Performance of fractional exhaled nitric oxide in predicting response to inhaled corticosteroids in chronic cough: a meta-analysis

Pasquale Ambrosino, Mariasofia Accardo, Marco Mosella, Antimo Papa, Salvatore Fuschillo, Giorgio Alfredo Spedicato, Andrea Motta and Mauro Maniscalco

Istituti Clinici Scientifici Maugeri IRCCS, Pavia, Italy; Department of Data Analytics and Actuarial Science, Unipol Group, Bologna, Italy; Institute of Biomolecular Chemistry, National Research Council, ICB-CNR, Naples, Italy

ABSTRACT

Background. Chronic cough is a disabling condition with a high proportion of diagnostic and therapeutic failures. Fractional exhaled nitric oxide (FeNO) has been considered a useful biomarker for predicting inhaled corticosteroids (ICS) response. We evaluated the relationship between FeNO and ICS response in chronic cough by performing a systematic review with meta-analysis.

Methods. PubMed, Web of Science, Scopus and EMBASE databases were systematically searched. Differences were expressed as Odds Ratio (OR) with 95% confidence intervals (95%CI). Pooled sensitivity, specificity, positive (PLR) and negative likelihood ratio (NLR), and area under the hierarchical summary receiver operating characteristic curve (HSROCAUC) were estimated.

Results. Nine articles on 740 chronic-cough patients showed that the response rate to ICS was 87.4% (95%CI: 83.8–91.0) in 317 patients with a high FeNO and 46.3% (95%CI: 41.6–51.0) in 423 controls, with an attributable proportion of 47.0% and a diagnostic OR of 9.1 (95%CI: 3.7–22.4, p < .001). The pooled estimate of diagnostic indexes resulted in a sensitivity of 68.5% (95%CI: 46.7–84.4) and specificity of 81.9% (95%CI: 63.0–92.3), with a HSROC AUC of 0.82 (95%CI: 0.64–0.90). In a realistic scenario with a pre-test probability set at 30%, based on a pooled PLR of 3.79 (95%CI: 1.24–7.47) and NLR of 0.38 (95%CI: 0.22–0.66), the post-test probability was 62% with a high FeNO and 14% if the test was negative. Subgroup analyses confirmed a better performance for the recommended FeNO cut-off greater than 25 ppb. Meta-regression and sensitivity analyses showed no impact of major demographic and clinic variables on results.

Conclusions. A high FeNO before starting ICS therapy may help identify chronic-cough patients responding to treatment, with a better performance of higher cut-off values. Further studies are needed to evaluate the real usefulness of this biomarker to guide cough therapy and optimise strategies in different healthcare settings (community, hospital, rehabilitation).

KEY MESSAGES

Chronic cough is a disabling condition with a high proportion of diagnostic and therapeutic failures. Fractional exhaled nitric oxide (FeNO) may be a useful biomarker for identifying chronic cough patients who respond to steroid treatment. A FeNO cut-off lower than 25 ppb should be considered irrelevant for this clinical application.

Introduction

With a worldwide prevalence of 9.6% [1], chronic cough is defined as a cough that lasts for more than 8 weeks [2], resulting in substantial disability and quality of life impairment [3]. Several diseases may be responsible for this clinical manifestation, including asthma, eosinophilic bronchitis, pulmonary fibrosis, gastro-esophageal reflux disease (GERD), postnasal drip syndrome (PNDS), chronic obstructive pulmonary disease (COPD), and respiratory tract infections (RTI) [4]. Exposure to cigarette smoke, environmental pollution and blood pressure medications are also involved in chronic cough pathogenesis [5].

Up to 44% of diagnostic and therapeutic failures have been documented among patients with chronic cough [3,6], thus leading to the new paradigm of a...
neuropathic syndrome with its own pathophysiology, known as cough hypersensitivity syndrome (CHS) [7]. In parallel, such diagnostic and therapeutic challenges called for the development of specific guidelines for chronic cough aimed at improving the current management strategies [4–6]. Such guidelines all rely on a common principle based on the identification and treatment of the disease potentially responsible for this clinical manifestation.

Among the peripheral stimuli for cough reflex, eosinophilic airway inflammation is one of the most frequent and, consequently, one of the most studied in terms of pathophysiology and clinical implications [7]. Having this airway inflammation a good response to inhaled corticosteroids (ICS) [8], it is crucial to identify potential ICS responders to enable tailored therapeutic strategies and avoid treatment failures or adverse drug reactions [2]. Conventionally, induced sputum eosinophil count has been used to diagnose eosinophilic airway inflammation, thus driving decisions on ICS treatment in patients with asthma, eosinophilic bronchitis and atopic cough [9]. However, this method is technically and logistically challenging, being currently restricted to a limited number of specialized centres.

Past observation that the nitric oxide (NO) concentration in exhaled air generally increases in chronic inflammatory airway diseases [10,11] has led the European Thoracic Society (ERS) and the American Thoracic Society (ATS) to agree on highly standardised procedures to measure fractional exhaled NO (FeNO) [12–14]. Thus, FeNO has become an important non-invasive support to monitor compliance and efficacy of steroids in chronic airway diseases, especially asthma [15,16]. More recently, the potential use of FeNO in predicting ICS response in chronic cough patients has attracted an increasing interest. In particular, some studies documented a higher response rate to ICS among chronic cough patients with a high FeNO as compared to those with a low FeNO [17,18], thus suggesting its potential role in guiding decisions on ICS initiation. However, recent data have challenged these results [19,20], and no meta-analytical data providing a comprehensive information about this issue are currently available.

