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REVIEW

The role of non-invasive biomarkers in detecting acute respiratory effects of traffic-related air pollution

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Summary

The role of non-invasive methods in the investigation of acute effects of traffic-related air pollution is not clearly established. We evaluated the usefulness of non-invasive biomarkers in detecting acute air pollution effects according to the age of participants, the disease status, their sensitivity compared with lung function tests and their specificity for a type of pollutant. Search terms lead to 535 titles, among them 128 had potentially relevant abstracts. Sixtynine full papers were reviewed, while 59 articles were excluded as they did not meet the selection criteria. Methods used to assess short-term effects of air pollution included analysis of nasal lavage (NAL) for the upper airways, and induced sputum (IS), exhaled breath condensate (EBC) and exhaled nitric oxide (FeNO) for central and lower airways. There is strong evidence that FeNO evaluation is useful independently from subject age, while IS analysis is suitable almost for adults. Biomarker changes are generally observed upon pollutant exposure irrespective of the disease status of the participants. None of the biomarkers identified are specific for a type of pollutant exposure. Based on experimental exposure studies, there is moderate evidence that IS analysis is more sensitive than lung function tests, whereas this is not the case for biomarkers obtained by NAL or EBC. Cells and some cytokines (IL-6, IL-8 and myeloperoxidase) have been measured both in the upper respiratory tract (NAL) and in the lower airways (IS). Overall, the response to traffic exposure seems different in the two compartments. In conclusion, this survey of current literature displays the complexity of this research field, highlights the significance of short-term studies on traffic pollution and gives important tips when planning studies to detect acute respiratory effects of air pollution in a non-invasive way.

Introduction

Millions of tons of harmful particles and gases are released into the air. Motor vehicles are a major source of air pollution followed by power stations, factories, offices and homes [1]. People who already have a lung disease, the elderly and children are more likely to be affected by short-term increase in air pollutants. The incidence of allergic respiratory diseases and bronchial asthma appears to be increasing world-wide, and people living in urban areas more frequently experience these conditions than those living in rural areas. One of the several causes of the rise in morbidity associated with allergic respiratory diseases is the augmented presence of outdoor air pollutants [2, 3].

Recent research suggests that air pollution contributes to the large global burden of respiratory and allergic diseases, including asthma, chronic obstructive pulmonary disease (COPD), pneumonia and possibly tuberculosis [4]. Increments of airborne particulate matter are associated with higher mortality and morbidity for cardiopulmonary diseases, including asthma, COPD, arrhythmias, congestive heart failure and myocardial infarction as shown in several epidemiological studies [5].

In developed countries, the major pollutants emitted into the atmosphere and currently measured include particulate matter (PM), gaseous pollutants (CO, SO_2, nitric oxides, (O_3), volatile organic compounds (VOC) and ammonia (NH_3). PM is a suspended mixture of solid and liquid particles of various sizes and composition. PM is classified according to its aerodynamic diameter into PM_{10} (diameter < 10 μm), $PM_{2.5}$ (fine particles with diameter < 2.5 μm) and $PM_{0.1}$ (ultrafine particles with diameter < 0.1 μm). The latter is considered as

potentially the most dangerous and represents a major component of exhaust from motor vehicles. Water-soluble gases (such as SO_2) react with the mucus layer of the upper airways, while less soluble gases (such as NO_2) are more likely to reach the alveoli. O_3 is a strong oxidizing substance synthesized, in absence of ventilation, by photochemical reaction due to the combination of ultraviolet sun radiation on exhaust gases such as NO_2 and hydrocarbons. Once emitted, pollutants interact with each other and with the environment in complex ways, which may depend on temperature, humidity and other environmental conditions. Thus, pollution concentration, composition and possibly toxicity are variable depending on the above-mentioned factors [2]. Humans enter in contact with different air pollutants primarily via inhalation and ingestion, while dermal contact represents a minor route of exposure [3].

During common human activities, individuals are subjected to high intensity and brief exposure to traffic-related air pollution. If other varying factors, such as weather conditions and day of the week, are properly controlled in the analyses, short-term studies are extremely powerful and efficient tools to explore the acute effects of air pollution. Indeed, individual characteristics (smoking status, diseases, genetic factors, etc.) do not change from day to day and do not act as confounder of the associations between air pollution and acute effects. Moreover, in short-term investigations, the subjects often serve as their own control [6].

The evidence that air pollution has adverse health effects is based on a broad range of research methods. Experimental and environmental approaches provide the two most important tools. Experimental studies involve controlled exposures in a chamber or laboratory, inhalation of a single pollutant or a well-defined mixture [e.g. diesel exhaust (DE)], and it is possible to build a dose–response curve depending on different levels of pollutants and the duration of exposure. Typical participants in controlled exposure studies are healthy adults or people with mild diseases, so experimental findings cannot be generalized to the potentially most susceptible groups such as children, elderly people or individuals with more severe diseases. On the other hand, in environmental studies, the exposure is always to a mixture of pollutants and neither composition nor level can be modified by the investigator. The role of single pollutants is difficult to interpret, but it is possible to observe subjects for longer exposure periods. Environmental approach allows to identify subgroups with increased or decreased susceptibility to the adverse effects of air pollution. The quantitative assessment of exposure can be achieved with environmental and/or personal sampling.

Non-invasive methods for assessing airway inflammation are increasingly used in the investigation of asthma, COPD, rhinitis and occupational respiratory diseases. Their role in the investigation of short-term effects of traffic-related air pollution on respiratory system, however, is not clearly established.

Aims

In this contest, the present article reviews investigations on the use of non-invasive methods in the assessment of airway inflammation due to acute exposure to air pollution. We examined both experimental and environmental human studies on traffic-related air pollution and highlighted biomarkers associated with the acute effects. In particular, the review addressed the following questions:

- Are there biomarkers more useful to detect acute effects of traffic-related pollution depending on the subjects age (children or adults)?
- Are the effects detected by biomarkers dependent on health or disease status of the subjects?
- Are there changes in biomarker levels influenced by a specific pollutant exposure?
- Are there biomarkers more sensitive than lung function parameters to detect effects of traffic pollution in the respiratory tract?

Methods

Appropriate terms were adapted to search Medline via PubMed (Table 1). The final literature search with PubMed was conducted in December 2013, excluding papers published before 1999. Additional papers were retrieved by cross-referencing. The abstract of each title was independently screened by two authors for consistency with the search aims (P.M., M.C.S.). Human studies regarding short-term air pollution exposure (defined as an exposure of up to 7 days) were selected for the evaluation. Only investigation on biomarkers obtained by non-invasive methods of sampling was included.

