): −0.49 (0.80); FEF_{25-75} (*z*-score): −0.46 (0.80)). Some *z*-scores of $FEV_{0.5}$/FVC were outside of the normal range (±1.96 *z*-scores). Thus, three infants showed *z*-scores of $FEV_{0.5}$/FVC above the ULN (≥1.96 *z*-scores), and five infants below the LLN (≤−1.96 *z*-scores). However, more than 95% of *z*-scores for $FEV_{0.5}$, FEF_{75}, and FEF_{25-75} were determined in the infants within the normal range (±1.96 *z*-scores), and less than 2.5% of *z*-scores for FVC, $FEV_{0.5}$, FEF_{75}, and FEF_{25-75} were below LLN (≤−1.96 *z*-scores).

TABLE 2 Correlation coefficients between characteristics of infants of this study (weight, height, and age) and lung function parameters or the features of volatile organic compounds in exhaled breath of the children

	Weight	Height	Age
FVC	**0.621**	**0.589**	**0.572**
$FEV_{0.5}$	**0.440**	**0.501**	**0.534**
$FEV_{0.5}$/FVC	**−0.397**	−0.256	−0.255
FEF_{75}	0.096	0.133	0.245
FEF_{25-75}	0.011	0.109	0.192
Acetone ($m/z = 58$)	−0.079	0.024	−0.118
Isoprene ($m/z = 67$)	0.127	−0.087	0.037
Decane ($m/z = 71$)	−0.034	0.007	0.056
Undecane ($m/z = 57$)	−0.097	−0.122	−0.021
Tetradecane ($m/z = 71$)	−0.240	−0.184	−0.193
Pentadecane ($m/z = 71$)	−0.117	−0.074	−0.015

Note: Bold characters mean statistically significant results ($p < 0.05$).
Abbreviations: FEF_{25-75}, forced expiratory flow at 25%–75% of FVC; FEF_{75}, forced expiratory flow at 75% of FVC; $FEV_{0.5}$, forced expiratory volume at 0.5 s; FVC, forced vital capacity.

3.2 | Relationship between lung function and exhaled volatilome in healthy infants

Significant correlations were found between three VOCs (acetone, tetradecane, and pentadecane) and several lung function parameters (Figure 2). Table 3 shows the Pearson correlation coefficients between all selected features measured in the exhaled breath and lung function

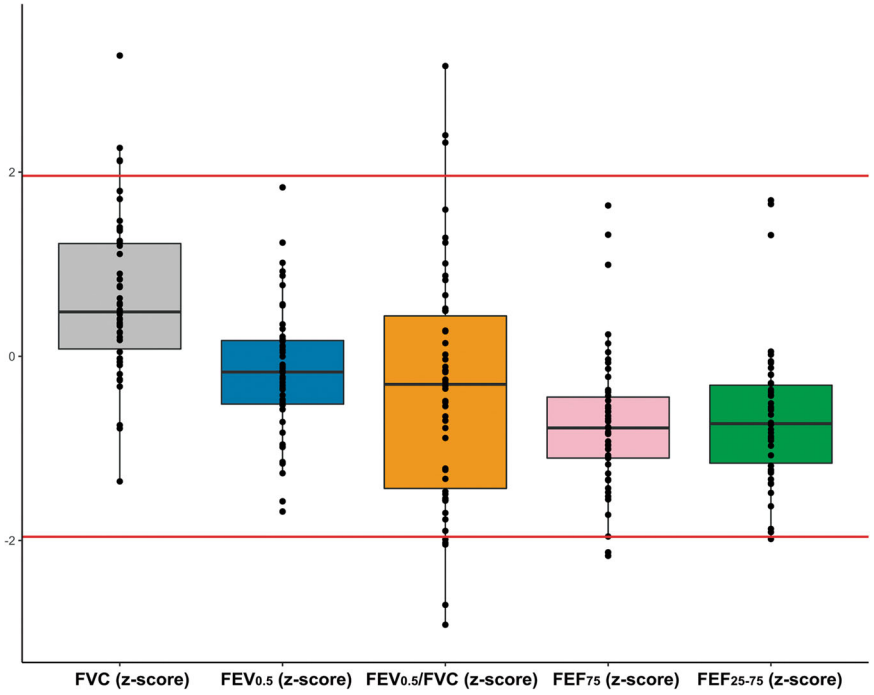

FIGURE 1 *Z*-scores for lung function parameters determined in infants. Red lines indicate the upper and lower limit of the normal range (±1.96 *z*-scores). FEF_{25-75}, forced expiratory flow at 25%–75% of FVC; FEF_{75}, forced expiratory flow at 75% of FVC; $FEV_{0.5}$, forced expiratory volume at 0.5 s; FVC, forced vital capacity [Color figure can be viewed at wileyonlinelibrary.com]

