

Changes in Exhaled Nitric Oxide After Inhalation Challenge With Occupational Agents

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■ Abstract

Background and objective: The use of fractional exhaled nitric oxide (FeNO) concentration has been proposed as a surrogate marker for monitoring airway response to specific inhalation challenge (SIC). We investigated the usefulness of FeNO measurements for monitoring airway response to SIC with occupational agents.

Material and methods: Workers with suspected occupational asthma were recruited to undergo SIC with occupational agents and subsequently FeNO testing at baseline and 24 hours.

Results: Sixty-eight patients were evaluated, 45 of whom had a positive SIC. SIC-positive patients showed a significant increase in FeNO 24 hours postchallenge, with an increase ratio of 1.25 (95% CI, 1.05-1.48; $P=.01$); no increase was seen in patients with a negative SIC ($P=.08$). The predictive capacity of variations in FeNO showed that for each unit increase in FeNO, the probability of a positive SIC rose by 4%. A baseline FeNO value of 25 ppb predicted a positive SIC with 60% sensitivity and 80% specificity. The increase in %FeNO cutoff point providing maximal sensitivity and specificity for predicting a positive SIC was 41% (sensitivity 50%, specificity 95%).

Conclusions: We demonstrated that asthmatic reactions induced by occupational agents during SICs are associated with a consistent increase in FeNO. However, the predictive diagnostic capacity of FeNO measurements is low. While FeNO may aid in the interpretation of SIC in some cases, it cannot be used as a general surrogate marker to predict or to assess SICs with occupational agents.

Key words: Occupational asthma. Fractional exhaled nitric oxide. Specific inhalation challenge. Bronchial inflammatory markers.

■ Resumen

Antecedentes y objetivo: Se ha sugerido la medición del óxido nítrico exhalado (FeNO) como un marcador en la monitorización de la respuesta de las vías respiratorias a provocaciones específicas bronquiales (SIC). Hemos investigado la utilidad de la medición del FeNO en la monitorización de la respuesta de la vía respiratoria a SIC con agentes ocupacionales.

Materiales y métodos: Se han reclutado trabajadores con sospecha de asma ocupacional sometidos a SIC y a los que se les determinó FeNO antes y a las 24 horas del SIC.

Resultados: Un total de 68 fueron evaluados, 45 de ellos tuvieron un SIC positivo. En los pacientes SIC-positivos el FeNO aumentó de forma significativa 24 horas tras el SIC con un incremento en el cociente de 1.25 (IC 1.05-1.48, $p=0.01$), pero no en el grupo de SIC-negativo ($p=0.08$). La capacidad predictiva de la variación del FeNO mostró que por cada unidad de incremento en FeNO, el riesgo de tener un SIC positivo se incrementó un 4%. Un valor de FeNO basal de 25 ppb predijo un SIC positivo con una sensibilidad del 60% y una especificidad del 80%. El punto de corte que dio la máxima sensibilidad y especificidad de incremento del %FeNO para predecir un SIC positivo fue del 41% (sensibilidad 50%, especificidad 95%).

Conclusiones: Se ha demostrado que las reacciones asmáticas inducidas por agentes ocupacionales durante SICs se asocian a un consistente aumento del FeNO. Sin embargo, su capacidad predictiva es baja. Aunque esta medición puede ayudar a interpretar los SICs, en algunos casos, no se puede generalizar su uso como marcador indirecto para predecir o valorar los SICs con agentes ocupacionales.

Palabras clave: Asma ocupacional. Fracción de óxido nítrico exhalado. Provocación bronquial específica. Marcadores de inflamación bronquial.

Introduction

The most widely used noninvasive methods for assessment of bronchial inflammation are induced sputum and fractional exhaled nitric oxide (FeNO) concentration. Compared with induced sputum, assessment of FeNO is totally noninvasive, quick, and relatively simple to perform. However, elevated FeNO is not specific for asthma or eosinophilic inflammation because it has been found in other diseases and several conditions may influence exhaled NO [1].

Recommendations for standardized FeNO measurement procedures have been published by the European Respiratory Society and the American Thoracic Society [2].