Table 1. Terms used for PubMed search

First term(s)	AND	AND
NAL	Air pollution exposure	Acute [or]
Nasal cytology	Traffic-related exposure	short term
Exhaled breath condensate	PM_{10}, $PM_{2.5}$ exposure	
Exhaled nitric oxide	NO, NO_2 exposure	
Induced sputum	CO exposure	
	SO_2 exposure	
	VOC exposure	

The present review is focused on inhalation of traffic-related pollutants; hence, studies on occupational exposure to dust or on indoor exposure to tobacco smoke, also called passive smoking, or concerning pollen exposure were not considered here. Studies related to cancer risk were excluded as well. The search results including the type of studies, pollutants, biomarker and population analysed and their major findings were displayed in evidence tables. The heterogeneity of the studies prevented the use of sophisticated analytical methods. The formulation of statements was graded according to the Royal College of General Practitioners (RCGP) three-star system [7].

Results

Results of literature search

The search terms lead to 535 article titles, among them 128 had potentially relevant abstract. Fifty-nine articles were excluded as not pertinent to the review aims (35 concerned chronic exposure, four occupational exposure, six indoor exposure, five tobacco smoke, five were *in vitro* studies and four animal studies). Eventually, 69 full papers were considered and included as final references.

Nasal biomarkers

Nasal lavage (NAL) consists of instillation of saline into the nasal cavity and its recovery after a predetermined dwell time. It has been used to investigate mucosal and intraluminal events in rhinitis [8], and nasal epithelial markers are receiving increased interest to evaluate airways damage caused by air pollution. The nose is an easily accessible mucosal surface for both challenge and sampling [9]. Repeated lavages may be made, allowing each subject to serve as his or her own control and the assessment of time course of a response. However, the dilution rate has to be considered when interpreting the results, and there is a risk of washout of material with repeated sampling.

Nine studies about short-term effects of air pollution exposure performing nasal biomarkers were identified [10–18] (Table 2). Using an experimental exposure to 0.2 ppm of O_3 for 4 h, Nightingale et al. [10] measured nasal NO in healthy volunteers and atopic asthmatics to assess acute effects of O_3. Similarly, Olin et al. [11] examined healthy subjects exposed to O_3, but for 2 h. Both studies concluded that nasal NO is not a useful marker to detect ozone-induced effects. On the other hand, using the same experimental conditions of Nightingale et al. [10] and Mudway et al. [16] suggest that uric acid in nasal respiratory tract lining fluid could confer protection to ozone exposure. Gomes et al. [17] studied also the impact of heat and humidity on ozone exposure. Indirect signs of epithelial damage and protective mechanism against the oxidative stress were observed only when ozone exposure was associated with high-intensity exercise, heat and humidity.

The effects of several pollutants on NAL cells and cytokines were considered in studies with environmental exposure at different lag time between pollution exposure and biomarker measurements. The populations more studied were represented by children [12–14]. Steerenberg et al. [12] observed an increase in inflammatory nasal markers measured in urban-located children compared with suburban-located children. An inflammatory mediator analysed in four papers was IL-8 [12, 13, 15, 18], and in three of them [12, 13, 18], it was positively associated with traffic-related air pollution. Larsson et al. [15] demonstrated IL-10, IL-12 and TNFα increase, but not IL-8 and cellularity changes, in NAL after inhalation of high concentration of air pollutants. The low number of subjects ($n = 5$) limits the strength of their finding. Moreover, compared with most investigations [12–14], Larsson et al. [15] found unaltered lung function after exposure. According to Barraza-Villarreal et al. [13], non-asthmatic and asthmatic children have a different susceptibility to traffic-related pollution: increased IL-8 levels were associated with exposure to PM and NO_2 in non-asthmatic, whereas they were associated with O_3 exposure in asthmatics. The study of Renzetti et al. [14] focused in particular on nasal eosinophil count and proved that it is a sensitive biomarker to monitor pollution-induced airway inflammation in allergic children, decreasing to virtually normal levels just 1 week after real-life relocation from a more polluted urban environment to a less polluted rural environment.

In summary, current published literature demonstrates that inflammatory mediator changes in NAL are influenced by traffic exposure and are confirmatory of respiratory worsening detected by lung function parameters. The findings are derived mainly from investigations in children as only one study with environmental exposure recruited adults with asthma [15]. The association between pollutants and NAL biomarker levels was observed at time lags ranging from 7 h to 7 days. A direct comparison of the time course of changes is prevented by a not homogeneous choice of lags among investigations and by different behaviour of each pollutant. Further studies on nasal biomarkers are needed in adults and healthy subjects.

Exhaled breath condensate (EBC)

Exhaled breath condensate is a biological fluid that is obtained by cooling exhaled air during tidal breathing. Collection of EBC is a method to sample airway lining

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Table 2. Summary table of studies on nasal biomarkers

Author, year	Biomarker	Exposure type	Pollutant(s)	Population (age)	Major findings	Lag
Nightingale, 1999 [10]	Nasal NO	Experimental	O_3 0.2 ppm for 4 h	10 healthy volunteers and 10 atopic asthmatics (18–39 years)	No nasal NO changes	
Mudway, 1999 [16]	Cell count MDA GSH Ascorbic acid UA α-tocopherol Extracellular-SOD Total protein Albumin MPO	Experimental	O_3 0.2 ppm for 2 h	13 healthy volunteers (20–31 years)	Depletion of nasal respiratory tract lining fluid UA	
Olin, 2001 [11]	Nasal NO	Experimental	O_3 0.2 ppm for 2 h	11 healthy non-smokers (20–29 year)	No nasal NO changes	
Gomes, 2011 [17]	IL-6 IL-8 CC16 Albumin UA GSH Total protein Total antioxidant	Experimental	O_3 0.1 ppm at 20°C and 50% rh; O_3 0.1 ppm at 31°C and 70% rh.	10 male runners (18–30 years)	Increase in CC16 and GSH/protein in the heat and O_3 trial	
Steerenberg, 2001 [12]	Cell count IL-8 Albumin UA Urea Nitrate, nitrite	Environmental	PM_{10} NO NO_2 CO SO_2 BS	126 children (8–13 years of age)	Increase in all inflammatory nasal markers analysed	1, 3 and 6 days
Barraza-Villarreal, 2008 [13]	IL-6 IL-8 IL-10	Environmental	$PM_{2.5}$ NO_2 O_3	158 asthmatic and 50 non-asthmatic schoolchildren (7–11 years)	Increased IL-8 associated with PM and NO_2 in non-asthmatic, associated with O_3 in asthmatics	0, 4 and 5 days
Romieu, 2008 [18]	IL-8	Environmental	$PM_{2.5}$ NO_2 SO_2 O_3	107 asthmatic children (mean age 9.5 years)	Augmented NAL IL8 levels related to MDA increase in EBC	0, 1 and 2 days