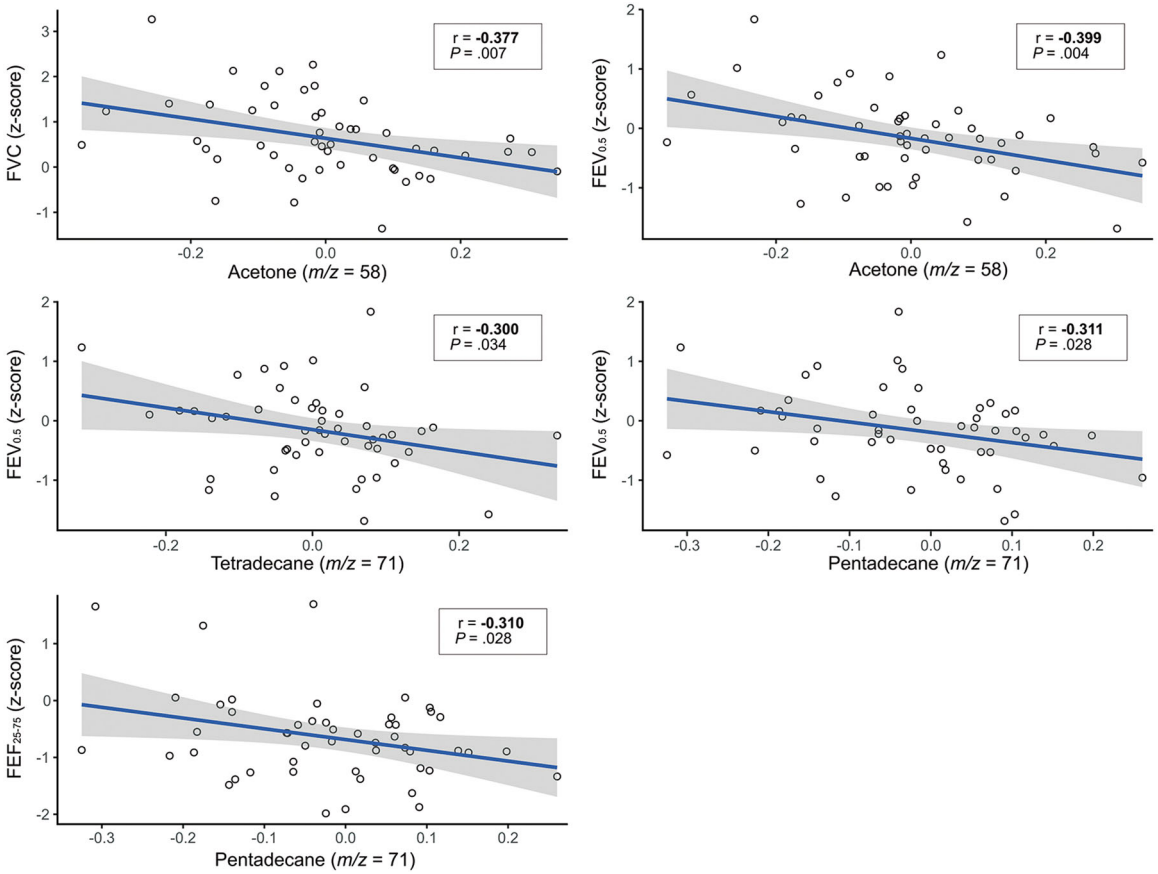

FIGURE 2 Significant correlations between lung function parameters (*z*-score) and features of volatile organic compounds determined in exhaled breath of infants. FEF_{25-75}, forced expiratory flow at 25%–75% of FVC; $FEV_{0.5}$, forced expiratory volume at 0.5 s; FVC, forced vital capacity [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Correlation coefficients between *z*-scores of lung function parameters and features of selected volatile organic compounds detected in the exhaled breath of infants

