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Fractional Exhaled Nitric Oxide (FeNO) in the Screening and Diagnosis Work-Up of Occupational Asthma

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Opinion Statement

FeNO is a simple non-invasive tool used as a surrogate marker of airway inflammation in the management of asthma. FeNO has been assessed in several populations of workers exposed to high- and low-molecular weight agents. However, there are many confounding factors to consider in the interpretation of FeNO. As such, its use in the investigation of occupational asthma (OA) has yielded inconsistent results. In screening studies of OA to high molecular weight (HMW) agents, an increase of FeNO over time has been associated with the development of bronchial hyperresponsiveness (BHR). When used in the investigation of OA, increases in FeNO show high positive predictive values for a positive specific inhalation challenge (SIC) to HMW agents. Subjects with high specific IgE to their workplace allergens seem to present higher increases in FeNO than subjects without specific IgE to causal agent. Changes in FeNO have less frequently been observed in studies of OA to low molecular weight (LMW) agents than OA to HMW agents, except for isocyanate-induced OA. Cluster analyses of patients exposed to various HMW and LMW agents have documented significant increases of FeNO in clusters of patients with OA to HMW agents, but not LMW. Recent studies have provided useful information for improving our understanding of the pathophysiology of exhaled NO in OA despite the previously reported conflicting results. Future studies are still required to clarify its role in the screening, investigation, and management of OA.

Introduction

Produced by inducible nitric oxide synthase (iNOS) in the lung, NO holds key functions such as vasodilator, bronchodilator, inflammatory mediator, and neurotransmitter. The measure of fractional exhaled nitric oxide (FeNO) has extensively been studied in asthma [1]. It is considered as a non-invasive proxy for the airway eosinophilic inflammation easy to perform with good within-subject repeatability. However, even if FeNO correlates with sputum eosinophils, the correlation remains moderate at best [2]. Its correlation to blood eosinophils is weak and is further weakened when patients are treated with inhaled corticosteroids [3]. In a dose-dependent manner, the treatment with corticosteroids weakens the strength of the correlation between FeNO levels and airway inflammation. Several other confounding factors such as smoking, atopy, and gender must be considered when interpreting the results of this test. Therefore, the reference ranges that have been proposed take these factors into account [4]. Despite these limitations, FeNO is currently widely used both in research and clinical settings.

FeNO has been increasingly measured in the screening and diagnosis of occupational asthma (OA). The recent ERS Task Force consensus statement on "Specific inhalation challenge in the diagnosis of occupational asthma" recommends the assessment of changes in FeNO levels in subjects who fail to provide suitable sputum during SIC, despite the conflicting data that have been published so far [5••]. The present article reviews recent studies and advancements regarding the use of FeNO in an occupational setting, specifically, during screening and investigating potential cases of OA.

FeNO in the screening of OA

Since FeNO can easily be measured at the workplace using a small portable device, its potential value as a screening tool for OA is appealing. Its value as a screening tool has been assessed in several populations of workers exposed to HMW and LMW agents.

Screening of OA in populations exposed to HMW agents

FeNO was measured among workers exposed to laboratory animals; the FeNO levels increased in two subjects throughout their workweek. These subjects had a high specific IgE to laboratory animal allergens. In contrast, subjects without specific IgE to laboratory animals did not show any increase in FeNO over time [6]. A recent study also attempted to identify predictors of sensitization to laboratory animals in new workers over the course of 2 years of exposure. There was no difference in the FeNO levels between study entry and after 24 months of employment [7].

Florentin et al. explored the use of FeNO as a screening tool in a population of workers at risk to develop OA: bakers and pastry-makers [8]. They identified an 8.5 ppb FeNO threshold as having a 78.9% sensitivity and a 42.8% specificity in identifying OA in their study population: 89.5% bakers or pastry-makers and 10.5% hairdressers. The addition of a questionnaire to this test substantially improved the specificity (80.5%) without changing its sensitivity. However, this retrospective study only included a small number of subjects with OA (n = 19) among whom, a third were on inhaled corticosteroids.