(continued)

Table 2 (continued)

Author, year	Biomarker	Exposure type	Pollutant(s)	Population (age)	Major findings	Lag
Renzetti, 2009 [14]	Nasal eosinophils	Environmental	PM_{10} NO_2 CO benzene	37 untreated allergic children (7 years of age or older) with mild persistent asthma	Decreased nasal eosinophils after 1 week relocation from urban to rural environmental	7 days
Larsson, 2010 [15]	Cell count IL-1 IL-2 IL-4 IL-5 IL-6 IL-8 IL-10 IL-12 IFNγ TNFα	Environmental	$PM_{2.5}$183 μg/m^3 PM_{10} 80 μg/m^3 NO 711 μg/m^3 NO_2 265 μg/m^3 CO 3.2 mg/m^3 for 2 h	14 mild asthmatic and the majority with allergy (24–38 years)	Increased IL-10, IL-12 and TNFα. Correlation between $PM_{2.5}$ exposure and increase in IL-10 concentration.	7 h

BS, black smoke; EBC, exhaled breath condensate; TSP, total suspended particles; MDA, malondialdehyde; GSH, reduced glutathione; UA, uric acid; SOD, superoxide dismutase; MPO, myeloperoxidase; rh, relative humidity.

fluid and can be performed repeatedly without adversely affecting a patient. Thus, it is feasible in studies involving large number of patients.

Six studies were conducted with an exposure chamber (Table 3). Nightingale et al. [10] measured nitrite in EBC in healthy volunteers and atopic asthmatics to assess acute effects of O_3, but no changes in nitrite levels were observed. Corradi et al. [19] studied ozone exposure in healthy non-smokers with intermittent exercise. In particular, they concentrated on the role of genotype for NAD(P)H:quinone oxidoreductase (NQO1) and glutathione-S-transferase (GSTM1). They observed in a subgroup of subjects characterized by NQO1wt and GSTM1-null genotypes that 2-h exposure to O_3, while performing mild physical exercise induced a sudden increase in EBC 8-isoprostane levels and a later increase (18 h) in EBC LTB-4 and thiobarbituric acid-reactive substances (TBARS) levels. Increased 8-isoprostane levels were associated with CAPs exposure also [20]. In the work of Hubbard et al. [21], healthy non-smokers were exposed to DE, and no significant changes in polar volatile organic compounds (PVOC) levels were detected in EBC despite of the application of an accurate analysis method. Hussain et al. [22] exposed adult asthmatics to 300 μg/m^3 $PM_{2.5}$ for 1 h and detected nitrite increase in EBC, but no changes in pH.

Despite pH evaluation is one of the simplest and better standardized measurement can be made in EBC, it gave controversial results. Four environmental studies [13, 23–25] with lag time of exposure up to 5 days demonstrated a pH decrease in asthmatic subjects (Table 3). Conversely, Epton et al. [26], Folino et al. [27] and Maestrelli et al. [28] did not find significant changes in EBC pH associated with air pollution 24 h after exposure in wheezing children, patients with myocardial infarction and asthmatic adults, respectively. The pH invariance might be due to technical differences – Epton et al. [26] did not degas EBC samples – to the ongoing disease or to the concomitant treatment and smoking status [28].

As demonstrated by two experimental and two environmental studies, malondialdehyde (MDA) may be a useful tool for investigating air pollution-related oxidative stress in both asthmatics and non-asthmatics regardless the subject age [18, 19, 29, 30].

An increase of nitrate and nitrite was observed associated with coarse particles levels, but not with $PM_{2.5}$ levels, both in asthmatic and COPD patients [31]. This study concluded that air pollution policies should take into account the effect of coarse particles. As stated by the authors, EBC nitric compounds may reflect an effect of PM exposure before such effects become marked enough to change airflow.

Major problems limiting the EBC usefulness are the variable dilution of solutes with water vapour and the

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Table 3. Summary table of studies on exhaled breath condensate

Author, year	Biomarker	Exposure type	Pollutant(s)	Population (age)	Major findings	Lag
Nightingale, 1999 [10]	Nitrite	Experimental	O_3 0.2 ppm for 4 h	10 healthy volunteers and 10 atopic asthmatics (18–39 years)	No changes in nitrite levels	
Corradi, 2002 [19]	H_2O_2 MDA 8-isoprostane LTB4 pH	Experimental	O_3 0.1 ppm for 2 h	22 healthy non-smokers (27–33 years)	Sudden increase in 8-isoprostane levels and a late increase in LTB4 and TBARS levels	
Mills, 2008 [20]	8-isoprostane nitrotyrosine	Experimental	CAPs (190 ± 37 μg/m^3) for 2 h	12 male patients with stable coronary heart disease and 12 male non-smoking volunteers	8-isoprostane levels increased 6 and 24 h after CAPs exposure, whereas nitrotyrosine levels did not significantly differ between exposures	
Rundell, 2008 [29]	MDA	Experimental	PM1	12 non-asthmatics males (20.5 ± 2.45 years)	MDA increase 40% after low exposure and 208% after high exposure	
Hubbard, 2009 [21]	PVOC	Experimental	DE 100 μg/m^3 for 1 h	11 healthy non-smokers (18–40 years)	No PVOC significant changes	
Hussain, 2012 [22]	pH Nitrite Nitrate	Experimental	DE 300 μg/m^3 for 1 h	16 non-smoking asthmatics (20–48 years)	Nitrite increase	
McCreanor, 2007 [23]	pH	Environmental	$PM_{2.5}$ NO_2 Pers.	60 asthmatics (19–55 years)	pH decrease	−2, 3 and 6 h
Barraza-Villarreal, 2008 [13]	pH	Environmental	$PM_{2.5}$ NO_2 O_3	158 asthmatic and 50 non-asthmatic schoolchildren (7–11 years)	pH decrease	0, 4 and 5 days
Epton, 2008 [26]	pH H_2O_2	Environmental	Wood burning PM_{10} $PM_{2.5}$ PM_1	93 schoolchildren (26 with asthma; 12–18 years)	No pH and H_2O_2 significant changes	0 h, 24 h and 1 week
Romieu, 2008 [18]	pH MDA	Environmental	$PM_{2.5}$ NO_2 SO_2 O_3	107 asthmatic children (mean age 9.5 years)	MDA increase related to air pollution exposure, lower lung function and augmented NAL IL8 levels	0, 1 and 2 days
Folino, 2009 [27]	pH LTB4	Environmental	PM_{10} $PM_{2.5}$ $PM_{0.25}$ Pers.	39 patients with myocardial infarction (45–65 years)	No pH and LTB4 significant changes	24 h
Zhang, 2009 [24]	pH free iron nitrite nitrate	Environmental	$PM_{2.5}$, UFP CO	60 non-smoking asthmatics (18–55 years)	pH decrease	2, 3 and 6 h