Here, we performed a systematic review with meta-analysis and meta-regression to evaluate the association between high FeNO and ICS response in chronic cough.

**Methods**

For this systematic review, we prospectively developed a protocol, specifying the objectives, the principles for the studies’ selection, the method for study quality assessment, the outcomes and statistical methods. The review protocol was registered on PROSPERO with identifier CRD42021261847.

**Search strategy**

In agreement with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [21], we performed a systematic search in electronic databases (PubMed, Web of Science, Scopus, EMBASE). The search terms *steroid, steroids, corticosteroid, corticosteroids, glucocorticoid, glucocorticoids, cough, and nitric oxide* were used in all possible combinations, without language restriction. The last search was performed on June 8, 2021.

In addition, the lists of the retrieved articles were manually reviewed. For missing data, we tried to contact the authors to obtain the original data. Two investigators (MMo and MA) independently analysed the studies and carried out data extraction. For divergent opinions, a third investigator was consulted (SF). Discrepancies were resolved by consensus. Selection results presented a high inter-reader agreement (κ = 0.97), and have been detailed according to PRISMA flowchart (Supplemental Figure 1).

**Data extraction and quality assessment**

According to the established protocol, we considered all studies reporting the rate of ICS response in chronic cough subjects with high and low FeNO. Case-reports, case-series without a control group, reviews and animal studies were not included. We also evaluated abstracts and citations from scientific conferences. The data extracted from the studies were study design, sample size, major clinical and demographic variables, number of subjects with a high FeNO (cases), number of subjects with a low FeNO (controls), and rate of ICS response in each of the two groups. Data on FeNO values (mean with standard deviation or standard error) among responders and non-responders were also collected. Devices and methods for FeNO assessment and FeNO cut-off values used in included studies are reported in Supplemental Table 1.

Quality Assessment of Diagnostic Accuracy Studies 2 (QUADAS-2) was used to evaluate the methodological quality of each study. This tool was specifically designed to judge the quality of diagnostic accuracy studies [22]. In brief, the scoring system considers 2 major domains: risk of bias (4 items) and applicability concerns (3 items). The items considered in both
domains are: patient selection, index test and reference standard. The flow and timing item is only evaluated in terms of risk of bias. According to the responses to the landmark questions, each item can be scored “low”, “high”, or “uncertain” based on the risk of bias or on the concerns about the degree of matching with the review question. QUADAS-2 assessment was performed by two independent reviewers (MMo and MA). When possible, disagreements were resolved by consensus or otherwise adjudicated by a third member of the review team (SF).

**Statistical analysis**

Statistical analyses were performed using Comprehensive Meta-analysis (Version 2, Biostat, Englewood NJ, 2005), Review Manager software (Version 5.4.1, The Cochrane Collaboration, Copenhagen, Denmark), R Statistical software (R Core Team 2021) and MetaDTA (Version 2.0) [23].

In each group, the response rate to ICS therapy in cases and controls was calculated as (number of responders)/(total number of subjects). The attributable proportion was defined as (response rate in cases – response rate in controls)/(response rate in cases) [24]. Differences in the response rates between cases and controls were expressed as diagnostic Odds Ratio (OR) with pertinent 95% confidence intervals (95%CI). Differences in FeNO values among responders and non-responders were expressed in parts per billion (ppb) as mean difference (MD) and 95%CI. The overall effect was tested using Z-scores, with a $p < .05$ being considered statistically significant. Since significant heterogeneity is to be expected in diagnostic accuracy studies [25], the random effects method was used whenever applicable to be as conservative as possible.

The bivariate random-effects binomial model was employed to obtain the summary point for sensitivity, specificity, positive (PLR) and negative likelihood ratio (NLR) with 95%CI [25]. The hierarchical summary receiver operating characteristic (HSROC) parameters and the area under the curve (AUC) were estimated using the bivariate model parameters and the equivalence equations of Harbord et al [26]. The Fagan’s nomogram was used to infer post-test probability from likelihood ratios and evaluate the clinical applicability of FeNO in a realistic scenario [27].

**Risk of bias assessment**

Based on the analysis of the funnel plot of the effect size vs. the inverse of the square root of the effective sample size, the presence of publication bias was tested with the Deeks funnel plot asymmetry test [30]. Possible small-study effect was also addressed by performing a visual inspection of the funnel plots of the logarithmic effect size vs. precision (1/standard error of the logarithmic effect size) or of the mean difference vs. precision (1/standard error of the mean difference). The Egger’s and the Begg and Mazumdar tests were used to test for funnel plot asymmetry over and above any subjective evaluation. [31]. Finally, the Duval and Tweedie’s trim-and-fill analysis was performed to evaluate an adjusted effect size after trimming and imputing studies [32]. A $p < .10$ was considered statistically significant when assessing the risk of bias [33].

**Sensitivity analyses**

Potential sources of heterogeneity were investigated by repeating the analyses after excluding retrospective studies. Because of the potential influence of smoking on FeNO values [34], we performed a further sensitivity analysis excluding the studies enrolling smokers. Moreover, we planned to separately analyse articles specifically using the recommended higher than 8-week limit for chronic cough definition [2]. Finally, we planned to repeat analyses after excluding any study with a high risk of bias and/or applicability concerns for patient selection according to QUADAS-2.

**Subgroup analyses**

The ATS strongly recommend that FeNO values lower than 25 ppb should be considered irrelevant for clinical applications, implying non-eosinophilic or no airway inflammation [35]. Thus, since included studies
used different thresholds, we separately analysed data based on the presence/absence of a FeNO cut-off greater than 25 ppb.