In a group of healthy baking and pastry-making apprentices, no relationship was identified between BHR and FeNO prior to the beginning of the apprenticeship [9]. Tossa et al. followed the same group of apprentices during their 15-

month training and showed that an increase in FeNO during the apprenticeship was highly correlated with de novo occurrence of BHR, regardless of the atopic status of the subjects [10, 11]. This relative increase in FeNO levels in both non-atopic (20% increase) and atopic subjects (16% increase) compared to baseline FeNO levels was the only factor associated with the development of BHR (OR 1.94; CI 95% 1.18–3.20). The correlation was observed among all occupational groups, independently of the molecular weight of the agent used. Bohadana et al. also studied FeNO and spirometry in apprentice bakers after their initial year of training. They found little agreement between the two tests [12]. Unfortunately, the incidence of BHR was not measured along with FEV1/FVC.

Screening of OA in populations exposed to LMW agents

Demange et al. completed a feasibility study in 39 lifeguards—employed in indoor swimming pools—to assess the relationship between FeNO levels and BHR in the context of occupational studies at the workplace [13]. The study showed a significant association between FeNO levels and BHR. Using a cutoff of a 60% predicted FeNO for abnormal values as previously suggested by Olin et al., FeNO identified subjects with and without BHR to methacholine in apparently healthy lifeguards, with a sensitivity and specificity of 80 and 42%, respectively [14]. This study suggested that FeNO could be a potential tool for the early detection of BHR in workers at risk of developing respiratory symptoms.

Hairdressers have often been studied in cohorts that also screened bakers and pastry-makers for OA [8, 11]. FeNO used as a single tool did not appear to be an effective screening tool to identify OA [8]. In other studies on hairdressers, an increase in FeNO by 16–20% from the baseline was identified as a predictor for the occurrence of BHR in apprentices [11].

Cement factory workers exposed to different levels of cement dust showed similar FeNO levels as water factory workers from the same region acting as controls [15]. While no significant variation of FeNO levels was observed, a large proportion of the subjects wore respiratory protection equipment. Furthermore, other potential exposures in the control group were not discussed. These results were consistent with the findings from a small study of American cement mason apprentices, which showed no difference between FeNO and spirometry in 11 masons when compared to 21 electricians acting as controls [16]. In 95 Norwegian cement production workers, FeNO levels did not rise significantly at 8 h post-shift, but a small decrease at 32 h post-shift was observed, along with statistically significant cross-shift reductions in FEV1 and $FEF_{25\%-75\%}$ [17]. However, this study did not indicate the time at which the FeNO measurements were taken in relation to the exposure itself. Furthermore, there was high variability of exposure, making interpretation more challenging.

These results contrast with the findings from earlier cement and underground construction worker studies in whom exhaled NO was found to be prominent among exposed workers when compared with controls [18, 19]. In Ulvestad's cohort of construction workers, underground workers had higher FeNO levels as well as an increase in lower airway symptoms when compared to outdoor workers, possibly suggestive of early signs of inflammation [19].

In a cohort of 186 aluminum potroom workers, although FeNO levels were 63% higher in non-smoking workers when compared to non-smoking controls, they remained within normal values in all groups [20]. Potroom workers also reported more asthma symptoms than controls (17 vs 12%), but the diagnosis of OA was not confirmed in this study.

These findings highlight the need for larger scale screening studies to improve our understanding of FeNO in the pathophysiology of OA.

FeNO in the diagnosis of OA

Changed in FeNO induced by exposure to occupational agents during specific inhalation challenges

There is conflicting evidence regarding the changes in FeNO after exposure to occupational agents in subjects with OA.