(continued)

Table 3 (continued)

Author, year	Biomarker	Exposure type	Pollutant(s)	Population (age)	Major findings	Lag
			EC NO_2 Pers.			
Liu, 2009 [30]	TBARS 8-isoprostane IL-6	Environmental	$PM_{2.5}$ NO_2 SO_2 O_3	182 asthmatic children (9–14 years)	Increased TBARS but no 8-isoprostane and IL-6 significant changes	1, 2 and 3 days
Maestrelli, 2011 [28]	pH	Environmental	PM_{10} $PM_{2.5}$ Pers.	32 adult asthma (15–44 years)	No pH significant changes	24 h
Martins, 2012 [25]	pH	Environmental	PM_{10} $PM_{2.5}$ NO NO_2 CO O_3 BTEX	51 wheezing children (5–8 years)	pH decrease associated with PM_{10}, NO_2, benzene and ethylbenzene	5 days
Rava, 2012 [32]	Nitrite Nitrate	Environmental	PM_{10} NO_2 O_3	949 adults	Nitrite/nitrate decrease with O_3 only	
Manney, 2012 [31]	Glutathione NOx	Environmental	PM_{10} $PM_{2.5}$	111 asthmatics or COPD (36–85 years)	NOx increase associated with coarse particles	0, 1 and 2 days

COPD, chronic obstructive pulmonary disease; PVOC, polar volatile organic compounds; MDA, malondialdehyde; NOx, total nitrite plus nitrate; TBARS, thiobarbituric acid-reactive substances; Pers., personal monitoring; BTEX, benzene, toluene, ethylbenzene and xylenes.

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concentration of biomarkers below detection limit of common assays. Due to the increasing sensitivity of new detection techniques, the purity of the samples also is becoming a major hurdle [33]. Recently published data have shown that metals, trace elements and specific chemical substances can be detected in EBC of occupationally exposed subjects [34]. EBC has the potential to be used for assessing the dose of inhaled environmental pollutants at the target organ. Except nitric compounds, the biomarkers assayed until now were associated with changes in lung function parameters. Thus, the identification of other EBC biomarkers more sensitive to acute traffic exposure represents a new challenge.

Exhaled NO

Among exhaled breath biomarkers, exhaled NO monitoring deserves a special attention, being applied by the majority of studies in this field. Exhaled NO is elevated in patients with untreated asthma, reduces with corticosteroid treatment and correlates with sputum eosinophils. The methodology for measuring fractional exhaled NO (FeNO) has been standardized based on international consensus. The determinants of exhaled NO levels are fairly well characterized, with the most important being cigarette smoking, nitrate intake, allergen sensitization and exposure, along with corticosteroid treatment, height, sex and age [35].

Among the 42 selected papers (Table 4), eight are referred to experimental exposure studies, and interestingly, seven did not detect any significant FeNO changes in response to $PM_{2.5}$ or O_3 exposure in asthmatic and healthy subjects [10, 11, 22, 36–39]. Rundell et al. [29] instead demonstrated a FeNO decrease related to lung function falls in healthy subjects after high PM exposure, as expected by the well-known effect of bronchoconstriction on FeNO [40].

On the other hand, most environmental studies showed FeNO increase associated with increase of a wide range of traffic-related air pollutants. Almost half of the published works recruited children [12–14, 27, 30, 41–50], and all observed a pollution-related FeNO increase except Liu et al. [30]. Berhane et al. [51] analysed 2240 schoolchildren and pointed out that ambient air pollution was associated with airway inflammation independent of asthma and allergy status. An increase in FeNO related to $PM_{2.5–0.25}$, $PM_{2.5}$, CO, NO_x, SO_2 and O_3 exposure was demonstrated in three investigations focusing on elderly people [52–54].

Four studies enrolled adult asthmatics [18, 19, 28, 55]. FeNO increase following traffic exposure was demonstrated by Zhang et al. [24] and Jansen et al. [55], whereas McCreanor et al. [23] and Maestrelli et al. [28] did not detect any significant change. Such controversial results suggest that underlying inflammation in asthma or anti-inflammatory treatment may obscure the effects of air pollution. Exercise during exposure to PM_{10} results in a modest FeNO increase according to Strak et al. [56]. Cyclists exposed to PM_{10}, $PM_{2.5}$ and ultrafine particles (UFP) investigated by Klepczynska et al. [57, 58] and Jacobs et al. [59] did not show significant variation of FeNO levels, but the authors recognize that a cause may be the small sample size of the study.

Following traffic-related air pollution exposure, five studies [10, 38, 39, 49, 52] detected FeNO increase without changes in lung function, while thirteen papers [12–14, 19, 25, 36, 43, 46, 47, 55, 60–62] reported FeNO changes concomitant to airflow worsening. Three studies observed no FeNO alteration or decreased lung function [27, 28, 59]. In nine studies, lung function parameters were not measured [43, 44, 48–51, 53, 63, 64]. Therefore, it remains controversial whether FeNO measurements allow to detect an air pollution effect before it is evident by lung function tests.

Overall, the majority of studies found correlation between FeNO and traffic-related pollutants irrespective of the lag time considered. Although the estimates of effect were small, inasmuch as FeNO is a marker of airway inflammation, this would suggest that air pollution increases lung inflammation. FeNO is often considered a surrogate for airway eosinophilia [33]. However, it has to be underlined that FeNO cannot be used interchangeably with sputum eosinophil cell count as an outcome measure as it is elevated in several other conditions [65]. Moreover, FeNO measurement does not differentiate between bronchial and alveolar components. Flow-independent parameters may provide more information of lung NO [66]. As suggested by the ERS task force [35], partitioning of exhaled NO into airway and peripheral fractions might be more useful for monitoring asthma and COPD, but the extended NO analysis has not been applied on studies concerning air pollution yet.