**Meta-regression analyses**

We also evaluated the impact of demographic variables (mean age, male gender) and clinical characteristics of the study population related to body composition [body mass index (BMI)], smoking habit, atopic status, concomitant respiratory diseases [asthma, recent respiratory tract infection (RTI)], pulmonary function [forced expiratory volume in 1 s (FEV1)], forced expiratory volume in 1 s/forced vital capacity (FEV1/FVC), use of medications [angiotensin converting enzyme-inhibitors (ACE-I)], length of follow-up after ICS initiation, and cough duration on differences in the prevalence of steroid response between patients with high and low FeNO. Thus, we performed meta-regression analyses after implementing a regression model with the rate of response as dependent variable (y), and the above reported covariates as independent variables (x). Comprehensive Meta-analysis (Version 2, Biostat, Englewood NJ, 2005) was used for meta-regressions.

**Results**

After excluding duplicate results, the search identified 2,237 articles. Of them, we excluded 1,993 because of topic after evaluating the title and the abstract, and 231 because were reviews/book chapters/editorials. Other 4 studies were excluded after the evaluation of the full length paper.

In the final analysis, we included 9 articles [17–20,36–40], in which a total of 740 chronic cough patients were enrolled (Supplemental Figure 1).

**Study characteristics**

Table 1 reports the baseline demographic and clinical data of patients with chronic cough treated with ICS from the included studies. Characteristics of the study design and steroid treatment are reported in Table 2.

The number of enrolled patients with chronic cough ranged between 39 and 244, with a mean-age ranging from 39.8 to 59.2 years, the mean BMI from 25.7 to 30.1 kg/m², and the male gender prevalence from 38.1 to 53.7%. Asthma was documented in 0–54.8% of patients, an atopic status in 5.2–100%, a recent RTI in 0–13.0%, and a smoking history in 0–51.9%. Mean values of FEV₁ ranged from 86.2 to

| Study | High FeNO (n) | Low FeNO (n) | Males (%) | Age (years) | BMI (kg/m²) | Smoking (%) | Recent RTI (%) | ACE-I (%) | Asthma (%) | Atopic status (%) | ICE naïve (%) | FEV₁ (% predicted) | FEV₁/FVC (%) |
|-------|--------------|--------------|-----------|-------------|-------------|-------------|---------------|-----------|------------|-----------------|---------------|------------------|-------------|
| Hahn 2007 | 41 | 23 | 40.6 | 46.8 | 28.9 | 15.6 | 0 | 48.4 | 95.6 | 90.7 | 81.4 |
| Koskela 2013a | 15 | 24 | 25.5 | 55.6 | 27.4 | 0 | 0 | 95.6 | 90.7 | 93.7 | 72.8 |
| Lamon 2019 | 41 | 22 | 38.1 | 59.2 | 25.7 | 30.1 | 9.5 | 50.6 | 95.7 | 90.8 | 61.3 |
| Price 2018 | 19 | 24 | 47.4 | 50.0 | 27.6 | 9.6 | 0 | 48.6 | 95.7 | 113.2 | 81.9 |
| Prieto 2009 | 41 | 24 | 43.4 | 48.3 | 28.1 | 30.1 | 0 | 48.6 | 95.7 | 81.9 | 95.7 |
| Y. 2016 | 139 | 105 | 53.7 | 39.8 | 10 | 0 | 0 | 95.7 | 90.7 | 93.7 | 54.7 |

Pop: population; BMI: body mass index; RTI: respiratory tract infection; ACE-I: angiotensin converting enzyme inhibitors; FEV₁: forced expiratory volume in 1 s; FVC: forced vital capacity. Continuous data are expressed as mean values, unless otherwise indicated. *Baseline demographic and clinical data refer to an original population of 43 patients for Koskela 2013 and 114 patients for Price 2018. **Any smoking history (previous or current). ***Asthma defined by methacholine challenge tests. ****Asthma defined by positive anamnesis. 

Only 95 patients with available atopy data.
113.2% predicted, while the FEV1/FVC ratio was between 72.8 and 88.9. Data on the use of ACE-I and on the proportion of steroid naïve patients lacked in most included studies.

Four studies were retrospective [17–19,37], four were prospective [20,36,38,40], and one had a randomised double-blind placebo-controlled design [39]. With the only exception of 2 articles [37,39], all included studies complied with the definition of chronic cough as the presence of the symptom for at least 8 weeks [2]. Mean follow-up after starting ICS therapy ranged from a minimum of 2 weeks to 5.3 months.

Although the standardised procedures for FeNO assessment were followed in all the included studies, different cut-off values (from 16.3 ppb to 44.5 ppb) were used to discriminate high and low FeNO (Supplemental Table 1).

