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Exhaled nitric oxide is of limited value in the diagnosis of occupational asthma

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Exhaled nitric oxide is of limited value in the diagnosis of occupational asthma

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Authorship

All authors participated in the conception and design of the study, analysis and interpretation of data, and drafting of the article. All approved the final version.

Key words: Exhaled nitric oxide, occupational asthma, specific inhalation challenge

Clinical Implications: exhaled nitric oxide has a limited value to interpret specific inhalation challenges in occupational asthma.

Running title: exhaled nitric oxide in specific inhalation challenge in occupational asthma.

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Conflict of interest

JS reports having served as a consultant to Thermofisher, MEDA, Novartis, Sanofi, Leti, Faes Farma, Mundipharma, and GSK; having been paid lecture fees by Novartis, GSK, Stallergenes, Leti, and Faes Farma; as well as having received grant support for research from Thermofisher, Sanofi, and ALK.

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48 Assessment of changes in exhaled nitric oxide fraction (FeNO) during specific
49 inhalation challenges (SIC) has been proposed as an inflammatory marker of response,
50 particularly in subjects with not enough change in FEV1 to identify an asthmatic
51 reaction and without suitable sputum samples as a means of evaluating a response. [1].
52 However, there is no consensus regarding the predictive capacity of changes in FeNO
53 after SIC with occupational agents or the percent change that predicts a positive or
54 negative response.

55 Aiming to assess the diagnostic accuracy of FeNO measurements before and
56 after SIC in a large population of subjects with suspected occupational asthma (OA), we
57 included consecutive subjects over 18 years of age who had been referred to our unit to
58 determine possible OA between 2005 and 2019. SICs were performed as previously
59 described, depending on the occupational agent tested (1). Briefly, subjects underwent
60 a challenge with a control substance on the first day, including FeNO measurement and
61 methacholine inhalation challenge at the end of the day. FeNO measurements and
62 methacholine challenge were then repeated 24 h after an asthmatic reaction occurred
63 (15% fall in FEV1 or a >2.5-fold decrease in PC20 methacholine) in response to a test
64 that exposed them to the offending agent or on the last day of exposure in case of a
65 negative result (between 1 to 3 days). FeNO levels were always measured before the
66 methacholine challenge. SIC was considered positive if a reproducible fall in FEV1 of
67 15% or more followed exposure to the offending agent or a decrease in PC20
68 methacholine of >2.5-fold. Inhaled corticosteroids and other medications were
69 withdrawn at least 5 days before the challenge [1]. FeNO was measured using the online
70 method (NiOX; AerocrineAB; Solna, Sweden).

Continuous variables were expressed as mean $\pm$ SD and categorical variables as percentages. ROC curve analysis examined FeNO absolute change in ppb and relative in rate. The area under the curve (AUC) for FeNO change as a predictor of OA was calculated for all SICs and stratified by high (HMW) and low (LMW) molecular weight agents. T-tests were used to compare FeNO outcomes. Statistical significance was set at a p-value of <0.05 . The Hospital Research Ethics Board approved the study.

A total of 219 individuals fulfilled the study criteria. Patient demographic and clinical characteristics are summarized in Table E1 of the supplementary material. More subjects were exposed to LMW agents (160 patients, 73.05%) than HMW agents. A total of 141 SICs (63.5%) were positive, of which 120 (85.1%) had an immediate or late decrease of 15% in FEV₁; in 18 (12.8%) patients SIC was considered positive based on a methacholine PC₂₀ decrease greater than 2.5-fold. Subjects with negative or doubtful SIC (n=3) but with an increase of >20 ppb in FeNO were evaluated individually. These three patients had a positive methacholine test, allowing eosinophilic bronchitis to be ruled out.

A statistically significant increase in FeNO level was found in patients with positive SIC ($p<0.0001$) and also when divided into HMW or LMW agents ($p=0.004$ and $p=0.03$ respectively). On the contrary, FeNo change was not statistically significant for negative SIC results ($p=0.11$) or when analyzed by HWM or LMW agents ($p=0.051$ and $p=0.39$, respectively) (Table 1). Patients with positive responses showed no significant difference in baseline FeNO from patients with negative SICs.

However, FeNO change by itself did not show good diagnostic accuracy in predicting a positive SIC, showing an area under the curve (AUC) of 0.59 (95% CI, 0.51-0.66) for absolute FeNO change and 0.58 (95% CI, 0.50-0.65) for relative change.

AUC was similar when divided into HMW or LMW challenge tests. For LMW agents, the AUC was 0.62 (95% CI, 0.53-0.71) for absolute and 0.61 (95% CI, 0.52-0.70) for relative FeNO change. For HMW agents, the AUC was 0.55 (95% CI, 0.40-0.70) for absolute and 0.49 (95% CI, 0.33-0.65) for relative FeNO change (*Figure E1 repository*).