Induced sputum

Sputum induction is a semi-invasive, safe and repeatable procedure which has been developed for examining healthy and diseased subjects. Sputum induction consists of inhaling an aerosol of saline solution over a determined time period and to produce timed, forced expectoration efforts [67]. Sputum induction requires a high degree of cooperation from the patient and it is difficult to be applied in children. It should be performed by adequately trained technologists or nurses with sufficient experience with the protocol, under the supervision of an experienced physician and resuscitation equipment should be available [68]. A remarkable

Table 4. Summary table of studies on exhaled NO

Author, year	Biomarker	Exposure type	Pollutant(s)	Population (age)	Major findings	Sampling time
Nightingale, 1999 [10]	FeNO	Experimental	O_3 0.2 ppm for 4 h	10 atopic asthmatics and 10 healthy volunteers (18–39 years)	No significant FeNO changes	
Newson, 2000 [36]	FeNO	Experimental	O_3 0.2 ppm for 2 h	9 atopic asthmatics (21–42 years)	No significant FeNO changes	
Olin, 2001 [11]	FeNO	Experimental	O_3 0.2 ppm for 2 h	11 healthy non-smoking volunteers (20–29 years)	No significant FeNO changes	
Frampton, 2004 [37]	FeNO	Experimental	UFP for 2 h Healthy+resting: 10 μg/m^3 Healthy+exercise: 10 then 25 μg/m^3 Asthma+exercise: 10 μg/m^3	16 mild-to-moderate asthmatics and 24 healthy controls (18–40 years)	No significant FeNO changes	
Gong, 2008 [38]	FeNO	Experimental	UFP 100 μg/m^3 for 2 h	17 healthy volunteers (24 ± 8 years), 14 asthmatics (34 ± 12 years)	No significant FeNO changes	
Rundell, 2008 [29]	FeNO	Experimental	PM1	12 non-asthmatics male	FeNO decrease after high exposure	
Behndig, 2011 [39]	FeNO	Experimental	PM_{10} 100 μg/m^3 for 2 h	32 asthmatics and 23 healthy controls (18–45 years)	No significant FeNO changes	
Hussain, 2012 [22]	FeNO	Experimental	$PM_{2.5}$ 300 μg/m^3 for 1 h	16 non-smoking asthmatics (20–48 years)	No significant FeNO changes	
Van Amsterdam, 1999 [63]	FeNO	Environmental	PM_{10}, NO, NO_2 CO SO_2	16 non-smoking healthy subjects (29–38 years)	FeNO increase	Day 0
Steerenberg, 2001 [12]	FeNO	Environmental	PM_{10}, NO, NO_2 CO, SO_2 BS	126 children (8–13 years of age)	FeNO increase	1, 3 and 6 days
Fischer, 2002 [41]	FeNO	Environmental	PM_{10} NO_2 NO CO	68 schoolchildren (10–11 years of age)	FeNO increase	1 and 2 days
Koenig, 2003 [42]	FeNO	Environmental	$PM_{2.5}$ Pers.	19 children (6–13 years of age)	FeNO increase	Day 0
Adamkiewicz, 2004 [52]	FeNO	Environmental	$PM_{2.5}$ NO_x SO_2 O_3	29 non-smoking elderly (53–91 years), among them 8 COPD and 7 asthmatics	FeNO increase	24 h
Jansen, 2005 [55]	FeNO	Environmental	PM_{10} $PM_{2.5}$ NO NO_2 Pers.	16 asthmatics or COPD or both (60–86 years of age)	FeNO increase	24 h
Koenig, 2005 [43]	FeNO	Environmental	$PM_{2.5}$ Pers.	19 asthmatic children (6–13 years)	FeNO increase	Day 0
Mar, 2005 [44]	FeNO	Environmental	$PM_{2.5}$	19 asthmatic children (6–13 years)	FeNO increase	10–12 h
Delfino, 2006 [45]	FeNO	Environmental	$PM_{2.5}$ $PM_{2.5}$ EC OC NO_2 Pers.	45 schoolchildren with persistent asthma (9–18 years)	FeNO increase	0, 1 and 2 days
Adar, 2007 [54]	FeNO	Environmental	$PM_{2.5}$ Pers.	44 non-smokers in bus trip (62–94 years of age)	FeNO increase	6 and 24 h

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Table 4 (continued)

Author, year	Biomarker	Exposure type	Pollutant(s)	Population (age)	Major findings	Sampling time
Holguin, 2007 [46]	FeNO	Environmental	$PM_{2.5}$ NO_2	200 children with or without asthma (6–12 years of age)	FeNO increase in asthmatics	2 days
McCreanor, 2007 [18]	FeNO	Environmental	$PM_{2.5}$ NO_2 Pers.	60 asthmatics (19–55 years)	No significant FeNO changes	0, 5 and 24 h
Murata, 2007 [47]	FeNO	Environmental	$PM_{2.5}$ NO NO_2 Pers.	19 children (5–10 years of age)	FeNO increase	24 h
Allen, 2008 [48]	FeNO	Environmental	PM_{10} $PM_{2.5}$ sulphur LAC LG Pers.	19 asthmatic children (6–13 years of age)	FeNO increase	1 day
Barraza-Villarreal, 2008 [13]	FeNO	Environmental	$PM_{2.5}$ NO_2 O_3	158 asthmatic and 50 non-asthmatic schoolchildren (7–11 years)	FeNO increase	0, 4 and 5 days
Folino, 2009 [27]	FeNO	Environmental	PM_{10} $PM_{2.5}$ $PM_{0.25}$ Pers.	39 patients with myocardial infarction (45–65 years)	No significant FeNO changes	24 h
Khatri, 2009 [62]	FeNO	Environmental	O_3	38 asthmatics and 13 healthy control subjects	Higher FeNO levels in asthmatics	2 days
Liu, 2009 [30]	FeNO	Environmental	$PM_{2.5}$ NO_2 SO_2 O_3	182 asthmatic children (9–14 years of age)	No significant FeNO changes	1, 2 and 3 days
Renzetti, 2009 [14]	FeNO	Environmental	PM_{10} NO_2 CO benzene	37 untreated allergic children with mild persistent asthma (7 years of age or older)	FeNO increase with decreased lung function	7 days
Zhang, 2009 [24]	FeNO	Environmental	$PM_{2.5}$ UFP CO EC NO_2 Pers.	60 non-smoking asthmatics (18–55 years of age)	FeNO increase associated with EC	2, 3, 4, 5, 6, 7 and 22 h after exposure
Delfino, 2010 [53]	FeNO	Environmental	$PM_{2.5–0.25}$ $PM_{0.25}$ CO NO_x O_3	60 elderly (65–90 years)	FeNO increase positively associated with $PM_{0.25–2.5}$ and O_3	5 days
Klepczynska Nystrom, 2010 [57]	FeNO	Environmental	$PM_{10}PM_{2.5}$ UFP NO NO_2 Pers.	20 healthy non-smokers (18–46 years)	No significant FeNO changes between subway and control exposures	14 h
Jacobs, 2010 [59]	FeNO	Environmental	$PM_{10}PM_{2.5}$ UFP Pers.	38 cyclist (18–65 years)	No significant FeNO changes	30 min
Larsson, 2010 [15]	FeNO	Environmental	$PM_{2.5}$ 183 $\mu g/m^3$ PM_{10} 80 $\mu g/m^3$ NO 711 $\mu g/m^3$ NO_2 265 $\mu g/m^3$ CO 3.2 mg/m^3 for 2 h	14 mild asthmatic and the majority with allergy (18–55 years)	No significant FeNO changes	7 h
Strak, 2010 [56]	FeNO	Environmental	PM_{10}	12 cyclists (23–57 years)	FeNO increase weakly	6 h
Maestrelli, 2011 [28]	FeNO	Environmental		32 adult asthma (15–44 years)	No significant FeNO changes	24 h