	FVC (*z*-score)	$FEV_{0.5}$ (*z*-score)	$FEV_{0.5}$/FVC (*z*-score)	FEF_{75} (*z*-score)	FEF_{25-75} (*z*-score)
Acetone (*m/z* = 58)	**−0.377**	**−0.399**	−0.011	−0.122	−0.157
Isoprene (*m/z* = 67)	−0.010	−0.081	−0.076	−0.086	−0.060
Decane (*m/z* = 71)	−0.135	−0.199	−0.055	−0.119	−0.092
Undecane (*m/z* = 57)	0.014	−0.127	−0.188	−0.135	−0.158
Tetradecane (*m/z* = 71)	−0.189	**−0.300**	−0.103	−0.202	−0.222
Pentadecane (*m/z* = 71)	−0.124	**−0.311**	−0.218	−0.190	**−0.310**

Note: Bold characters mean statistically significant results ($p < 0.05$).

Abbreviations: FEF_{25-75}, forced expiratory flow at 25%–75% of FVC; FEF_{75}, forced expiratory flow at 75% of FVC; $FEV_{0.5}$, forced expiratory volume at 0.5 s; FVC, forced vital capacity.

parameters (*z*-score). The feature *m/z* = 58 of acetone was significantly negatively correlated with FVC and $FEV_{0.5}$. In addition, the levels in exhaled breath of a couple of linear alkanes, tetradecane (feature *m/z* = 71) and pentadecane (feature *m/z* = 71) had a significant negative correlation with $FEV_{0.5}$. Moreover, pentadecane (feature *m/z* = 71) was also negatively correlated with FEF_{25-75} (*z*-score). Furthermore, multiple linear regression analyses also showed that lower FVC values were significantly associated with higher levels of acetone (feature *m/z* = 58) in exhaled air; lower $FEV_{0.5}$ values with higher levels in exhaled breath of acetone (feature *m/z* = 58), tetradecane (feature *m/z* = 71), and pentadecane (feature *m/z* = 71); and FEF_{25-75} values and pentadecane (feature *m/z* = 71) levels in exhaled air were significantly negatively associated. Table 4 shows multiple linear regression analyses performed on lung function parameters without converted into *z*-score and Table S3 on *z*-scores of the lung function parameters.

3.3 | Principal component analysis

PCA was implemented to study the overall behavior of the lung function parameters. The first two components obtained in PCA accounted for more than 80% of data variability (Principal Component 1 accounted for 58.5% and Principal Component 2 for 37.3%). In this sense, almost all the information provided by the five lung function parameters can be represented in a single graph (Figure 3A). The parameters that contribute most to Principal Component 1 were $FEV_{0.5}$, FEF_{75}, and FEF_{25-75} (Figure 3C). On the other hand, as shown in Figure 3B and Table S4, Principal Component 1 was significantly negatively correlated with tetradecane (feature *m/z* = 71) and pentadecane (feature *m/z* = 71).

4 | DISCUSSION

In this study, an association between volatilome and lung function in healthy infants has been demonstrated. Some lung function parameters (FVC, $FEV_{0.5}$, and FEF_{25-75}) were negatively correlated with the levels of acetone, tetradecane, and pentadecane detected in exhaled breath (Table 3 and Figure 2). Although the correlations found between lung function parameters and VOC levels were weak (*r* around −0.3), the findings were significant because the probability of a relationship by chance was extremely low ($p < 0.05$). In addition, the same results have been obtained by other approaches such as multiple linear regression analysis (Table 4 and Table S3). Furthermore, the results of multiple linear regression analysis and of assessment of the relationship between exhaled volatilome and demographic characteristics of infants confirm that the relationship between these three compounds and lung function in infants is independent of factors such as weight, height, age, gender, and maternal smoking during pregnancy (Tables 2 and 4, Table S3 and Figure S2).