**Quality of the included studies**

Figure 1 shows the QUADAS-2 report for each included study. Only one study presented a low risk of bias or low applicability concerns in all QUADAS-2 domains [20]. Four studies [17–19,37] with a retrospective design had an unclear risk of bias regarding patient selection. One prospective study [38] did not avoid a case-control design and was considered at high risk of bias for this item. Applicability concerns for patient selection were raised for two studies [37,39], which included patients with non-specific respiratory symptoms and/or used a lower than 8-week limit for chronic cough definition. Considering that baseline FeNO was assessed before starting ICS therapy, the index test results were interpreted without knowledge of the results of the reference standard in all included studies. With only one exception [36], all studies clearly described the devices and procedures for FeNO assessment. However, the FeNO cut-off value was not pre-specified in five articles [19,36–38,40], which were considered at unclear risk of bias for the index test item. Two studies [20,39] used validated scores as reference standard in blinded conditions, thus were judged at low risk of bias and with no applicability concerns for this item. Two further studies using validated scores [36,40] and four studies relying on the judgement of physicians to define ICS...

| Study Design | Definition of Chronic Cough | Cough Duration | ICS Type/Dose | Follow-up | Definition of ICS Response |
|--------------|-----------------------------|---------------|--------------|-----------|----------------------------|
| Hahn 2007    | Retrospective               | ≥8 weeks      | 40.3 months  | 5.3 months| Physician-documented significant improvement in cough, no further diagnostic study ordered for assessment of cough, and no alteration in ICS dose |
| Hsu 2013     | Retrospective               | ≥8 weeks      | 15.3 months  | ≥2 weeks  | Complete control of cough determined by physician |
| Koskela 2013 | Prospective                 | ≥8 weeks      | 8.5 years    | 12 weeks  | Improvement of > 1.3 points in the Leicester Cough Questionnaire score |
| Lamon 2019   | Retrospective               | ≥8 weeks      | 43.1 months  | ≥3 months | Self-reported reduction of cough frequency |
| Price 2018   | Double-blind randomised placebo-controlled trial | ≥6 weeks | – | 4 weeks | Improvement of ≥ 20 mm in the VAS cough score |
| Prieto 2009  | Prospective                 | ≥8 weeks      | –            | 4 weeks   | Reduction of > 50% in the mean daily cough symptom scores |
| Shebl 2020   | Prospective                 | ≥8 weeks      | –            | 4 weeks   | Complete cough disappearance according to a cough symptom scorea |
| Watanabe 2016| Retrospective               | ≥3 weeks      | 15.4 months  | 3 months  | Significant improvement in cough with ICS declared by the patient and confirmed by the physician |
| Yi 2016      | Prospective                 | ≥8 weeks      | –            | –         | Complete control of cough determined by physicianb |

LCQ: Leicester Cough Questionnaire; VAS: Visual Analogue Scale. Continuous data are expressed as mean values, unless otherwise indicated.

aInformation from another reference by the same authors [41].
bInformation from another reference by the same authors [42].
response [17,19,37,38] did not clearly report if the reference standard results were interpreted without knowledge of the results of the index test. Thus, we scored an unclear risk of bias for this item. Furthermore, unclear applicability concerns were raised for studies [17,19,37,38] not using a validated and repeatable tool to assess ICS response. Only one study [18] based the effectiveness of steroids on a patient declaration of cough frequency reduction, thus being scored at high risk of bias with high concerns in terms of applicability. Exclusion rates among retrospective studies were high in two studies [17,19], which were scored at unclear risk of bias in terms of flow and timing.

**Performance of FeNO in predicting ICS response**

The analysis of the nine studies [17–20,36–40] showed that the response rate to ICS was 87.4% (95%CI: 83.8–91.0) in 317 chronic-cough patients with a high FeNO, and 46.3% (95%CI: 41.6–51.0) in 423 controls, with an attributable proportion of 47.0% and a corresponding OR of 9.1 (95%CI: 3.7–22.4, \( p < .001 \), Figure 2(A)). The heterogeneity among the studies was significant (\( I^2: 69.0\% \), \( p = .001 \)) and was not reduced by the exclusion of one study at a time.

The pooled analysis of diagnostic indexes resulted in a sensitivity of 68.5% (95%CI: 46.7–84.4) and a specificity of 81.9% (95%CI: 63.0–92.3), with a PLR of 3.79 (95%CI: 1.24–7.47) and a NLR of 0.38 (95%CI: 0.22–0.66). Based on pooled likelihood ratios and a pre-test probability set at 30% by default, Fagan’s nomogram revealed that the post-test probability increased to 62% if the patient had a high FeNO and decreased to 14% if the test was negative (Figure 3).

The analysis of the HSROC curve revealed a pooled AUC of 0.82 (95%CI: 0.64–0.90) (Figure 4(A)). No threshold effect was detected by diagnostic threshold
analysis, confirmed by a Spearman correlation coefficient between sensitivity and false positive rate of 0.498 ($p = .173$).

**Publication bias**

Since publication bias has been reported to affect results of meta-analyses, this potential bias was assessed by using funnel plots analysis. The Deeks funnel plot asymmetry test suggested the absence of publication bias, confirmed by a $p$-value of 0.760 (Figure 4(B)). Accordingly, the funnel plot of the effect size vs. precision for studies evaluating the difference in steroid response between chronic-cough patients with a high and low FeNO in each study (A) or the Mean Difference in FeNO between responders and non-responders to corticosteroids. Lines are the 95% confidence intervals. The black diamond represents the cumulative Odds Ratio (A) or the cumulative Mean Difference (B) for analysed studies.

**Sensitivity analyses**

Study design can potentially influence the observed results. Therefore, we repeated the analysis after excluding the four retrospective studies [17–19,37], confirming, however, the results (OR: 6.3; 95%CI: 1.9–20.8, $p < .001$; $I^2$: 76.5%, $p = .002$). Similarly, when specifically considering the studies on non-smokers [19,20,36,38,40], we obtained an OR of 7.8 (95%CI: 2.4–25.0, $p < .001$; $I^2$: 66.8%, $p = .017$) for the association between high FeNO and steroid response. A diagnostic OR of 10.5 (95%CI: 4.0–27.5, $p < .001$; $I^2$: 66.0%, $p = .007$) was documented after excluding 2 studies [37–39] using a lower than 8-week limit for chronic cough definition. Overall, after excluding 3 studies [37–39] with a high risk of bias and/or applicability concerns for patient selection, an OR of 10.0
was confirmed. Results substantially comparable to those of the overall analysis with no threshold effect were obtained when estimating the pooled diagnostic indexes for these sensitivity analyses (Table 3).