Some studies have examined the usefulness of FeNO in the investigation of occupational asthma, but with inconsistent results [3-12]. A recent study showed that isocyanate-induced asthmatic reactions were associated with a consistent increase in FeNO, which was maximal 24 to 48 hours following exposure; FeNO, by contrast, did not vary with isocyanate exposure in occupational rhinitis or in nonsensitized individuals [12]. Other studies have suggested that an increase in FeNO is found not only after exposure to sensitizers, but also after exposure to irritant agents such as solvents [13] or organic dusts in a swine confinement building [14].

Several issues should be considered in the interpretation of the conflicting results obtained by studies that have analyzed FeNO after specific inhalation challenge (SIC) with occupational agents. One is the insufficient duration of patient monitoring and another is the fact that corticosteroids inhibit the induction of NO synthase and FeNO falls after treatment with oral or inhaled corticosteroids in individuals with asthma [1]. In studies including patients undergoing steroid treatment at the time of testing, FeNO response might have been blunted. Finally, an increase in NO production in the presence of bronchoconstriction might have been underestimated [1].

We investigated the potential usefulness of FeNO testing for the purpose of monitoring airway response after SIC with occupational agents in a large sample of patients with positive and negative SIC results to these agents.

Materials and Methods

Participants

Consecutive workers with suspected occupational asthma referred to the Occupational Health Unit of Fundación Jiménez Díaz in Madrid, Spain between 2005 and 2011 and who had undergone exhaled nitric oxide measurement (FeNO) and SIC with occupational agents were recruited for the study. A full medical and occupational history was obtained.

Characteristics of the workers, such as smoking history, atopy (defined as at least 1 positive skin prick test [>3 mm wheal] to a common environmental allergen using saline and histamine as negative and positive controls), and treatment, were recorded.

Study Protocol

For patients who were receiving inhaled corticosteroids (n=32), the drugs were withdrawn for at least 1 week prior to

SIC. None of the patients were receiving leukotriene modifiers or nonsteroidal anti-inflammatory drugs. Methacholine challenges were performed, as described elsewhere [15], the day before the challenge and in the case of doubtful challenge results, 24 hours after the challenge. Airway responsiveness to methacholine was expressed as the cumulative provocative dose (in milligrams of methacholine) causing a 20% fall in forced expiratory volume in the first second (FEV₁) (PC₂₀).

FeNO measurements (NIOX MINO, Aerocrine) were carried out at baseline (day before SIC) and 24 hours after SIC with occupational agents.

The study was approved by our institution's ethics committee, and all participants provided written informed consent.

Specific Inhalation Challenges

SICs with occupational agents were performed as previously described [15-19]. Forced vital capacity (FVC) and FEV₁ were measured every 10 minutes for the first hour and from that moment, peak expiratory flow (PEF) and FEV₁ were measured hourly with a computerized flowmeter (Amos, Jaeger, Germany) for 24 hours after the challenge, not counting sleep time. An asthmatic reaction was considered to occur when FEV₁ decreased by at least 20% from baseline within 1 hour (immediate) or more than 2 hours (late) after exposure.

Data Analysis

Individuals' characteristics were described using mean and standard deviation (SD) or median and CI for quantitative variables and frequency tables for qualitative variables.

Data were analyzed using FeNO as a continuous variable. This variable was not normally distributed (Kolmogorov-Smirnov test) and was therefore log transformed.

The differences in FeNO levels before and after SIC were evaluated by the relative change and 95% CI, and values before and after SIC were compared using the *t* test for paired samples.

Differences in physiological parameters between groups were assessed using the χ^2 test for qualitative variables and the Mann-Whitney U test, independent *t* test, or one-way analysis of variance for quantitative variables (age, FEV₁ percent predicted, and log-transformed FeNO).

The association between changes in FeNO levels pre- and post-SIC and the outcome of the challenge was assessed using receiver operating characteristic (ROC) curves.

A difference was considered to be significant when the *P* value was less than .05.

Multiple linear regression was used to control for variables potentially confounding the relation between FeNO and bronchial hyperresponsiveness. The Pearson correlation was used to compare reactivity to methacholine and NO levels with both used as continuous data.