Acetone, which is generated from acetoacetate, is one of the endogenous compounds found in the highest concentrations in exhaled human breath.[8] Linear alkanes can be provided by endogenous sources as well as exogenous sources.[34] (Figure 4). Therefore, for an accurate determination of endogenous VOCs in exhaled breath, it is essential to analyze the ambient air, as it has been performed in this study (Figure S3). Both acetone[35,36] and several linear alkanes[8,37,38] have previously been suggested as inflammation biomarkers because they are subproducts of lipid peroxidation (Figure 4). In fact, acetone[39] and several linear alkanes[13] have been reported as possible biomarkers in exhaled breath for respiratory diseases such as asthma. The levels of features from acetone, tetradecane, and pentadecane were higher in children with lower lung function, suggesting that poor lung function could be linked to inflammatory processes. Paredi et al.[40] observed in adults that ethane levels in exhaled breath were negatively correlated with FEV_1 values.

The infants included in the present study from the NELA cohort will be followed up during childhood (follow-up visits at 18 months, 5 years, and 7 years of age).[16,18] In this regard, a prospective assessment will be performed to check the association of the outcomes of lung function parameters and exhaled VOCs with the future development of respiratory/atopic diseases such as asthma. Thus, it would be possible to determine if lung function tests together with

TABLE 4 Multiple linear regression analysis of the relationship between lung function parameters and features of selected volatile organic compounds detected in the exhaled breath of infants

		β	95% CI	p
FVC	Acetone (*m/z* = 58)	**−69.415**	**−125.815, −13.015**	**0.017**
	Isoprene (*m/z* = 67)	−38.022	−106.165, 30.122	0.267
	Decane (*m/z* = 71)	−27.967	−78.426, 22.492	0.270
	Undecane (*m/z* = 57)	−10.275	−80.182, 59.631	0.768
	Tetradecane (*m/z* = 71)	−68.959	−148.135, 10.217	0.086
	Pentadecane (*m/z* = 71)	−39.909	−111.545, 31.727	0.268
$FEV_{0.5}$	Acetone (*m/z* = 58)	**−57.285**	**−98.934, −15.636**	**0.008**
	Isoprene (*m/z* = 67)	−18.096	−69.643, 33.452	0.483
	Decane (*m/z* = 71)	−27.438	−64.883, 10.007	0.147
	Undecane (*m/z* = 57)	−31.040	−82.640, 20.560	0.232
	Tetradecane (*m/z* = 71)	**−74.022**	**−131.117, −16.927**	**0.012**
	Pentadecane (*m/z* = 71)	**−69.026**	**−119.223, −18.829**	**0.008**
$FEV_{0.5}$/FVC	Acetone (*m/z* = 58)	−0.004	−0.163, 0.156	0.964
	Isoprene (*m/z* = 67)	0.028	−0.150, 0.205	0.755
	Decane (*m/z* = 71)	−0.001	−0.134, 0.132	0.991
	Undecane (*m/z* = 57)	−0.086	−0.268, 0.096	0.345
	Tetradecane (*m/z* = 71)	−0.114	−0.323, 0.095	0.278
	Pentadecane (*m/z* = 71)	−0.156	−0.337, 0.025	0.089
FEF_{75}	Acetone (*m/z* = 58)	−51.187	−157.287, 54.913	0.336
	Isoprene (*m/z* = 67)	−17.929	−140.781, 104.922	0.770
	Decane (*m/z* = 71)	−46.586	−136.495, 43.323	0.302
	Undecane (*m/z* = 57)	−78.426	−200.533, 43.681	0.202
	Tetradecane (*m/z* = 71)	−123.164	−263.948, 17.621	0.085
	Pentadecane (*m/z* = 71)	−112.806	−237.336, 11.723	0.075
FEF_{25-75}	Acetone (*m/z* = 58)	−87.814	−234.817, 59.188	0.235
	Isoprene (*m/z* = 67)	−21.096	−192.331, 150.139	0.805
	Decane (*m/z* = 71)	−54.734	−180.476, 71.007	0.385
	Undecane (*m/z* = 57)	−119.516	−289.012, 49.981	0.162
	Tetradecane (*m/z* = 71)	−192.948	−387.239, 1.342	0.052
	Pentadecane (*m/z* = 71)	**−227.382**	**−393.386, −61.378**	**0.008**

Note: Models adjusted for sex, height, weight, and age of the infant at the time of lung function testing, and maternal smoking during pregnancy.

Bold characters mean statistically significant results ($p < 0.05$).