(continued)

Table 4 (continued)

Author, year	Biomarker	Exposure type	Pollutant(s)	Population (age)	Major findings	Sampling time
			PM_{10} $PM_{2.5}$ Pers.			
Zuurbier, 2011 [60]	FeNO	Environmental	PM_{10} $PM_{2.5}$	34 healthy non-smoking adults (18–56 years)	FeNO increase modestly	6 h
Lin, 2011 [49]	FeNO	Environmental	$PM_{2.5}$ BC	8 asthmatic and 28 non-asthmatic children (9–12 years)	FeNO increase associated with BC	24 h
Berhane, 2011 [51]	FeNO	Environmental	$PM_{2.5}$ PM_{10} NO_2 O_3	2240 subjects	FeNO increase	1 and 7 days
Graveland, 2011 [64]	FeNO	Environmental	PM_{10}	812 children (7–11 years)	FeNO increase	0, 1, 2 and 3 days
Klepczynska Nystrom, 2012 [58]	FeNO	Environmental	$PM_{10}$$PM_{2.5}$ UFP NO NO_2 Pers.	16 mild asthmatics (18–52 years)	No significant FeNO changes between subway and control exposures	14 h
Weichental, 2012 [61]	FeNO	Environmental	VOC Pers.	42 healthy non-smoking adults (19–58 years)	FeNO increase	1–4 h
Martins, 2012 [25]	FeNO	Environmental	PM_{10} $PM_{2.5}$ NO NO_2 CO O_3 BTEX	51 wheezing children (5–8 years)	FeNO increase associated only with ethylbenzene	5 days
Sarnat, 2012 [50]	FeNO	Environmental	$PM_{2.5-10}$ $PM_{2.5}$ BC NO_2	58 asthmatic children (6–12 years)	FeNO increase	2 and 4 days

BS, black smoke; COPD, chronic obstructive pulmonary disease; EC, elemental carbon; LAC, light absorbing carbon; LG, levoglucosan; UFP, ultra-fine particulate; OC, organic carbon; BTEX, benzene, toluene, ethylbenzene and xylenes; Pers., personal monitoring.

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issue is the possibility that induction itself or subsequent sputum processing activates airway inflammatory cells. In addition, the interference by sulfhydryl-reducing reagents, such as dithiothreitol (DTT), is important when soluble mediators are measured [69].

Eighteen papers reported IS analysis to study short-term effects of traffic-related pollution (Table 5), and fifteen of them were performed with controlled chamber exposures.

Many studies analysed O_3 effects on asthmatics and healthy volunteers [10, 36, 70–73]. Hiltermann et al. [70] suggest that eosinophils rather than neutrophils play a pathogenetic role in ozone-induced airway hyperresponsiveness. Nightingale et al. [10] show that ozone exposure leads to a neutrophil inflammatory response in both normal and asthmatic subjects. Newson et al. [36] concluded that short-term ozone exposure induces firstly polymorphonuclear leucocytes influx followed by plasma extravasation and activation of both eosinophils and neutrophils.

Three other experimental exposure studies focused on diesel exhaust particles (DEP) effects exclusively [74–76]. Interestingly, the number of neutrophils increased in healthy volunteers following DEP exposure [74, 75]; instead, no changes in neutrophils count emerged in atopic asthmatics [76]. Enhanced levels of IL-6 were observed both in healthy volunteers [75] and asthmatics [76].

Controlled CAPs exposure was investigated by two successive works of Gong et al. [77, 78] in asthmatic and COPD patients. Only the asthmatics exhibited a 50% decrease of columnar epithelial cells in IS upon exposure without any change in inflammatory leucocytes. The authors believe that the discordant results among these two studies could reflect differences in susceptibility of the two clinical groups. As remarked by Frampton [37], despite the efforts to use similar protocols, the comparison of his and Gong [38] studies is challenging because the studies involved small numbers of participants exposed to different types of particles in different locations. Nonetheless, they both observed that inflammatory effects in the airways detected by IS are minimal. Alexis and co-workers [79] demonstrated that endotoxin on $PM_{10–2.5}$ is not necessary to induce neutrophil responses upon PM exposure, but is essential to induce cytokine responses from alveolar macrophages.

The work of Bosson et al. [80] considered for the first time the effect of adding O_3 to exposure of DEP. It demonstrated that O_3 exposure could amplify the inflammatory response induced by DEP as they found raised sputum neutrophils and levels of myeloperoxidase (MPO) after the combined exposure.

Among the exposure studies, both McCreanor et al. [18] and Zhang et al. [19] measured IL-8, MPO and eosinophilic cationic protein in addition to cell count in asthmatic subjects. They both observed neutrophilic inflammation after urban diesel traffic exposure. Exercise and environmental PM_{10} and O_3 exposure affect apoptosis of airway cells according to Chimenti et al. [81]. Apoptosis of neutrophils increased with inhalation of environmental pollutants, while apoptosis of bronchial epithelial cells augmented after intense exercise.