Changes in FeNO during specific inhalation challenges to high-molecular-weight agents

Significant changes in FeNO during SIC have mostly been observed in studies with agents inducing an IgE-mediated sensitization. A 12% or greater increase in FeNO levels showed a high positive predictive value for predicting a positive SIC in a small group of subjects challenged with common allergens or HMW agents [21]. This cutoff of a 12% increase in FeNO achieved a sensitivity of 81% and a specificity of 92% for predicting a positive SIC. Another study in 42 workers exposed to HMW agents—farmers, bakers, health care workers—reported significant elevations in FeNO 24 h after exposure in 19 subjects with a positive SIC [22, 23].

Other studies investigating latex-induced OA during SIC have demonstrated a positive correlation between asthmatic responses induced by latex and increases in FeNO values [24–26]. In 45 health care workers—31 sensitized and 14 not sensitized to latex—who had undergone a SIC to latex gloves, only sensitized subjects presented an increase in FeNO levels greater than 50%, 22 h post-SIC, as compared to none of the 12 non-responders. Overall, the specificity and sensitivity of an increase in FeNO greater than 50% of the baseline values in asthmatics were respectively 70 and 79% [26].

Among 18 subjects who underwent a natural rubber latex challenge, eight subjects presented increases in FeNO levels of 10 ppb or greater 22 h after the challenge. All had a positive skin prick test to latex and seven had specific IgE antibodies to latex [24]. However, only five of the subjects with significant FeNO changes had a positive SIC. Among the 12 subjects with a positive SIC, only five had significant changes in FeNO levels. Although there was a trend toward an association between IgE-positive subjects showing positive SIC and increase in FeNO levels, no clear relationship was found between responses to SIC and FeNO levels. Another study did not identify a clear relationship between the results of specific inhalation challenges, high specific IgE antibodies and increases in FeNO levels in 16 subjects exposed to latex. However, this was a small study that was composed of only eight subjects with self-reported symptoms to latex undergoing an SIC and eight controls, which did not show any significant changes in FeNO after a positive challenge in sensitized subjects [25].

Pedrosa's [21] and Lemière's [27••] studies reported significant FeNO elevations in subgroups of subjects with positive SIC to HMW agents. In a cluster analysis of workers with OA to LMW and HMW agents documented by SIC, only the clusters of patients with OA to HMW agents had an increase in FeNO [27••]. The cluster of patients with OA to LMW agents did not present increases in FeNO. The increase in FeNO with a positive SIC was only associated to the molecular weight of the agents, which may in fact be correlated to the underlying IgE-dependent mechanism. Accordingly, subjects with IgE to isocyanates showed significant increases in FeNO after positive challenge in Barbinova's study [28].

Changes in FeNO during SIC to mixed or low-molecular-weight agents

FeNO was measured in 40 workers with suspected OA to HMW (n = 20) and LMW agents (n = 20) [29]. A significant increase in FeNO levels was only noted in workers with a late response to SIC and low baseline FeNO levels (cutoff <12 ppb). Subjects who had a baseline level of FeNO greater than 14.5 ppb did not have a significant increase in FeNO during SIC despite significant bronchoconstriction. Patients with a negative response to SIC did not show a significant increase in FeNO values. Ten patients had a positive specific IgE, but the analysis of their response to SIC as a group was lacking as well as data on corticosteroid use. Thirty-three subjects exposed to HMW and 44 exposed to LMW agents also showed an increase in FeNO levels 24 h after SIC in subjects with positive SIC, but not in those with a negative SIC [30]. FeNO levels did neither achieve a discriminative sensitivity nor specificity for predicting a positive SIC.

Sastre et al. investigated the use of FeNO to monitor the airway response to SIC with occupational agents [31]. Two-thirds of the 68 subjects had a positive SIC, and 72% of positive challenges were with LMW agents. After adjusting for variables such as atopy, smoking, and type of agents, only LMW agents were found to induce a significant increase in FeNO after SIC, irrespective of the type of response (immediate, dual, or delayed). A FeNO of 25 ppb had a sensitivity of 60% and specificity of 80% for a positive SIC. This was consistent with Ferrazzoni's study on isocyanate-induced asthma, which also reported significant increases in FeNO 24–48 h after positive SIC [32]. However, contrary to Sastre et al., this study reported that a greater increase in FeNO values was observed in subjects with an immediate response to SIC compared to subjects with a dual or late reaction. Interestingly, this study also showed an early decrease in FeNO that was correlated to the degree of FEV_1 reduction.