Abbreviations: 95% CI, 95% confidence interval; β, regression coefficient; FEF_{25-75}, forced expiratory flow at 25%–75% of FVC; FEF_{75}, forced expiratory flow at 75% of FVC; $FEV_{0.5}$, forced expiratory volume at 0.5 s; FVC, forced vital capacity.

monitoring of oxidative stress by exhaled breath analysis in healthy infants during the first months of life could be a useful strategy for early respiratory disease detection.

The main limitation of this study was the small number of subjects ($n = 50$). Thus, some relationships close to significance ($p < 0.10$) between the function parameters and some VOCs such as tetradecane or pentadecane (Table 4) could be significant if more subjects were included. Consequently, a PCA was conducted to compare the levels of selected VOCs and lung function globally, and to check whether relationships close to significance have a greater impact when all function parameters are considered as a whole. The PCA reduces a large number of variables to a much smaller number of components, allowing us to identify representative components of the data and study the global lung function of infants. A negative

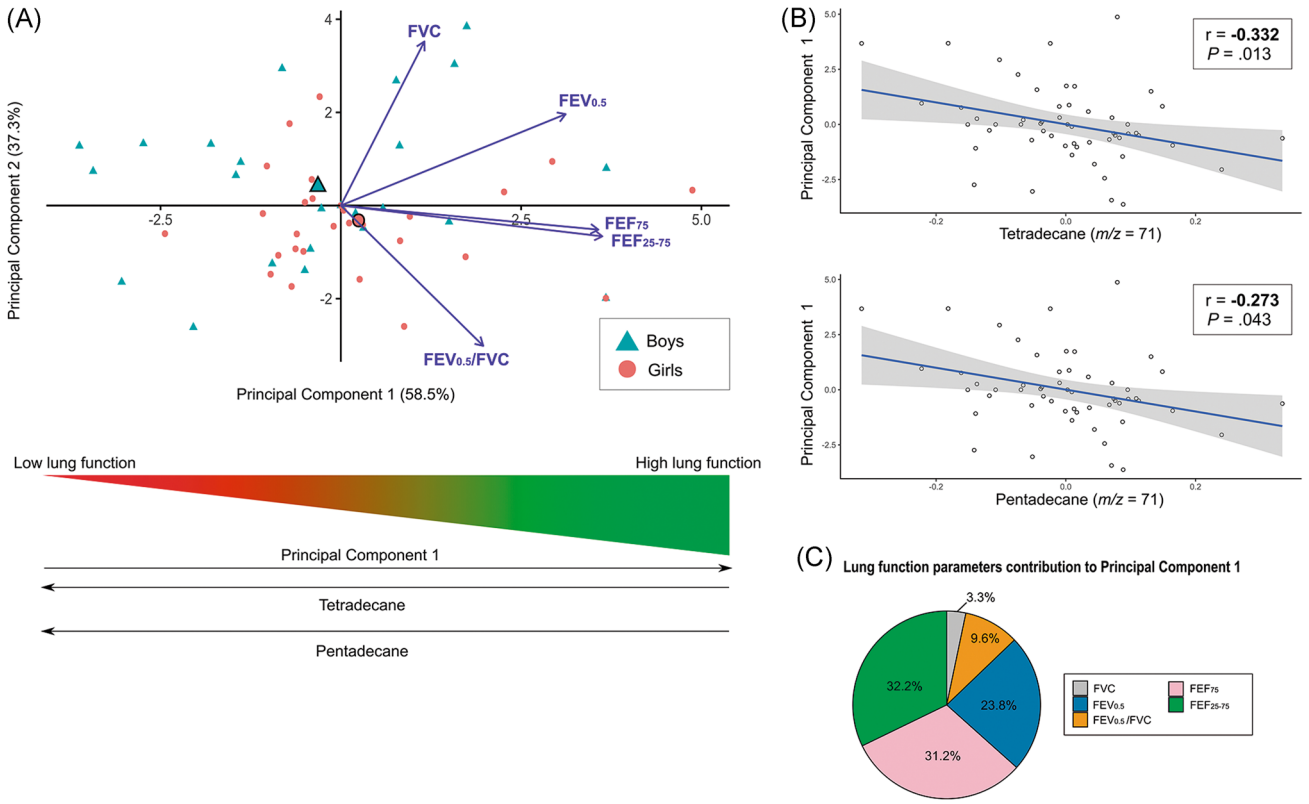

FIGURE 3 Principal component analysis (PCA) was performed on the lung function parameters in infants. (A) Biplot of the first two components. The purple vectors correspond to the variables involved in the PCA. (B) Negative significant correlations between the first component, and tetradecane (feature $m/z = 71$) and pentadecane (feature $m/z = 71$) were determined in the exhaled breath of the infants. (C) Contribution of the variables in the first component. FEF_{25-75}, forced expiratory flow at 25%–75% of FVC; FEF_{75}, forced expiratory flow at 75% of FVC; $FEV_{0.5}$, forced expiratory volume at 0.5 s; FVC, forced vital capacity [Color figure can be viewed at wileyonlinelibrary.com]