This study shows that FeNO was increased in subjects with a positive SIC after exposure to occupational agents, which was not the case in subjects with negative SICs. This finding supports previous research in patients who underwent challenges with HMW or LMW agents [2-5]. However, the accuracy of FeNO in predicting a positive SIC remains controversial, as is the issue of a cut-off point associated with a positive response. Some authors found a cut-off for change in FeNO showing acceptable sensitivity and specificity associated with positive SIC. Nevertheless, these cut-offs vary greatly: our group described a 25-ppb or 41% increase in 86 patients [6], while Florentin, et al [7] reported that a cut-off of 8.5 ppb provided 80.5% specificity for OA and 79.0% sensitivity in 178 patients, finding only 10% with positive SICs. Pedrosa [8] reported that a 12% FeNO increase was associated with high sensitivity (0.81) and specificity (0.92) in 34 patients challenged only with HMW agents. A 50% increase was determined to be a significant response by Barbinova et al. [4] in patients tested with isocyanates, and 13 ppb [9] showed high specificity (0.9) but low sensitivity (0.5) in 148 workers studied, although only 21% of SICs were positive. On the contrary, other authors were unable to find an acceptable cut-off based only on FeNO changes. In general, changes in FeNO were less discriminant than differences in sputum eosinophils [2],

It should be noted that 62% of patients in the current study had negative methacholine tests at baseline, which may have an influence on their response to the SIC and can be considered as a limitation of the study. However, the study's protocol

included a methacholine test 24 hours after last SIC to exclude false negative SIC. FeNO levels are affected by smoking, but only 14% of our patients were current smokers.

In conclusion, despite significant efforts to identify a relevant, clinically non-invasive inflammatory marker for SIC interpretation, at least in patients with little or no change in FEV1, we believe that FeNO is of limited value. Although it is well established that a positive SIC is associated with increased FeNO, FeNO by itself cannot be used to interpret SICs as positive or negative.

Word count: 1000

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163 Table I. Exhaled Nitric Oxide (FeNO) values on different population studied.

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	Total studied population (n=219)		By molecular weight			
		p value	HMW (n=59)	p value	LMW (n=160)	p value
Baseline FeNO, mean (SD)	31.74 (33.8)	Negative SIC: p = 0.11	41.43 (32.02) (All) Negative SIC: 22.7 (12.6) Positive SIC: 44.9 (30.06)	Negative SIC: p=0.051	29.23 (34.04) (All) Negative SIC: 30.5 (49) Positive SIC: 28.3 (20)	Negative SIC: p =0.39
FeNO after SIC, mean (SD)	39.22 (46.58)	Positive SIC: p <0.001	51.13 (36.9) (All) Negative SIC: 29.9 (21.6) Positive SIC: 61.7 (38.6)	Positive SIC: p=0.0004	35.07 (48.93) (All) Negative SIC: 31.7 (65.12) Positive SIC: 37.05 (36.22)	Positive SIC p =0.03

FeNO change in ppb, mean (SD)	6.33 (23.6)		9.7 (30.9) (All) Negative SIC: 7.2 (14.6) Positive SIC: 14.45 (25.21)		6.19 (24.24) (All) Negative SIC: 2.1 (18.8) Positive SIC: 8.6 (26.7)	
Percent FeNO change, mean (SD)	22.5 (0.89)		11.99 (47.04) Negative SIC: 32.5 (0.6) Positive SIC: 32.1 (0.8)		18.92 (0.65) Negative SIC: 3.7 (0.52) Positive SIC: 28.36 (0.7)	

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Table E1 (repository). Demographic and clinical characteristics of patients (N=219)

<i>Epidemiologic data</i>	
Age, mean (SD)	41.32 (10.1)
Sex, male, No. (%)	120 (54.79%)
Smoking habit, active smoking No. (%)	33 (14.85%)
Smoking habit, never smokers No. (%)	147 (67.12%)
Atopy ¹ , No. (%)	93 (41.89%)
Rhinitis and/or conjunctivitis, No. (%)	114 (52,05%)
<i>General characteristics of SIC</i>	
Not currently working, No. (%)	161 (72.52%)
LMW of the suspected agent, No. (%)	160 (73,05%)
HMW of the suspected agent, No. (%)	59 (26,9%)
Positive SIC, No. (%)	141 (63.5%)
Positive baseline methacholine challenge ² , No. (%)	137 (61.7%)
<i>Characteristics depending on MW</i>	
- HMW agents (n=59)	
Atopy ¹ , No. (%)	29 (48.33%)
Rhinitis and/or conjunctivitis, No. (%)	38 (63.5%)
Positive SIC, No. (%)	42 (70%)
Positive baseline methacholine challenge ² , No. (%)	40 (66.67%)
Immediate FEV1 fall in SIC, No. (%)	29 (48.33%)
Late FEV1 fall in SIC, No. (%)	26 (43.33%)
Positive post-SIC methacholine challenge ² , No. (%)	30 (50%)
>2.5 increase PC20 after challenge	14 (23,3%)
- LMW agents (n=160)	
Atopy ¹ , No. (%)	
Rhinitis and/or conjunctivitis, No. (%)	64 (39.5%)
Positive SIC, No. (%)	76 (47.5%)
Positive baseline methacholine challenge ² , No. (%)	99 (61.87%)
Immediate FEV1 fall in SIC, No. (%)	86 (53.08%)
Late FEV1 fall in SIC, No. (%)	41 (25.30)
Positive post-SIC methacholine challenge ² , No. (%)	60 (37.03%)
>2.5 increase PC20 after challenge	92 (56.7%)
	30 (18,51%)