Several other studies have shown an increase in FeNO levels after exposure to isocyanates [28, 32, 33]. Barbinova and Baur investigated 55 subjects suspected of having OA due to isocyanates [28]. They measured FeNO levels before, during, and after SIC to isocyanates. Twelve subjects had a positive SIC and a greater increase in FeNO than subjects with a negative challenge. Sixty-seven percent of the subjects with a positive inhalation challenge had an increase of FeNO greater than 50%, whereas only 28% of the non-responders had the same increase. Two-thirds of the subjects had a FeNO rise of more than 50% when measured 22 h after an isocyanate challenge. Interestingly, a few non-responders with BHR and a significant FeNO increase had specific resistance changes marginally below the threshold for asthma (range 65–100%).

Although Allmers et al. did not find a clear relationship between specific inhalation challenges, elevated specific IgE antibodies and increases in FeNO levels in subjects exposed to isocyanates or latex, their study only included nine patients assessed for OA to 4,4-diphenylmethane diisocyanates (MDI). Two of the three patients with a positive challenge test for isocyanates had MDI-specific IgE antibodies [24].

Walters et al. also studied FeNO during SIC to a variety of LMW agents in 16 subjects to determine whether or not changes in FeNO could predict a positive SIC [34]. The increase in FeNO values did not differ between subjects with a positive SIC (7% change in FeNO) and those with a negative SIC (9% change in FeNO). Only two of the 16 subjects with positive SIC to LMW agents had a significant FeNO increase after SIC.

Older studies on suspected OA to western red cedar have also reported increases in FeNO after SIC. Obata et al. challenged 17 subjects with plicatic acid [35]. The mean pre-test FeNO was 40.9 ppb in responders and 34.1 ppb in non-responders. However, FeNO significantly increased only in non-responders 24 h after the challenge.

Changes in FeNO observed in cohort studies of workers exposed to occupational agents

Changes in FeNO in cohort studies of workers exposed to HMW agents

Early studies suggested an increase of FeNO levels in subjects with symptoms of asthma when exposed to laboratory animals, whereas normal levels were found in asymptomatic subjects exposed to the same animals [6]. In a recent study including 77 health care workers exposed to latex, 38% of subjects reported asthma symptoms. However, no difference in FeNO values was noted between the symptomatic and asymptomatic subjects [36].

Van der Walt et al. studied 150 spice mill workers exposed to garlic and chili pepper dust. In a first study, they categorized 4 to 8% of subjects as having probable asthma based on airflow reversibility and FeNO levels greater than 50 ppb. In their study, atopy was strongly correlated to sensitization and FeNO levels greater than 50 ppb [37]. In a later study, FeNO levels were measured before the work shift (after 48 h without exposure), as well as after the work shift and across a 24-h period [38••]. An increase in FeNO greater than 12% was noted in 23% of subjects, but the sensitization to spice dust allergens was correlated to baseline FeNO levels greater than 50 ppb alone, not to FeNO increases.

Baatjies et al. studied FeNO in bakers and found a strong relationship between FeNO levels, diagnosed or probable baker's asthma, and sensitization to wheat or rye. The association between high FeNO and sensitization to wheat was stronger in non-atopic than atopic subjects [39]. The presence of serum specific IgE to wheat was the most important determinant of FeNO variability. Smoking and atopy were also important determinants of FeNO levels. In this population of bakers sensitized to flour allergens, the effect of smoking on FeNO counterbalanced the effect of atopy in a dose-dependent manner.