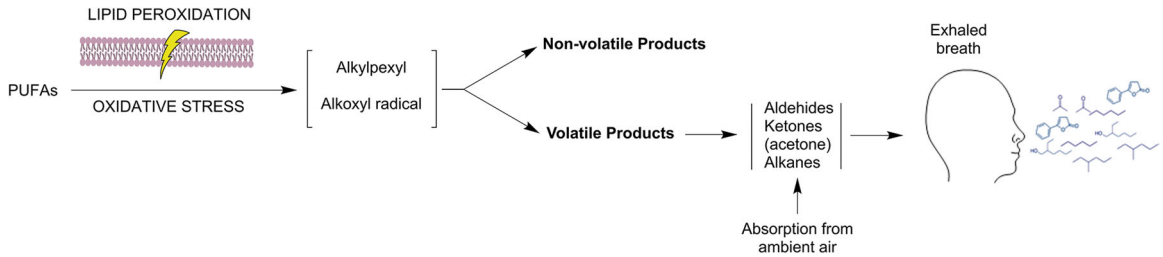

FIGURE 4 Oxidative stress monitoring by volatile organic compounds analysis in exhaled breath. PUFAs, polyunsaturated fatty acids [Color figure can be viewed at wileyonlinelibrary.com]

significant correlation has been observed between tetradecane (feature $m/z = 71$) and pentadecane (feature $m/z = 71$) in exhaled air, and the first principal component obtained in the PCA performed on lung function parameters (Figure 3B). Principal Component 1 (58.5% of the variability) explains the lung function level of individuals, owing to higher values of the five lung function parameters will result in a higher value of Principal Component 1. So, infants with higher values in Principal Component 1 (placed on the left of the graph in Figure 3A) and lower levels of tetradecane (feature $m/z = 71$) and pentadecane (feature $m/z = 71$) had higher lung function, and those with lower values (placed on the right of the graph in Figure 3A) and higher levels of tetradecane (feature $m/z = 71$) and pentadecane (feature $m/z = 71$) presented lower lung function. These results also support that lung function, oxidative stress, and lipid peroxidation are closely associated. To our knowledge, no association between lung function parameters and levels of tetradecane and pentadecane in exhaled breath has previously been reported. However, these compounds, mainly tetradecane, have been found to be characteristic of volatilome from children with allergic asthma.[41,42]

In conclusion, the findings of this study show, for the first time to the best of our knowledge, the relationship between lung function and VOCs associated with lipid peroxidation and oxidative stress in

healthy infants. In addition, our results contribute to lung function and volatilome characterization in healthy infants.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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DATA AVAILABILITY STATEMENT

The NELA study protocol including all questionnaires and measurements of all visits already performed is available on the NELA website (https://nela.imib.es/) upon request. The NELA data, including de-identified individual participant data, will be made available to interested researchers by the NELA Steering Committee. Access will require a formal request, a written proposal, and a signed data access agreement.

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REFERENCES

1. Xie M, Liu X, Cao X, Guo M, Li X. Trends in prevalence and incidence of chronic respiratory diseases from 1990 to 2017. *Respir Res*. 2020; 21(1):49.