Results

A total of 68 patients were evaluated. Their clinical characteristics are shown in Table 1. Forty-five had positive SIC results. The implicated agents were isocyanates (n=13),

Table 1. Clinical Characteristics of Patients^a

Variable	All Patients	Patients With Positive SIC (n=45)	Patients With Negative SIC (n=23)
Sex			
Male	38 (55.9)	26 (57.8)	12 (52.2)
Female	30 (44.2)	19 (42.2)	11 (47.8)
Atopy			
No	23 (33.8)	13 (28.8)	10 (43.4)
Yes	45 (66.2)	32 (71.2)	13 (56.6)
Tobacco			
No	46 (68)	26 (57.7)	20 (82.6)
Yes	15 (21)	12 (26.7)	3 (17.4)
Ex-smoker	7 (11)	7 (15.6)	0 (0.0)
Molecular weight			
HMW agents	16 (27.9)	13 (35.6)	3 (13.0)
LMW agents	52 (72.1)	32 (64.4)	20 (87.0)
Type of asthmatic reactions during SIC			
Immediate		19 (44)	
Dual		8 (18)	
Late		18 (40)	
Age, mean (SD), y	39.5 (10.3)		
Fall in FEV ₁ during immediate asthmatic reactions, mean (SD)		25.39 (10.69)	
Fall in FEV ₁ during late asthmatic reactions, mean (SD)		23.88 (11.21)	
Baseline FeNO, median (IQR), ppb	23.0 (15.0-32.9)	26.7 (18.0-36.0)	16.9 (13.0-23.5)
Post SIC FeNO, median (IQR), ppb	23.0 (15.7-49.4)	30.1 (17.2-61.7)	17.8 (13.2-23.5)
Baseline methacholine PC ₂₀	1.71 (0.62-3.50)	1.18 (0.54-3.31)	3.49 (1.40-4.45)

Abbreviations: FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in the first second; HMW, high molecular weight; IQR, interquartile range; LMW, low molecular weight; SIC, specific inhalation challenge.

^aData are expressed as number (%) unless otherwise specified.

Table 2. Exhaled Nitric Oxide (ppb) at Baseline and After Specific Inhalation Challenge (SIC) in Patients With Positive SIC Results

	No.	Baseline		After SIC		Relative Change		P Value
		Mean ^a	(95% CI)	Mean ^a	(95% CI)	Ratio	(95% CI)	
HMW agent	13	31.73	(22.40-44.95)	38.53	(23.21-63.98)	1.21	(0.80-1.85)	.33
LMW agent	32	24.73	(19.84-30.83)	31.16	(22.21-43.73)	1.26	(1.05-1.52)	.01
Nonsmoker	26	24.04	(19.15-30.18)	28.88	(21.01-39.69)	1.20	(1.00-1.45)	.05
Smoker	12	24.06	(14.60-39.66)	23.72	(11.97-47.01)	0.99	(0.54-1.79)	.95
No atopy	13	22.91	(16.35-32.12)	25.36	(13.60-47.26)	1.11	(0.76-1.60)	.55
Atopy	32	28.69	(23.33-35.28)	37.23	(27.86-49.75)	1.30	(1.05-1.61)	.01
Total	45	26.72	(22.28-32.04)	33.28	(25.37-43.65)	1.25	(1.05-1.48)	.01

Abbreviations: HMW, high molecular weight; LMW, low molecular weight.

^aP < .05

flours (n=10), acrylates (n=6), formaldehyde (n=3), spiramycin (n=3), persulfates (n=3), and eugenol, styrene, 5-aminosalicylic acid, latex, wood, anhydride, and fish (1 case each). Twenty-three patients had a negative SIC with isocyanates (n=3), flours (n=3), acrylates (n=5), spiramycin (n=2), persulfates (n=4), and 1 case each of formaldehyde, eugenol, styrene, glutaldehyde, anhydrides, and potassium dichromate.

A comparison of FeNO values in patients with positive and negative SIC at both baseline and postchallenge revealed a nonsignificant difference ($P=.23$); however, we did find significant differences for smokers ($P=.02$) and for patients

challenged with low-molecular-weight (LMW) agents ($P=.04$) with positive SIC. In patients with positive SIC results, there was a significant increase in FeNO after positive testing, with an increase ratio of 1.25 (95% CI, 1.05-1.48; $P=.01$ (Table 2). Only 1 patient in the negative SIC group had more than a 1-fold increase in FeNO, but with no change in methacholine PC₂₀.