Carbon load in IS macrophages has been used by some researchers to assess individual exposure to biomass fuels [82, 83] and PM_{10} [84–86]. While most studies were conducted in children [82, 84, 85], a study evaluated carbon load in healthy adults [83] and another one was performed in non-smoking adults with diabetes [86]. Overall, the authors agree that carbon load in IS macrophages is a promising marker to assess individual intake of inhaled carbonaceous particles. As observed by Nwokoro et al. [87], however, it is still unknown whether carbon in alveolar macrophages reflects black carbon dose inhaled over weeks, months or even years.

All together these studies demonstrate that IS as good source of both cells and extracellular mediators in the supernatant, but involved only adults. Based on experimental exposure studies, there is limited evidence that IS analysis is more sensitive than lung function tests. Given that sputum leucocytes reach peak values 24 h after experimental exposure, environmental studies performing IS [18, 19, 81] adopted a lag time of 20–24 h uniformly. A single lag time in each study design is also justified by the risk that the induction itself causes some degree of airway inflammation. Therefore, a proper time course of inflammatory response to pollutants exposure has not been assessed.

Conclusions

The methods used to assess short-term effects of traffic-related air pollution vary according to the compartment of respiratory tract to be examined. Cells and soluble mediators to study upper airways are obtained by NAL. Central and lower airways can be examined using IS, EBC and exhaled gas.

A summary of the usefulness of main biomarkers in detecting acute air pollution effects according to the age of participants (adults vs. children), the disease status (healthy vs. asthma–atopy), their sensitivity compared with lung function tests and their specificity for a type of pollutant is displayed in Table 6.

In NAL samples, increased levels of IL-8 are positively associated with PM and gaseous pollutants inhalation in children [12, 13, 18], and alteration in NAL cell number or cytokines levels is concomitant with respiratory worsening observed by lung function parameters.

Table 5. Summary table of studies on Induced sputum

Author, year	Biomarker	Exposure type	Pollutant(s)	Population (age)	Major findings	Lag
Hiltermann, 1999 [70]	Cell count Albumin ECP Elastase IL-8	Experimental	O_3 0.4 ppm for 2 h	16 non-smoking subjects with intermittent asthma (19–35 years)	Total cell number, ECP and elastase increased	
Nightingale, 1999 [10]	Cell count TNFα IL-8 GM-CSF	Experimental	O_3 0.2 ppm for 4 h	10 healthy volunteers and 10 atopic asthmatics (18–39 years)	Increased neutrophils	
Newson, 2000 [36]	Cell count Albumin hist MPO ECP IL-8 Gro-[alpha]	Experimental	O_3 0.2 ppm for 2 h	9 atopic asthmatics (21–42 years)	PMNL influx at 6 h after exposure; increase of albumin, total protein, MPO and ECP at 24 h after exposure	
Nightingale, 2000 [74]	Cell count MPO TNFα IL-8	Experimental	DEP 200 μg/m^3 for 2 h	10 healthy non-smoking volunteers (25–31 years)	Increased neutrophils and MPO	
Nordenhall, 2000 [75]	Cell count M-hist MPO ECP TNFα IL-6 IL-8 Gro-α	Experimental	DEP 300 μg/m^3 for 1 h	15 healthy non-smoking volunteers (22–33 years)	Increased neutrophils, IL-6 and M-hist	
Nordenhall, 2001 [76]	Cell count M-hist MPO ECP IL-6 IL-8 Il-10	Experimental	DEP 300 μg/m^3 for 1 h	14 non-smoking atopic asthmatics with stable disease (22–57 years)	Increased IL-6, but no changes in neutrophils count	
Gong, 2003 [77]	Cell count IL-6 IL-8	Experimental	Fine CAPs average 174 μg/m^3 for 2 h	12 mild asthmatics and 12 healthy controls (18–45 years)	Decrease of 50% in columnar epithelial cells, but no changes in IL-6 and IL-8	
Gong, 2004 [78]	Cell count IL-6 IL-8	Experimental	CAPs 200 μg/m^3 for 2 h	13 elderly with COPD (73 ± 8 years) and 6 healthy controls (68 ± 11 years)	Not significant changes	

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Table 5 (continued)

Author, year	Biomarker	Exposure type	Pollutant(s)	Population (age)	Major findings	Lag
Frampton, 2004 [37]	Cell count IL-6 IL-8	Experimental	UFP for 2 h Healthy+resting: 10 μg/m^3 Healthy+exercise: 10 then 25 μg/m^3 Asthma+exercise: 10 μg/m^3	16 mild-to-moderate asthmatics and 24 healthy controls (18–40 years)	Increased macrophage % in asthmatics but IL-6 and IL-8 unaffected by UFP exposure	
Arjomandi, 2005 [71]	Cell count	Experimental	O_3 0.2 ppm for 4 h	13 asthmatics (21–45 years)	Increases in both the concentration of neutrophils and the %PMNs	
Alexis, 2006 [79]	mCD14 CD11b HLA-DR CD64 CD16	Experimental	$PM_{2.5–10}$ 70 μg/m^3 for 24 h	9 healthy volunteers (18–35 years)	Biological material on coarse fraction of PM increases % of PMN, macrophage mRNA TNF-a, eotaxin, up-regulated immune surface phenotypes on macrophages and monocytes phagocytosis	
Ratto, 2006 [72]	Cell count Total protein LDH Fibrinogen IL-6 IL-8	Experimental	O_3 0.4 ppm for 2 h	15 healthy non-smoking volunteers (20–31 years)	Increase in the neutrophils %	
Bosson, 2007 [80]	Cell count MPO MMP-9 IL-6	Experimental	PM 300 μg/m^3 for 1 h O_3 0.2 ppm for 2 h	16 healthy non-smoking volunteers (20–28 years)	Increased neutrophils and MPO	
Gong, 2008 [38]	Cell count Total protein IL-4 IL-6 IL-8 IL-10 IL-13 GM-CSF Interferon TNF	Experimental	UFP 100 μg/m^3 for 2 h	17 healthy volunteers (24 ± 8 years), 14 asthmatics (34 ± 12 years)	Not significant changes	
Hernandez, 2010 [73]	Cell count CD11b CD14 CD86	Experimental	O_3 0.4 ppm for 2 h	25 healthy volunteers, 14 atopic non-asthmatic subjects and 11 atopic asthmatic subjects	Atopic asthmatics have increased airway inflammatory response	

(continued)

Table 5 (continued)