Subgroup analyses

The subgroup analysis of the three studies [18,20,36] using a lower FeNO threshold (≤ 25 ppb) suggested a lower performance in predicting ICS response (OR: 3.9; 95%CI: 2.7–36.4, \( p < .001 \); \( \chi^2 \): 71.1%, \( p = .004 \)) was confirmed.

In contrast, when separately analysing studies [17,19,37–40] with a FeNO cut-off greater than 25 ppb, a better performance was documented (OR: 13.5; 95%CI: 4.1–44.2, \( p < .001 \); \( \chi^2 \): 71.6%, \( p = .003 \)) with an AUC of 0.83 (95%CI: 0.62–0.94) (Table 3). To assess the clinical utility of the recommended FeNO threshold [35], the Fagan’s nomogram was used, revealing a post-test probability of 64% if the patient had a high FeNO and 11% if the test was negative.

Meta-regression analyses

As shown in Supplemental Table 3, no demographic or clinical variable related to body composition, smoking habit, atopic status, concomitant respiratory
diseases, pulmonary function, length of follow-up and cough duration affected the difference in steroid response rate between chronic cough patients with high and low FeNO values.

**FeNO values among responders and non-responders**

In line with the above results, in six studies [17,18,20,37,38,40] we found that 321 chronic cough patients responding to ICS therapy showed significantly higher FeNO values as compared to 240 non-responders (MD: 23.0 ppb; 95%CI: 15.2–30.9, p < .001; I² = 83.0%; p < .001, Figure 2(B)). Of interest, when excluding the study by Prieto et al. [20], an even higher difference between responders and non-responders was observed (MD: 26.7 ppb; 95%CI: 23.5–29.4, p < .001) without heterogeneity (I² = 0%; p = .440).

The analysis of the funnel plot for studies evaluating FeNO levels among responders and non-responders to ICS suggested the absence of publication bias for this additional analysis, also confirmed by the Egger’s test (p = .917).

**Discussion**

The results of our meta-analysis suggest that high FeNO values before starting ICS therapy may be associated with higher response rates in chronic cough patients, with an acceptable performance in predicting steroid responsiveness. These findings are supported by sensitivity and meta-regression analyses, showing no impact of several clinical and demographic variables on the observed results, including those related to cough aetiology and duration. Furthermore, this meta-analysis offers interesting insights into the identification of the optimal FeNO cut-off for predicting response to steroids. In particular, in line with the ATS recommendations [35], we documented a better performance of FeNO values higher than 25 ppb, with a difference of 23 ppb between responders and non-responders.
In recent years, FeNO has become a useful and non-invasive method to identify chronic inflammation of airway diseases [43], thus being successfully used to monitor adherence to steroid treatment in asthmatic patients [44]. Considering that steroids are able to reduce eosinophilic inflammation documented in most asthma phenotypes, FeNO is the perfect biomarker for monitoring steroid response in this clinical setting [44]. In patients with mild-to-moderate asthma, a recent meta-analysis demonstrated that a gradual ICS reduction when FeNO is below 50 ppb does not increase exacerbations [45]. The effectiveness of FeNO for asthma management was also confirmed when specifically evaluating paediatric populations [46]. Moreover, FeNO has been considered for asthma diagnostics, with values greater than 40 ppb showing the best performance (OR: 9.8) [47]. More recently, the need for a safe and simple method driving the decisional process of steroid prescription in patients with non-specific respiratory symptoms has led to a growing focus on this biomarker [7]. Thus, randomised studies with a more robust design have been carried out to better address this issue [39].

Considering that cough-variant asthma, eosinophilic bronchitis and atopic cough are characterised by a T helper 2-mediated airway inflammation, they more often tend to respond to ICS treatment [44]. However, ICS responsiveness can depend upon several factors, including direct and indirect smoking exposure [48] and cough duration [49]. In keeping with this, a number of studies failed in demonstrating a correlation between airway hyperresponsiveness and ICS response in patients with subacute or chronic cough [48,50]. A recent meta-analysis of randomised clinical trials reported only a modest efficacy of ICS over placebo on chronic cough, thus concluding that a more rigorous patient selection is needed to identify those who may certainly respond to ICS [51]. Overall, the diagnostic uncertainty often leads to inappropriate ICS prescription, particularly in subjects with undiagnosed non-specific respiratory symptoms [52], and in those with recent RTI and otherwise self-limiting cough [53].

The rationale for using FeNO in the decisional process of ICS prescription is complex and only partially elucidated [44]. In brief, eosinophilic airway inflammation derives from the activation of mast cells and antigen-specific T helper 2 cells, with the concomitant production of cytokines, such as interleukin (IL)-4, IL-5 and IL-13 [10]. Their release regulates the expression of the inducible isoform of NO synthase (iNOS), thus determining an increased NO production in an allergic environment [11]. This implies that FeNO assessment may be able to accurately predict eosinophilic airway inflammation - a peripheral trigger of cough reflex with a good ICS response - in a rapid, non-demanding and cost-effective manner [54]. In a previous cost-effectiveness analysis, Sabatelli et al found that combining FeNO monitoring with standard asthma care saved €62.53 ($74.40) per patient-year in Spain, with potential annual savings of €129 million ($153 million) [55]. A similar conclusion was obtained for the asthma management in paediatric population [56].