On analyzing different variables (atopy, smoking, and type of agent), we observed that only LMW agents induced a significant increase in FeNO ($P=.01$) after SIC. In patients with a negative SIC no significant difference was observed ($P=.08$) in FeNO values, although these were significantly

Table 3. Exhaled Nitric Oxide (ppb) at Baseline and After Specific Inhalation Challenge (SIC) in Patients With Negative SIC Results

	No.	Baseline		After SIC		Relative Change		P Value
		Mean ^a	(95% CI)	Mean ^a	(95% CI)	Ratio	(95% CI)	
HMW agent	3	19.74	(9.47-41.16)	28.39	(4.57-176.2)	1.44	(0.22-9.56)	.49
LMW agent	20	17.91	(13.65-23.51)	17.28	(13.50-22.11)	0.96	(0.83-1.11)	.60
Nonsmoker	20	18.53	(13.87-24.77)	18.41	(13.65-24.84)	0.99	(0.81-1.22)	.94
Smoker	3	16.39	(13.69-19.63)	18.53	(14.51-23.66)	1.13	(1.00-1.27)	.04
No atopy	10	17.90	(12.16-26.35)	17.24	(11.31-26.28)	0.96	(0.72-1.29)	.77
Atopy	13	19.50	(13.85-27.45)	20.40	(14.45-28.81)	1.05	(0.81-1.36)	.70
Total	23	18.14	(14.31-22.99)	18.43	(14.44-23.53)	1.02	(0.86-1.20)	.08

Abbreviations: HMW, high molecular weight; LMW, low molecular weight.

^aGeometric mean.

higher in smokers ($P=.04$) (Table 3). All the patients in the positive SIC group had a methacholine PC₂₀ of 16 mg/mL or lower; therefore, none of the patients fulfilled the criteria of eosinophilic bronchitis. In the negative SIC group, 8 patients had a negative PC₂₀ (>16 mg/mL) at baseline and postchallenge. In these patients no significant increase in FeNO was observed, ruling out the possibility of occupational eosinophilic bronchitis.

No significant correlation was found between a fall in FEV₁ (maximal, immediate, or late) and baseline FeNO or PC₂₀ values. However, a maximum fall in FEV₁ and FeNO came close to statistical significance ($r=0.28$, $P=.06$).

A linear association was found between FeNO values and maximum FEV₁ fall (0.190, $r=0.10$; $P=.02$), which means that when baseline FeNO increases by 1%, the maximum fall in FEV₁ increases by 0.19%. However, the association between the 2 variables is too weak. Thus, FeNO is not useful for predicting fall in FEV₁.

The predictive capacity of baseline FeNO was analyzed using logistic regression. The odds ratio (OR) for FeNO was 1.04 (95%

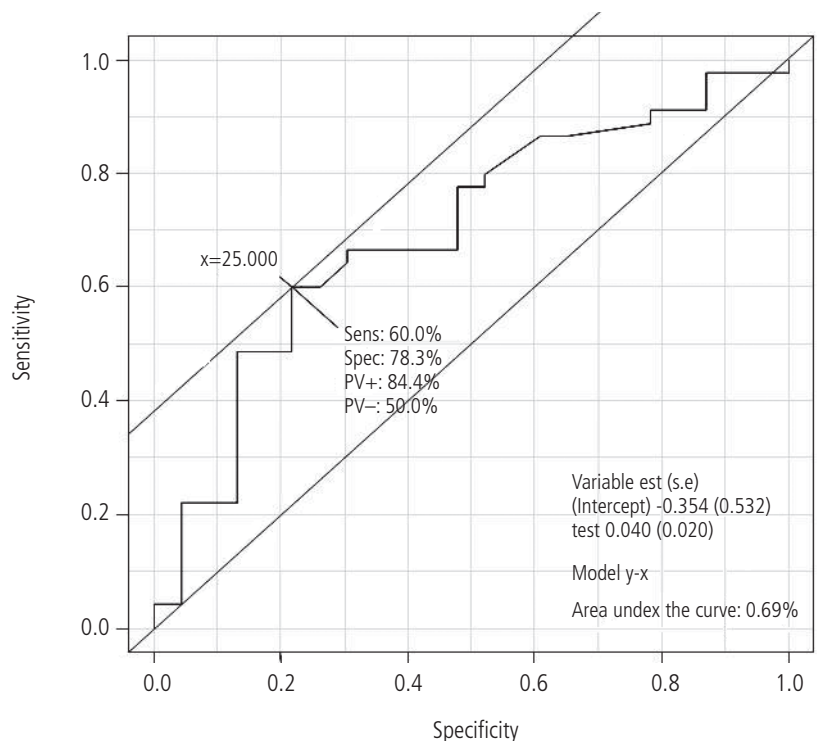


Figure 1. Receiver operating characteristics for baseline fractional exhaled nitric oxide values for predicting a positive outcome in the specific inhalation challenge test. Sens indicates sensitivity; spec, specificity; PV+, positive predictive value; PV-, negative predictive value.