Changes in FeNO in cohorts of workers exposed to LMW agents

Jonaid et al. described a small group of seven subjects with specific IgE antibodies to hexamethylenediisocyanate (HDI) within a cohort of 201 workers

Table 1. Selected studies on FeNO in the screening and investigation of OA

Agents	*n*	Results
SCREENING		
HMW agents		
Laboratory animals [6]	50 exposed workers	No significant changes in FeNO in seronegative subjects.
Laboratory animals [7]	70 exposed workers	Higher FeNO in sensitized group at 6 and 12 months but not at 24 months after employment. Sensitization rates of 19% at two years were predicted by atopy and increased IgE levels.
LMW agents		
Lifeguards [13]	39; 15 with positive methacholine test	FENO values ≥60% of predicted values were 80% sensitive and 42% specific to identify subjects with positive methacholine challenge.
Cement dust [16]	11 exposed masons	No differences in FeNO or spirometry when compared to controls (24 electricians).
Cement dust [18]	95 exposed workers	Small cross-shift reduction in FeNO (2 ppb) associated with decrease of FEV1 (37 mL), no control group.
Cement dust [18]	121 (90 exposed)	Increase in FeNO in exposed workers, with significant correlation with duration of exposure.
Underground dust, gas [19]	55 (29 exposed)	Increase in FeNO in underground workers exposed to dust, quartz and NO2, independent of symptoms.
Aluminum [20]	186 exposed	Non-smoker symptomatic subjects had higher FeNO despite comparable spirometry to controls ($n = 40$).
Mixed agents		
LMW (hairdressers) and HMW (bakers, pastry-makers) agents [8]	178 (74 hairdressers 104 bakers/pastry-makers), incl. 19 OA	Using FeNO threshold > 8.5 ppb has a sensitivity around 80% and cannot be used alone for screening of OA. This study used a telephone screening questionnaire to assess symptoms.
LMW (hairdressers) and HMW (bakers, pastry-makers) agents [9, 11]	441 (122 bakers, 87 pastry makers, 132 hairdressers)	Higher FeNO was associated to atopy but not the BHR at the start of the apprenticeship. During follow up, FeNO increased in subject who had occurrence or aggravation of BHR, regardless of the molecular weight.
INVESTIGATION		
HMW agents		
Latex [47]	22 (11 hospital workers, 11 controls)	No increase in FeNO in group with self-reported latex sensitivity ($n = 11$) compared with controls ($n = 11$).
Latex [36]	77 exposed workers (29 symptomatic)	The difference in FeNO values between symptomatic and asymptomatic workers with suspected latex allergy was related to atopy (prick test or IgE confirmed). Once adjusted for atopy, there was no difference in FeNO.
Latex [26]	45 workers (31 latex-sensitized)	Increase in FeNO 1 h after challenge in sensitized and non-sensitized subjects. Increase in FeNO 22 h after challenge in sensitized subjects only.

Table 1. (Continued)