¹ Positive skin prick test to 1 or >1 to usual pneumoallergens. 2 Positive methacholine challenge is considered when PC20 <8 mg/ml.

Figure E1

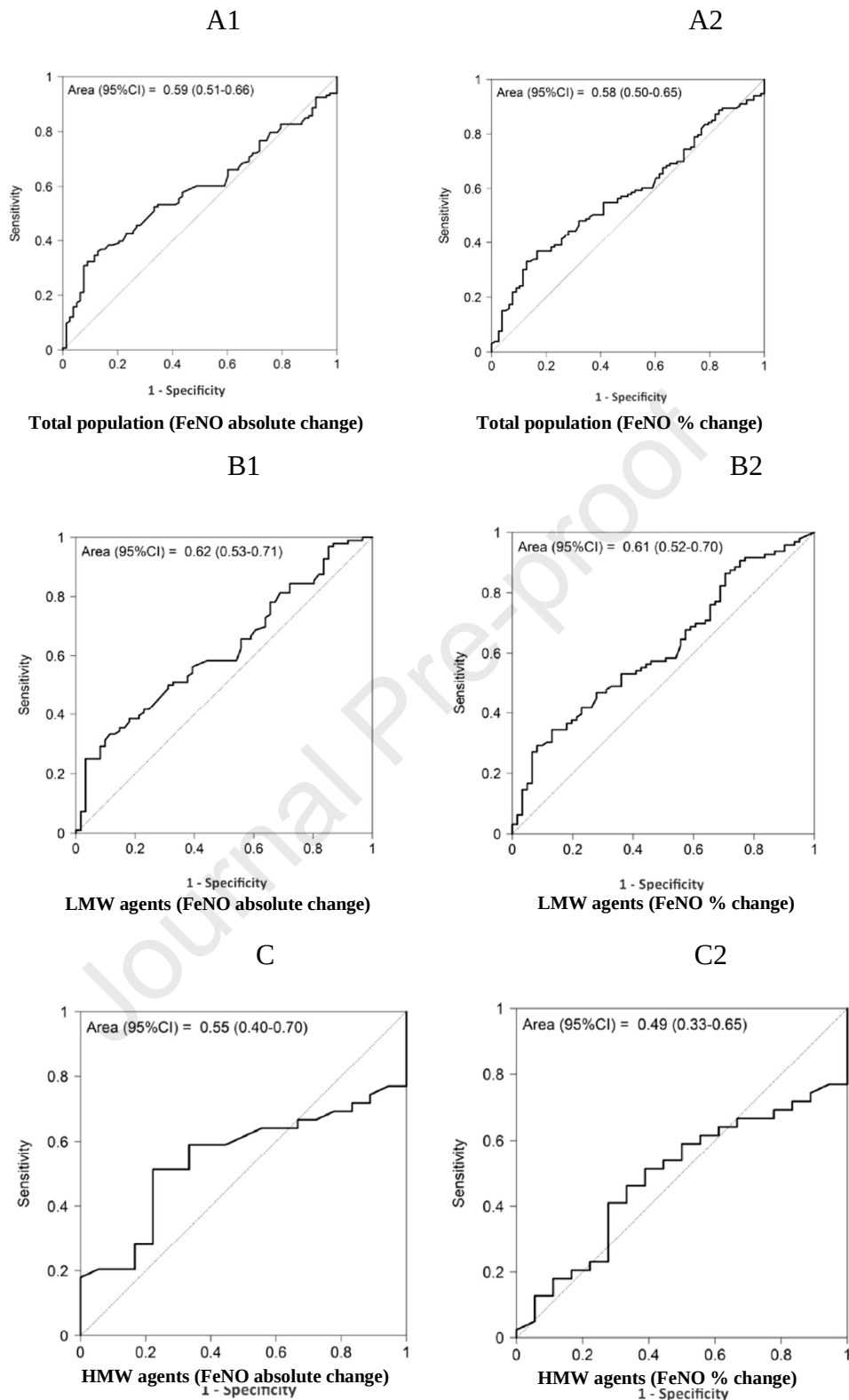


Figure E1: ROC curve analysis for FeNO changes after specific inhalation challenges

A= total studied population (n=219), B= LMW agents SIC (n=160), C= HMW agents SIC (n=59) 1 = absolute eNO change (measured in ppb), 2= relative eNO change (measured in %).