CI, 1.01-1.09; $P=.01$). It follows from this that higher baseline FeNO values increase the risk of a positive SIC and that for each unit increase in FeNO, the risk of a positive SIC rises by 4%. Using ROC, the highest achievable combination of sensitivity and specificity for baseline FeNO values to predict a positive SIC response was reached at a derived threshold of 25 ppb, which yielded 60% sensitivity and 80% specificity (positive predictive value [PPV], 84.4%; negative predictive value [NPV], 50%; and area under the curve [AUC], 0.69) (Figure 1).

The cutoff point providing maximal sensitivity and specificity for increase in %FeNO postchallenge was 41%. This change in FeNO levels had a sensitivity of 50% and a specificity of 95% (PPV, 94.4% and NPV, 46.8%) for predicting a positive outcome in the challenge test (Figure 2). The AUC was 0.62, thus making predictive capacity low. For high-molecular-weight (HMW) agents, maximal sensitivity and specificity for increase in %FeNO after challenge was 21% (sensitivity, 61.5%; specificity 66.7%; PPV, 88.9%; NPV, 28.6%; AUC, 0.46) vs 41.7% for LMW agents (sensitivity, 37.9%; specificity, 100%; PPV, 100%; NPV, 52.6%; AUC, 0.63). On analyzing smokers and nonsmokers separately, the smoker cutoff providing maximal sensitivity and specificity for increase in %FeNO postchallenge was 94% (vs 41% for nonsmokers).

Discussion

Some studies have examined the usefulness of FeNO in the investigation of occupational asthma, although their results have not been consistent [3-12]. The present study describes changes in FeNO values at baseline and after SIC with occupational agents in a large sample of patients. We found a significant increase in FeNO values with respect to baseline in patients with a positive SIC, revealing an increase ratio of 1.25 (95% CI, 1.05-1.48; $P=.01$), but not in those with a negative SIC. Therefore, the mean FeNO increase was 25% after positive challenges with occupational agents. However, when FeNO values obtained from patients with positive and negative SIC results were compared, this difference was not statistically significant. Nevertheless, the difference was significant in patients challenged with LMW agents ($P=.04$), probably due to a larger number of patients included, and in smokers ($P=.02$).

Our results are consistent with findings from a recent study by Ferrazzoni et al [12], which showed that isocyanate-induced asthmatic reactions were associated with a consistent increase in FeNO, which was maximal 24 to 48 hours following exposure; they were not, however, associated with isocyanate exposure in patients with occupational rhinitis or in nonsensitized subjects individuals [12]. The authors also found a positive correlation between sputum eosinophil levels