Agents	*n*	Results
Fish processing [48]	139 salmon workers, 127 trawler workers	Higher FeNO levels in trawler workers ($n = 139$) than salmon workers ($n = 127$). Higher FeNO in asthmatic trawler workers when compared to non-asthmatics, but no differences within the salmon workers group.
Swine dust [49]	33 healthy exposed	Increase in FeNO only in workers not wearing a respiratory mask.
Swine dust [50]	15 exposed workers	No increase in FeNO in swine confinement workers after 1 week, compared with controls ($n = 9$).
Dairy or swine farming [51]	81 farmers with OA	Decrease of FeNO in farmers with OA after educational program on OA.
Dairy or swine farming [52]	43 farmers with OA	Persistent decrease of FeNO in farmers 1 year after intervention with educational program on OA.
Cereal flour [39]	424 supermarket bakery workers	Increased FeNO is strongly associated with wheat IgE, independently of atopy. The effect of smoking on FeNO overrides that of atopy, in a dose-dependent manner.
Flour dust [53]	167 (59 with OAR)	FeNO was associated with OA/rhinitis more than with atopy, and correlated with IL-8 levels, responsible for neutrophilic recruitment in the lung.
Spice dusts (garlic and chili) [37]	150 exposed workers	Subjects sensitized to both garlic and chili pepper showed a stronger association with elevated FeNO > 50 ppb than those with monosensitization.
Spice dusts (garlic and chili) [38]	150 exposed workers	Sensitization to chili pepper showed a stronger association with elevated FeNO > 50 ppb than garlic. Atopy was also associated with high FeNO.
Coffee dust, endotoxins [54]	117 exposed workers	Positive association between elevated FeNO levels and numbers of years of experience in coffee handpicking.
Coffee dust, endotoxins [55]	138 workers and 120 controls	No association found between FeNO levels and coffee workers' exposure ($n = 138$) when compared to controls ($n = 120$). No association of FeNO levels with cumulative exposure to coffee dust and endotoxins.
Various HMW agents [22]	42 (17 farmers, 15 bakers, 10 healthcare workers)	Patients with positive SIC had increased FeNO levels 24 h post-SIC, compared to none of the SIC-negative patients.
Various HMW agents [21]	34 subjects with asthma, sensitized to inhalant allergens	Increase in FeNO levels 24 h after positive SIC in OA; not in those with negative SIC. A 12% change in FeNO had maximal sensitivity and specificity for predicting positive SIC, 81% and 92% respectively. Stopping ICS 72 h prior to challenge was sufficient to avoid FeNO levels suppression.
Various HMW agents [29]	40 (suspected OA)	Increase in FeNO after SIC in workers with low baseline level of FeNO, with a late component.

Table 1. (Continued)

Agents	*n*	Results
LMW agents		
Aluminum [46]	45 (35 exposed)	Non-smoker asthmatic workers had higher FeNO, correlated inflammatory changes of lamina propia.
Red cedar/plicatic acid [35]	17 (suspected OA)	Increase in FeNO 24 h after SIC in non-responders.
Red cedar/ plicatic acid [40]	71 (OA to red cedar)	No change in FeNO, no relationship between FeNO and degree of respiratory impairment between the 21 patients with OA to red cedar with ongoing exposition compared to the 50 patients no longer exposed.
Isocyanates [32]	42 (suspected OA)	In the SIC-positive group ($n = 15$), initial decrease of FeNO followed by peak at 48 h. The initial decrease in FeNO correlated with early FEV1 reduction. The magnitude of FeNO increase was greater in late or dual reactions to SIC. No change in SIC-negative group ($n = 24$).
Isocyanates [28]	55(suspected OA)	Increase of FeNO in subjects with positive challenge and even higher with IgE-mediated sensitization.
Isocyanates [33]	80 spray painters, 121 controls	Increase of FeNO related to exposure in small group of non-atopic non-smokers subjects with IgG for HDI.
Isocyanate [56]	1 case report	Case report of OA with specific immediate asthmatic reaction to triglycidylisocyanurate. A 20% fall in FEV1 was elicited by SIC, without any change in FeNO at 24 h, in an IgE-negative subject.
Organic solvents [57]	17 shoe and leather workers	Increase in FeNO in workers at the end of the shift, in smokers and non-smokers.
Ammonium Persulfate [58]	1 case report	Case report of OA with isolated late response to SIC and positive patch test but negative prick test. Elevated FeNO and eosinophilic sputum associated with isolated late response to SIC.
Various LMW agents [34]	16 subjects with positive SIC. 10 on ICS	No significant increase of post-SIC FeNO in subjects when compared to controls.
Mixed agents		
Isocyanates and latex [24]	27 (9 MDI, 18 latex)	Subjects with specific IgE and positive bronchial challenge showed a tendency of increase in FeNO.
Various LMW and HMW agents [30]	68 (26 positive SIC; 12 HMW, 14 LMW)	Significant increase in FeNO only at 24 h, sputum eosinophils more sensitive to detect positive SIC than 10 ppb change in FeNO.
Various LMW and HMW agents [31]	68 (45 with positive SIC)	Increase in FeNO in SIC positive subjects, not correlated to the type of SIC response. A FeNO of 25 ppb had a sensitivity of 60% and specificity of 80% for positive SIC.