Overall, our findings partly respond to the urgent need of rapid and reliable methods to identify chronic cough patients who could benefit from steroid therapy. In a previous systematic review, Song et al concluded that there is no strong evidence to support the use of FeNO testing for predicting ICS responsiveness in chronic cough [7]. However, this interesting study that first appeared online in 2016, although rigorously conducted according to PRISMA guidelines, identified articles published in peer-reviewed journals up to February 2015, thus including ≈50% of currently available studies. Thus, given the low number of studies and the considerable heterogeneity among the study designs and outcome measurements, the Authors conveniently decided not to carry out any meta-analytical evaluation. Considering the higher number of available articles in 2021, an attempt was made to perform a meta-analysis, although aware of the relevant limitations due to the high heterogeneity and the impossibility of drawing definitive conclusions. In fact, statistical heterogeneity was found to be generally significant as a result of our analyses, potentially depending on a different study design and different inclusion and exclusion criteria in considered studies. For example, a diagnostic accuracy study should ideally enrol a random or consecutive sample of eligible patients with the suspected condition to avoid the case-control design and, therefore, a potential bias. Thus, the inclusion of studies enrolling patients with the target condition and a control group without it may overestimate diagnostic accuracy [57]. To improve the critical evaluation of published studies, the QUADAS-2 tool was specifically designed, thus ensuring greater transparency in the classification of bias and applicability for diagnostic accuracy meta-analyses [22]. In our meta-analysis, based on this quality assessment of included studies, we planned a number of additional analyses to investigate potential sources of such heterogeneity, including a sensitivity analysis without the studies at high risk of bias for patient selection. Of interest, our results were substantially confirmed in each of these additional analyses.
Similarly, meta-regression analyses were also planned to evaluate the influence of a number of clinical and demographic variables on the observed results. For example, the length of follow-up after ICS initiation and the mean duration of chronic cough were quite heterogeneous in different studies and this may have significantly affected our findings [46]. The observation that such demographic and clinical covariates – even those related to cough aetiology (e.g. asthma, atopy, smoking) and duration – had no impact on our results confirms the potential usefulness of FeNO in predicting ICS response.

Overall, in our meta-analysis, we reported a significantly higher response rate in high FeNO patients as compared to those with a low FeNO (87.4% vs. 46.3%), corresponding to a diagnostic OR of 9.1. Of interest, in line with the ATS recommendation [35], the performance of this biomarker was even higher (OR: 13.5) when specifically considering studies with a FeNO cut-off greater than 25 ppb. Unfortunately, because of the relatively low number of included studies (n = 9), no further stratification according to different cut-off values could be performed. In our opinion, the fact that different devices for FeNO assessment were used in different studies had no effect on our results, since all included studies substantially followed ERS/ATS standardised procedures [12–14], although with different thresholds. Interestingly, the threshold effect was not the source of heterogeneity of our meta-analysis. Thus, considering that likelihood ratios do not change with the pre-test probability [58], the estimated post-test probabilities in a more realistic scenario suggested that the test could be considered suitable for routine practice. The usefulness of FeNO in the decisional process of ICS prescription has been recently reported also in chronic respiratory diseases [59,60], thus suggesting that this biomarker could be considered in the near future for a routine use in a wide range of clinical and healthcare settings (i.e. community, hospital, rehabilitation) [61,62].

As reported above, our meta-analysis has relevant limitations, mainly due to the considerable heterogeneity among the study designs and outcome measurements, with no objective information regarding the baseline severity of cough or magnitude of improvement of cough. Since a meta-analysis is performed on aggregate data and some missing information is present in each study, our sensitivity, subgroup and meta-regression analyses were able to refine results, allowing for the adjustment for some – but not all – potential confounders. Moreover, the presence of publication bias was consistently excluded with different methods. Overall, despite the additional analyses substantially confirming our findings, caution is required when interpreting the results of this meta-analysis. Over and above the aforementioned limitations, the relatively low number of included studies with a small sample should be considered as a major weakness of our pooled analyses, confirmed by the large confidence intervals reflecting the uncertainty in the estimation of the diagnostic test accuracy.

In conclusion, our results suggest that a high FeNO before starting ICS therapy may help identify chronic cough patients responding to treatment, with a better performance for higher cut-off values. This further supports the need for large clinical trials with a robust design aimed at defining more refined prediction strategies before ICS initiation, in order to guide decisions and reduce the risk of adverse events and cost-ineffective strategies. In this regard, FeNO may have the potential to be used as an additional tool to identify combined prediction strategies allowing for more individualised approaches.

**Author contributors**

PA conceived and designed the study, performed statistical analysis, interpreted results and drafted the manuscript. MA conceived the study, interpreted results, and drafted the manuscript. MMO, AP, and SF acquired clinical data and drafted the manuscript. GAS performed statistical analyses and critical revision. MM and AM supervised the project, interpreted results, drafted the manuscript and performed critical revisions. All Authors read and approved the final version of the manuscript.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

**Funding**

The author(s) reported there is no funding associated with the work featured in this article.