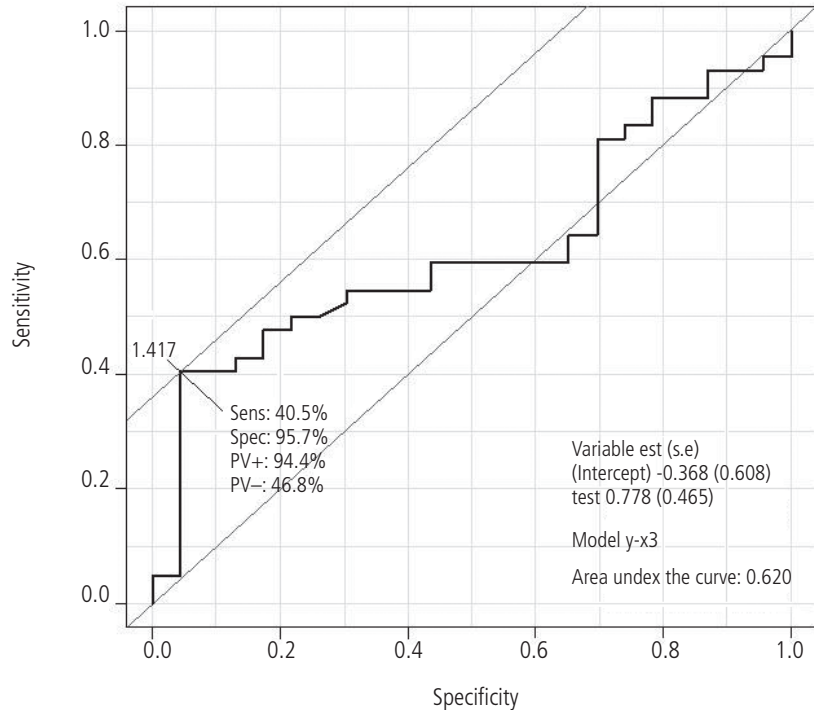


Figure 2. Receiver operating characteristics for % increase in fractional exhaled nitric oxide values for predicting a positive outcome in the specific inhalation challenge test. Sens indicates sensitivity; spec, specificity; PV+, positive predictive value; PV-, negative predictive value.

and FeNO levels before and after SIC, confirming that FeNO levels reflect the degree of airway eosinophilia. Nevertheless, our results differ from those of other researchers [3-5,8,9]. On analyzing different variables (atopy, smoking, and type of agent), we saw that only LMW agents induced a significant increase in FeNO ($P=.01$) after a positive SIC. In patients with a negative SIC, we found a significant increase only in smokers ($P=.04$). It is difficult to explain this finding, but tobacco smoke is known to have an effect on FeNO [1]. Additionally, our study included only patients who had abstained from inhaled or oral corticosteroids for at least 7 days at the time of the test, which arguably increases the consistency of our results by eliminating the influence of the substance on FeNO response [20].

We did not find that FeNO increase depended on the type of asthmatic reaction during SIC; this finding is contrary to that of Ferrazzoni et al [12], who reported that the magnitude of FeNO increase was greater in patients who experienced an immediate asthmatic reaction. The authors also detected a strong positive correlation between fall in FEV₁ during the early asthmatic response in the SIC and the corresponding decrease in FeNO ($r = -0.76$, $P < .001$). We, by contrast, found a weak linear association between FeNO and fall in FEV₁, and therefore, the capacity of baseline FeNO to predict this fall was very low. When FeNO increased by 1% from baseline, the maximal fall in FEV₁ was 0.19%. Moore et al [21] studied whether the magnitude of PEF response to occupational exposure was related to FeNO. They found that the group with a FeNO level of less than 15 ppb exhibited less reactivity in methacholine challenge testing, but similar results for PEF changes in relation to work. The capacity to predict a positive

SIC using baseline FeNO and PC₂₀ was also analyzed. For FeNO, the OR was 1.04 (1.01-1.09) ($P=0.01$). This means that for each unit increase in FeNO, the risk of a positive SIC is 4%. Using ROC, the highest achievable combination of sensitivity and specificity for baseline FeNO values was reached at a derived threshold of 25 ppb, which yielded 60% sensitivity and 80% specificity. The cutoff point providing maximal sensitivity and specificity for an increase in %FeNO postchallenge was 41%. This change in FeNO levels had a sensitivity of 50% and a specificity of 95% for predicting a positive challenge test. The AUC was 0.62, meaning that the predictive capacity is low. When HMW and LMW agents were analyzed separately, sensitivity did not increase significantly, and high rates of specificity were maintained. Recently, Pedrosa et al [22] found that an increase in FeNO levels of 12% after SIC achieved good discriminating sensitivity and specificity for cases with positive SIC results. However, this study only included SIC with HMW occupational and nonoccupational agents.

In conclusion, we have demonstrated that asthmatic reactions induced by occupational agents during SIC are associated with a consistent increase in FeNO postchallenge. This increase was significantly higher in atopic patients and in cases where LMW agents were used, although the predictive diagnostic capacity of FeNO remains low. While FeNO may aid in the interpretation of SIC in some cases, it cannot be used in a generalized fashion as a surrogate marker to predict or to assess SIC with occupational agents.

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Conflicts of Interest

Erika Aguado, Manuela García del Potro, Ignacio Mahillo, Cristina Costa and Mar Fernández-Nieto declare that they have no conflicts of interest.

Joaquín Sastre has served as a consultant to Phadia, Schering-Plough, Merck, FAES Farma, and GSK, has been paid speaker's fees by Novartis, GSK, Stallergenes, FAES FARMA, and UCB, and has received grant support from Phadia, GSK, and ALK-Abello. None of these disclosures had any influence on this research.

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