Author, year	Biomarker	Exposure type	Pollutant(s)	Population (age)	Major findings	Lag
	TLR4 FcεRI CD23 HLA-DRHA					
McCreanor, 2007 [18]	Cell count IL-8 MPO ECP	Environmental	$PM_{2.5}$ NO_2 Pers.	60 asthmatics (19–55 years)	Increased neutrophils	24 h
Zhang, 2009 [24]	Cell count IL-8 MPO ECP	Environmental	$PM_{2.5}$ UFP CO EC NO_2 Pers.	60 non-smoking asthmatics (18–55 years)	Increased neutrophils and MPO	22 h
Chimenti, 2009 [81]	Cell count TUNEL TNFα IL-8	Environmental	PM_{10} NO_2 O_3	9 runners (36–44 years)	Increased neutrophils apoptosis	20 h

COPD, chronic obstructive pulmonary disease; MPO, myeloperoxidase; Gro-α, growth-related oncogene-α; ECP, eosinophilic cationic protein; TNFα, tumour necrosis factor-α; EC, elemental carbon; M-hist, methylhistamine; DEP, diesel exhaust particles; GM-CSF, granulocyte-macrophage colony-stimulating factor; HA, hyaluronic acid; LDH, lactate dehydrogenase; Pers., personal monitoring; UFP, ultra-fine particulate.

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Table 6. Summary of the usefulness of main biomarkers in detecting acute air pollution effects according to the age of participants (adults vs. children), the disease status (healthy vs. asthma-atopy), their sensitivity compared with lung function tests and their specificity for a type of pollutant

Biomarker	Suitability in children	Suitability in adults	Healthy vs. asthma-atopy	More sensitive than lung function	Specificity for a type of pollutant
NAL† (cells, cytokines)	Yes (***: strong evidence)	Yes (*: limited evidence)	No disease specific (**: moderate evidence)	No (***: strong evidence)	No (**: moderate evidence)
EBC pH†	Yes (**:moderate evidence)	No (*:contradictory evidence)	No disease specific (***: strong evidence)	No (***: strong evidence)	No (***: strong evidence)
FeNO†	Yes (***: strong evidence)	Yes (***: strong evidence)	No disease specific (***: strong evidence)	No (*: contradictory evidence)	No (***: strong evidence)
IS‡ (cells, cytokines)	No (–: no evidence)	Yes (***: strong evidence)	No disease specific (**: moderate evidence)	Yes (**:moderate evidence)	No (**: moderate evidence)

The statements were graded according to the Royal College of General Practitioners (RCGP) three-star system (–: no scientific evidence; *: limited/contradictory evidence; **: moderate evidence; ***: strong evidence).
†Based on environmental exposure studies.
‡Based on experimental exposure studies.

Exhaled breath condensate collection is practical in studies involving large number of patients. Traffic-related exposure results in EBC pH acidification, but the identification of more specific EBC molecules to acute air pollution exposure still represents a challenge. MDA seems a useful tool for investigating air pollution-related oxidative stress in both asthmatics and non-asthmatics regardless the subject age [18, 19, 29, 30].

Many evidences support the usefulness of FeNO measurement for both adults and children. However, it is controversial whether FeNO changes are detectable before the occurrence of lung function deterioration. Partitioning of exhaled NO into airway and alveolar fractions might be a more sensible tool to identify the site of lung damage due to augmented level of air pollution.

Reviewed studies showed association between traffic pollution and increase of sputum neutrophils. Based on experimental exposure studies, there is moderate evidence that IS analysis is more sensitive than lung function tests. Moreover, IS may be useful in assessment of exposure to PM in terms of lung particles load by measuring alveolar macrophage carbon content [82–87].

Cells and some cytokines (IL-6, IL-8 and MPO) have been measured both in the upper respiratory tract (NAL) and in the lower airways (IS). Overall, the response to traffic exposure seems different in the two compartments. No relevant changes were detected in NAL cell count, in contrast, in the lower respiratory tract neutrophilic response predominates [24, 75, 80]. IL-6 measurements do not give consistent results after air pollution exposure: only one study reports augmented IL-6 concentration in IS of healthy volunteers after DEP exposure [75]. Augmented IL-8 levels were detected in some environmental studies in NAL [13, 18], but not in IS samples. Finally, while NAL MPO levels were unaffected by ozone exposure, MPO increased in the majority of studies adopting IS sampling.

Although COPD subjects were generally considered at higher risk of adverse effects of air pollutants, they were recruited only in few studies [31, 52, 60, 78]. According to Manney et al. [31], increase of EBC nitrate and nitrite was associated with coarse particles exposure in COPD subjects and asthmatic without significant differences between the two diseases. FeNO measured in other two studies gave opposite results: one found a relationship with $PM_{2.5}$ in subjects with COPD but not with asthma [52], while in the other [60], FeNO was associated with levels of PM air pollution in study participants with asthma but not in those with COPD. According to Gong et al. [78] induced sputum cellularity in COPD subjects is not affected by CAPs exposure. New efforts are thus required to elucidate traffic-related air pollution acute effect in subjects suffering lung diseases other than asthma.

Short-term monitoring of exposure may provide insights into long-term inhaled air pollutants dose when behaviour is habitual [87]. However, the acute

health effects are difficult to be attributed to one particular pollutant in most investigations of environmental exposure. As pointed out by Zhang [19], the measured pollutant concentrations may simply represent the entire roadside diesel–traffic exposure that comprises not only the pollutants measured in this study but also other pollutants in the complex DEP mixture and in resuspended coarse particles from road dust, engine debris and tyre debris. The accuracy of an exposure–response relationship may be reduced by a misclassification of exposure [28]. In fact, many investigators have relied on fixed-site measurements of PM, which may not reflect individual exposures. Only a limited number of studies have examined the association between personal exposure to PM and health effects in adult asthmatics [23, 24, 28, 43, 45, 48, 55, 58]. Further research should be directed to promote a better standardization of the methods to monitor traffic pollutants. It has to be reminded that under real-life conditions 'unexposed' subjects do not exist and the difference between the lowest and highest levels of air pollution is often less than threefold [6]. Thus, particularly when planning observational studies, a baseline for the levels of inflammatory markers needs to be established in subjects exposed to low levels of pollutants to be able to detect an effect at higher exposures.

This survey of the current literature displays the complexity of this research field, highlights the significance of short-term studies on traffic-related air pollution and gives important tips when planning studies to detect acute respiratory effects of pollution in a non-invasive way.

Conflict of interests

The authors declare no conflict of interest.

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