2. Garcia-Marcos L, Edwards J, Kennington E, et al. Priorities for future research into asthma diagnostic tools: a PAN-EU consensus exercise from the European asthma research innovation partnership (EARIP). *Clin Exp Allergy*. 2018;48(2):104-120.
3. Oliva Hernández C, Gómez Pastrana D, Sirvent Gómez J, et al. Estudio de la función pulmonar en el paciente colaborador. Parte I. *An Pediatr*. 2007;66(4):393-406.
4. Mallol J, Aguirre V. Advances in the study of infant lung function: forced expiratory maneuvers from an increased lung volume. *Arch Bronconeumol*. 2007;43(4):233-238.
5. Tenero L, Zaffanello M, Piazza M, Piacentini G. Measuring airway inflammation in asthmatic children. *Front Pediatr*. 2018;6:196.
6. Ochs-Balcom HM, Grant BJB, Muti P, et al. Oxidative stress and pulmonary function in the general population. *Am J Epidemiol*. 2005;162(12):1137-1145.
7. van de Kant KDG, van der Sande LJTM, et al. Clinical use of exhaled volatile organic compounds in pulmonary diseases: a systematic review. *Respir Res*. 2012;13(1):117.
8. Miekisch W, Schubert JK, Noeldge-Schomburg GF. Diagnostic potential of breath analysis—focus on volatile organic compounds. *Clin Chim Acta*. 2004;347(1–2):25-39.
9. Amann A, de Lacy Costello B, Miekisch W, et al. The human volatilome: volatile organic compounds (VOCs) in exhaled breath, skin emanations, urine, feces and saliva. *J Breath Res*. 2014;8(3):034001.
10. Smolinska A, Klaassen EMM, Dallinga JW, et al. Profiling of volatile organic compounds in exhaled breath as a strategy to find early predictive signatures of asthma in children. *PLoS One*. 2014;9(4):e95668.
11. Muñoz-Lucas MÁ, Jareño-Esteban J, Gutiérrez-Ortega C, et al. Influence of chronic obstructive pulmonary disease on volatile organic compounds in patients with non-small cell lung cancer. *Arch Bronconeumol*. 2020;56(12):801-805.
12. Azim A, Barber C, Dennison P, Riley J, Howarth P. Exhaled volatile organic compounds in adult asthma: a systematic review. *Eur Respir J*. 2019;54(3):1900056.
13. Sola-Martínez RA, Pastor Hernández JM, Yanes Torrado Ó, et al. Exhaled volatile organic compounds analysis in clinical pediatrics: a systematic review. *Pediatr Res*. 2021;89(6):1352-1363.
14. Sanchez-Solis M, Garcia-Marcos L. Lung function in wheezing infants. *Front Biosci - Elit*. 2014;6 E(1):185-197.
15. Fuglsbjerg MG, Rasmussen MA, Hansen KS, et al. Limited clinical value of exhaled volatile organic compound measurements in childhood asthma. *ERJ Open Res*. 2018;4(4):00026-02018.
16. Morales E, Alcantara-Lopez MV, Cabezas-Herrera J, et al. The Nutrition in Early Life and Asthma (NELA) birth cohort study: rationale, design, and methods. *Paediatr Perinat Epidemiol*. 2021;00:1-15.
17. Sola-Martínez RA, Pastor Hernández JM, Lozano Terol G, et al. Data preprocessing workflow for exhaled breath analysis by GC/MS using open sources. *Sci Rep*. 2020;10(1):22008.
18. Sola-Martínez RA, Lozano-Terol G, Gallego-Jara J, et al. Exhaled volatilome analysis as a useful tool to discriminate asthma with other coexisting atopic diseases in women of childbearing age. *Sci Rep*. 2021;11(1):13823.
19. Domingo-Salvany A, Regidor E, Alonso J, Alvarez-Dardet C. Proposal for a social class measure. Working Group of the Spanish Society of Epidemiology and the Spanish Society of Family and Community Medicine. *Aten Primaria*. 2000;25(5):350-363.
20. Culver BH, Graham BL, Coates AL, et al. Recommendations for a standardized pulmonary function report. An official American Thoracic Society technical statement. *Am J Respir Crit Care Med*. 2017;196(11):1463-1472.
21. American Thoracic Society (ATS) and the European Respiratory Society (ERS). ATS/ERS statement: raised volume forced expirations in infants—guidelines for current practice. *Am J Respir Crit Care Med*. 2005;172(11):1463-1471.
22. Sanchez-Solis M, Perez-Fernandez V, Bosch-Gimenez V, Quesada J, Garcia-Marcos L. Lung function gain in preterm infants with and without bronchopulmonary dysplasia. *Pediatr Pulmonol*. 2016;51(9):936-942.