**ORCID**

Pasquale Ambrosino  http://orcid.org/0000-0002-9398-0428
Antimo Papa  http://orcid.org/0000-0003-4248-2784
Salvatore Fuschillo  http://orcid.org/0000-0002-1393-4473
Giorgio Alfredo Spedicato  http://orcid.org/0000-0002-0315-8888
Andrea Motta  http://orcid.org/0000-0002-8643-658X
Mauro Maniscalco  http://orcid.org/0000-0001-6751-9921
Data availability statement

The data that support the findings of this study are available from the corresponding author [M.M.] upon reasonable request.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the position of the institution to which they belong.

References

[1] Song WJ, Chang YS, Faruqi S, et al. The global epidemiology of chronic cough in adults: a systematic review and Meta-analysis. Eur Respir J. 2015;45(5):1479–1481.
[2] Chung KF, Pavord ID. Prevalence, pathogenesis, and causes of chronic cough. Lancet. 2008;371(9621):1364–1374.
[3] Song WJ, Chang YS, Faruqi S, et al. Defining chronic cough: a systematic review of the epidemiological literature. Allergy Asthma Immunol Res. 2016;8(2):146–155.
[4] Morice AH, Fontana GA, Sovijarvi AR, et al.; ERS Task Force. The diagnosis and management of chronic cough. Eur Respir J. 2004;24(3):481–492.
[5] Irwin RS, Baumann MH, Bolser DC, et al. Diagnosis and management of cough executive summary: ACCP evidence-based clinical practice guidelines. Chest. 2006;129(1 Suppl):15–235.
[6] Irwin RS, French CT, Lewis SZ, et al. Methodologies for the development of CHEST guidelines and expert panel reports. Chest. 2014;146(1):182–889.
[7] Song WJ, Won HK, Moon SD, et al. Could fractional exhaled nitric oxide test be useful in predicting inhaled corticosteroid responsiveness in chronic cough? A systematic review. J Allergy Clin Immunol Pract. 2017;5(1):135–143 e131.
[8] Berry M, Morgan A, Shaw DE, et al. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. Thorax. 2007;62(12):1043–1049.
[9] Gibson PG, Dolovich J, Denburg J, et al. Chronic cough: eosinophilic bronchitis without asthma. Lancet. 1989;1(8651):1346–1348.
[10] Ambrosino P, Parrella P, Formisano R, et al. Clinical application of nasal nitric oxide measurement in allergic rhinitis: a systematic review and Meta-analysis. Ann Allergy Asthma Immunol. 2020;125(4):447–459 e445.
[11] Ambrosino P, Molino A, Spedicato GA, et al. Nasal nitric oxide in chronic rhinosinusitis with or without nasal polyps: a systematic review with Meta-Analysis. J Clin Med. 2020;9(1):200
[12] Kharitonov S, Alving K, Barnes PJ. Exhaled and nasal nitric oxide measurements: recommendations. The european respiratory society task force. Eur Respir J. 1997;10(7):1683–1693.
[13] Recommendations for standardized procedures for the on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. This official statement of the american thoracic society was adopted by the ATS board of directors, july 1999. Am J Respir Crit Care Med. 1999;160(6):2104–2117.
[14] American Thoracic S, European Respiratory S; ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med. 2005;171(8):912–930.
[15] Dweik RA, Sorkness RL, Wenzel S, et al; National Heart, Lung, and Blood Institute Severe Asthma Research Program. Use of exhaled nitric oxide measurement to identify a reactive, at-risk phenotype among patients with asthma. Am J Respir Crit Care Med. 2010;181(10):1033–1041.
[16] Maniscalco M, Bianco A, Mazzarella G, et al. Recent advances on nitric oxide in the upper airways. Curr Med Chem. 2016;23(24):2736–2745.
[17] Hahn PY, Morgenthaler TY, Lim KG. Use of exhaled nitric oxide in predicting response to inhaled corticosteroids for chronic cough. Mayo Clin Proc. 2007;82(11):1350–1355.
[18] Lamon T, Didier A, Brouquieres D, et al. Exhaled nitric oxide in chronic cough: a good tool in a multi-step approach. Respir Med Res. 2019;7:64–9.
[19] Hsu JY, Wang CY, Cheng YW, Chou MC. Optimal value of fractional exhaled nitric oxide in inhaled corticosteroid treatment for patients with chronic cough of unknown cause. J Chin Med Assoc. 2013;76(1):15–19.
[20] Prieto L, Ferrer A, Ponce S, et al. Exhaled nitric oxide measurement is not useful for predicting the response to inhaled corticosteroids in subjects with chronic cough. Chest. 2009;136(3):816–822.
[21] Moher D, Liberati A, Tetzlaff J, et al.; PRISMA Group. Preferred reporting items for systematic reviews and Meta-analyses: the PRISMA statement. PLoS Med. 2009;6(7):e1000097.
[22] Whiting PF, Rutjes AW, Westwood ME, et al.; QUADAS-2 Group. Group Q: QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. Ann Intern Med. 2011;155(8):529–536.
[23] Patel A, Cooper N, Freeman S, et al. Graphical enhancements to summary receiver operating characteristic plots to facilitate the analysis and reporting of Meta-analysis of diagnostic test accuracy data. Res Synth Methods. 2021;12(1):34–44.
[24] Walter SD. The estimation and interpretation of attributable risk in health research. Biometrics. 1976;32(4):829–849.
[25] Reitsma JB, Glas AS, Rutjes AW, et al. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. J Clin Epidemiol. 2005;58(10):982–990.
[26] Harbord RM, Deeks JJ, Egger M, et al. A unification of models for Meta-analysis of diagnostic accuracy studies. Biostatistics. 2007;8(2):239–251.
[27] Caraguel CG, Vanderstichel R. The two-step fagan’s nomogram: ad hoc interpretation of a diagnostic test
result without calculation. Evid Based Med. 2013;18(4):125–128.