Table 1. (Continued)

Agents	*n*	Results
Various LMW and HMW agents [27]	178 (98 positive SIC)	Increases in FeNO is subjects mainly exposed to HMW; no changes in FeNO in subjects exclusively exposed to LMW.
Various LMW and HMW agents [59]	60 (8 HMW, 52 LMW)	Increase in FeNO in subgroup of OA subjects, unrelated to atopy or agent.

exposed to isocyanates [33]. Their FeNO levels were significantly increased after the exposure to isocyanates. FeNO levels were also correlated with IgG antibodies to HDI, but contrary to workers with positive IgE to isocyanates, no significant association was found between FeNO and exposure to HDI in subjects with positive specific IgG to HDI.

In a large follow-up study involving 71 patients with documented OA to western red cedar, no correlation was found between FeNO and FEV_1, PC_{20}, and respiratory impairment irrespective of the corticosteroid treatment [40]. In fact, no difference was noted in FeNO levels in subjects who had ceased exposure to red cedar when compared to subjects with continued exposure. More than half of the subjects in each group were still treated with corticosteroids many years after removal from exposure. Specific IgE antibodies were found in less than half (25–44%) of the subjects with proven OA to western red cedar and were not reported in neither of these two studies [41, 42].

In cleaning workers, variable results have been reported. A cohort study of 95 cleaning workers used a questionnaire to distinguish two groups of patients: 42 cleaning workers with asthma-like symptoms and/or a history of asthma (cases) and 53 cleaning workers without any symptoms or any history of asthma (controls) [43]. There was no difference in FeNO levels between the two groups of workers. High FeNO values were associated to the use of multiuse products, glass cleaners and polishes, but only in the control group [44, 45]. In this study, the use of multiuse products was associated with increased levels of FeNO and elevated total serum IgE.

Sjaheim et al. studied 20 potroom workers with confirmed OA as well as 15 healthy potroom workers and 10 controls [46]. Non-smoking workers with OA had significantly higher FeNO levels than healthy non-smoking exposed workers and non-exposed controls. A bronchial biopsy sampling was performed in all participants. Interestingly, both asthmatics and healthy asymptomatic exposed workers showed significant subclinical inflammation compared to controls, as revealed by elevated FeNO as well as significantly increased reticular basement membrane thickening (RBM) and by the increased number of eosinophils in the lamina propia under the RBM.

In Table 1, a short description of relevant studies of FeNO levels in the screening and investigation of OA is provided.

Factors influencing the measured levels of FeNO

The time of collection of FeNO certainly plays an important role when interpreting data regarding FeNO. In studies with dual asthmatic

Table 2. Influence of confounding factors on FeNO levels

Confounding factors	Effect on FeNO levels	References
Time of collection	Increase in FeNO peaks between 24 to 48 h after exposure	[21, 29–32, 60, 61]
Use of corticosteroids	Decrease in FeNO	[67, 71–74]
Exposure to HMW agent/agent-specific IgE	Increase in FeNO	[21, 27, 37, 39]
Smoking	Decrease in FeNO	[4, 33, 69, 71, 75, 76]
Atopy	Increase in FeNO	[33, 69, 77, 78]
Current upper respiratory infection	Increase in FeNO	[32, 69, 72]
Nitrate-rich diet	Increase in FeNO	[79, 80]

responses, a maximal FeNO increase has been reported to be 10 h after exposure [23]. However, in most studies, the peak of increase in FeNO levels generally occurs between 24 [21, 30, 60] and 48 h [29, 31, 32, 61] after exposure to the offending agent. As opposed to sputum eosinophil counts, the rise in FeNO levels 7 h after exposure to the offending agent is limited. This may explain in part why some earlier studies could not detect changes in FeNO shortly after the end of exposure [25]. A prolonged increase in FeNO after exposure or challenge in sensitized patients reaffirms the importance of extended FeNO levels monitoring after SIC. Sufficient time should be allowed between recent exposure at work and performance of the SIC, to avoid high basal FeNO values that could potentially mask significant increases in FeNO values after SIC [29].