23. Sanchez-Solis M, Garcia-Marcos PW, Agüera-Arenas J, Mondejar-Lopez P, Garcia-Marcos L. Impact of early caffeine therapy in preterm newborns on infant lung function. *Pediatr Pulmonol*. 2020;55(1):102-107.
24. Smith CA, Want EJ, O'Maille G, Abagyan R, Siuzdak G. XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. *Anal Chem*. 2006;78(3):779-787.
25. Senan O, Aguilar-Mogas A, Navarro M, et al. CliqueMS: a computational tool for annotating in-source metabolite ions from LC-MS untargeted metabolomics data based on a coelution similarity network. *Bioinformatics*. 2019;35(20):4089-4097.
26. Domingo-Almenara X, Brezmes J, Vinaixa M, et al. eRah: a computational tool integrating spectral deconvolution and alignment with quantification and identification of metabolites in GC/MS-based metabolomics. *Anal Chem*. 2016;88(19):9821-9829.
27. Smilde AK, Jansen JJ, Hoefsloot HCJ, Lamers R-JAN, van der Greef J, Timmerman ME. ANOVA-simultaneous component analysis (ASCA): a new tool for analyzing designed metabolomics data. *Bioinformatics*. 2005;21(13):3043-3048.
28. Bertinetto C, Engel J, Jansen J. ANOVA simultaneous component analysis: a tutorial review. *Anal Chim Acta X*. 2020;6:100061.
29. García-Serna AM, Hernández-Caselles T, Jiménez-Guerrero P, et al. Air pollution from traffic during pregnancy impairs newborn's cord blood immune cells: the NELA cohort. *Environ Res*. 2021;198:110468.
30. Skamarock WC, Klemp JB, Dudhi J, et al. *A Description of the Advanced Research WRF Version 3*. 2008.
31. Lum S, Bountziouka V, Wade A, et al. New reference ranges for interpreting forced expiratory manoeuvres in infants and implications for clinical interpretation: a multicentre collaboration. *Thorax*. 2016;71(3):276-283.
32. Quanjer PH, Stanojevic S, Cole TJ, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J*. 2012;40(6):1324-1343.
33. Lê S, Josse J, Husson F. FactoMineR: an R package for multivariate analysis. *J Stat Softw*. 2008;25(1):1-18.
34. Mitsui T, Kondo T, Phillips M, Cataneo RN, Greenberg J. Inadequacy of theoretical basis of breath methylated alkane contour for assessing oxidative stress. *Clin Chim Acta*. 2003;333(1–2):91-94.
35. Silva CL, Perestrelo R, Silva P, Tomás H, Câmara JS. Volatile metabolomic signature of human breast cancer cell lines. *Sci Rep*. 2017;7:43969.
36. Scholpp J, Schubert JK, Miekisch W, Geiger K. Breath markers and soluble lipid peroxidation markers in critically III patients. *Clin Chem Lab Med*. 2002;40(6):587-594.
37. Calenic B, Miricescu D, Greabu M, et al. Oxidative stress and volatile organic compounds: interplay in pulmonary, cardio-vascular, digestive tract systems and cancer. *Open Chem*. 2015;13(1):1020-1030.
38. Phillips M, Cataneo RN, Greenberg J, Gunawardena R, Naidu A, Rahbari-Oskoui F. Effect of age on the breath methylated alkane contour, a display of apparent new markers of oxidative stress. *J Lab Clin Med*. 2000;136(3):243-249.
39. Dragonieri S, Schot R, Mertens BJA, et al. An electronic nose in the discrimination of patients with asthma and controls. *J Allergy Clin Immunol*. 2007;120(4):856-862.
40. Paredi P, Kharitonov SA, Leak D, Ward S, Cramer D, Barnes PJ. Exhaled ethane, a marker of lipid peroxidation, is elevated chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2000;162(2):369-373.
41. Caldeira M, Barros AS, Bilelo MJ, Parada A, Câmara JS, Rocha SM. Profiling allergic asthma volatile metabolic patterns using a headspace-solid phase microextraction/gas chromatography based methodology. *J Chromatogr A*. 2011;1218(24):3771-3780.

42. Caldeira M, Perestrelo R, Barros AS, et al. Allergic asthma exhaled breath metabolome: a challenge for comprehensive two-dimensional gas chromatography. *J Chromatogr A*. 2012;1254:87-97.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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