The airway caliber may have a significant impact on the levels of measured FeNO [62, 63]. During bronchoconstriction, the reduction of volume in conduction airways leads to an increase in airflow, resulting in a reduced time for inflammatory cells to release NO into the airways expirate [64]. However, a recent study of NO dynamics in subjects sensitized to isocyanates failed to find differences in alveolar FeNO between positive and SIC negative groups, suggesting that changes in FeNO are independent of the pattern and magnitude of the bronchoconstriction [60]. Others have suggested that inhomogeneous ventilations during bronchoconstriction leads to focal NO trapping in the airways or that the reduction of NO diffusion is caused by airway wall thickening [65, 66].

The use of corticosteroids which inhibits induction of NO synthase is certainly an important confounding factor. Falls in FeNO are expected after treatment with inhaled or systemic corticosteroids in patients with asthma [67]. The lack of increase in FeNO after exposure to the suspected agent in patients with suspected OA has been reported many times. The use of corticosteroids was not always considered in older studies. The increase in FeNO after exposure to occupational exposures may be blunted in patients undergoing active treatment.

FeNO elevations were significant in subgroups of subjects with a positive SIC to HMW agents in Pedrosa's and Lemière's studies [21, 27••]. In a

large recent study investigating 150 workers exposed to spice dusts, atopy was strongly associated with sensitization to occupational allergens as well as high NO levels [37]. In another study on 424 supermarket bakery workers, elevated FeNO values were strongly correlated to IgE to wheat, independently of atopy and smoking status [39]. A cluster analysis performed in workers with OA to LMW and HMW agents [27••] identified an increase in FeNO after SIC only in the clusters of workers with OA to HMW agents. The only factor associated with an increase in FeNO after positive SIC was the molecular weight of the agent. The association may be due to the underlying IgE-dependent mechanism rather than to the type of agent itself.

Smoking decreases FeNO levels in healthy adults as well as in asthmatics. The effect is reported in short-term smoking (conventional and electronic cigarettes) as well as chronic smoking, and a dose-effect relationship has been observed [68–70]. These effects have a confounding influence on the interpretation of FeNO among smoking workers. Significantly lower FeNO values have repeatedly been reported in smokers when compared to non-smokers in the occupational context [33, 38••, 39]. There are few studies reporting FeNO values in response to SIC in smokers. Most of these studies have not observed a blunted response of FeNO after SIC compared to non-smokers [26–28, 30]. Sastre et al. evaluated changes in FeNO after SIC in 68 subjects. In subjects with a positive challenge (n = 45), smokers did not present a significant increase in FeNO compared to their baseline, whereas non-smokers did. A summary of the factors influencing FeNO levels can be found in Table 2.

Conclusion

The use of non-invasive markers of airway inflammation such as FeNO is increasingly studied in OA. Although FeNO is easy to perform and has many potential applications in occupational settings, its interpretation must consider the influence of many confounders such as atopy, corticosteroid treatment, timing of measurement, and tobacco use. The use of FeNO has been shown to be useful, particularly in subjects exposed to HMW agents. Further studies are needed, especially regarding the screening and follow-up of patients with suspected or confirmed OA.

Compliance with Ethical Standards

Conflict of Interest

Dr. Isabel Coman declares that she has no conflicts of interest. Dr. Catherine Lemière reports grants and personal fees from AstraZeneca and TEVA and personal fees from GlaxoSmithKline, outside the submitted work.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

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