[28] Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in Meta-analyses. BMJ. 2003;327(7414):557–560.

[29] Deville WL, Buntinx F, Bouter LM, et al. Conducting systematic reviews of diagnostic studies: didactic guidelines. BMC Med Res Methodol. 2002;2:9.

[30] Deeks JJ, Macaskill P, Irwig L. The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. J Clin Epidemiol. 2005;58(9):882–893.

[31] Burkner PC, Doebler P. Testing for publication bias in diagnostic Meta-analysis: a simulation study. Stat Med. 2014;33(18):3061–3077.

[32] Duval S, Tweedie R. Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in Meta-analysis. Biometrics. 2000;56(2):455–463.

[33] Sedgwick P, Marston L. How to read a funnel plot in a Meta-analysis. BMJ. 2015;351:h4718.

[34] Malinovschi A, Janson C, Holmkvist T, et al. Effect of smoking on exhaled nitric oxide and flow-independent nitric oxide exchange parameters. Eur Respir J. 2006;28(2):339–345.

[35] Dweik RA, Boggs PB, Erzurum SC, et al.; American Thoracic Society Committee on Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Applications. American thoracic society committee on interpretation of exhaled nitric oxide levels for clinical A: an official ATS clinical practice guideline: interpretation of exhaled nitric oxide levels (FENO) for clinical applications. Am J Respir Crit Care Med. 2011;184(5):602–615.

[36] Koskela HO, Purokivi MK. Capability of hypertonic saline cough provocation test to predict the response to inhaled corticosteroids in chronic cough: a prospective, open-label study. Cough. 2013;9:15.

[37] Watanabe K, Shinkai M, Shinoda M, et al. Measurement of eNO with portable analyser might improve the management of persistent cough at primary care practice in Japan. Clin Respir J. 2016;10(3):380–388.

[38] Yi F, Chen R, Luo W, et al. Validity of fractional exhaled nitric oxide in diagnosis of Corticosteroid-Responsive cough. Chest. 2016;149(4):1042–1051.

[39] Price DB, Buhl R, Chan A, et al. Fractional exhaled nitric oxide as a predictor of response to inhaled corticosteroids in patients with non-specific respiratory symptoms and insignificant bronchodilator reversibility: a randomised controlled trial. Lancet Respir Med. 2018;6(1):29–39.

[40] Shebl E, Abdel-Moety H. Assessment of the role of fractional exhaled nitric oxide as a predictor of airway eosinophilia and corticosteroid responsiveness in patients with chronic cough. Egypt J Bronchol. 2020;14(1):15.

[41] Li Y, Zhongmin Q, Hanjing L, et al. Clinical benefit of sequential three-step empirical therapy in the management of chronic cough. Respiriology. 2008;13:353–358.

[42] Lai K, Chen R, Lin J, et al. A prospective, multicenter survey on causes of chronic cough in China. Chest. 2013;143:613–620.

[43] Molino A, Fuschillo S, Mosella M, et al. Comparison of three different exhaled nitric oxide analyzers in chronic respiratory disorders. J Breath Res. 2019;13(2):021002.

[44] Heffler E, Carugatto GE, Favero E, et al. Fractional exhaled nitric oxide (FENO) in the management of asthma: a position paper of the italian respiratory society (SIP/IRS) and italian society of allergy, asthma and clinical immunology (SIAAIC). Multidiscip Respir Med. 2020;15(1):36.

[45] Wang K, Verbakel JY, Oke J, et al. Using fractional exhaled nitric oxide to guide step-down treatment decisions in patients with asthma: a systematic review and individual patient data Meta-analysis. Eur Respir J. 2020;55(5):1902150.

[46] Wang X, Tan X, Li Q. Effectiveness of fractional exhaled nitric oxide for asthma management in children: a systematic review and Meta-analysis. Pediatr Pulmonol. 2020;55(8):1936–1945.

[47] Wang Z, Pianosi PT, Keogh KA, et al. The diagnostic accuracy of fractional exhaled nitric oxide testing in asthma: a systematic review and Meta-analyses. Mayo Clin Proc. 2018;93(2):191–198.

[48] Ponsioen BP, Hop WC, Vermue NA, et al. Efficacy of fluticasone on cough: a randomised controlled trial. Eur Respir J. 2005;25(1):147–152.

[49] Ye Q, He X-O, D’Urzo A. A review on the safety and efficacy of inhaled corticosteroids in the management of asthma. Pulm Ther. 2017;3(1):1–18.

[50] Rytila P, Ghaly L, Varghese S, et al.; Airway Inflammation Study Group. Airway inflammation study G: Treatment with inhaled steroids in patients with symptoms suggestive of asthma but with normal lung function. Eur Respir J. 2008;32(4):989–996.

[51] Lee SE, Lee JH, Kim HJ, et al. Inhaled corticosteroids and placebo treatment effects in adult patients with cough: a systematic review and Meta-analysis. Allergy Asthma Immunol Res. 2019;11(6):856–870.

[52] Morice AH, McGarvey L, Pavord I.; British Thoracic Society Cough Guideline Group. British thoracic society cough guideline G: Recommendations for the management of cough in adults. Thorax. 2006;61(Suppl 1):i1–i24.

[53] Visca D, Beghe B, Fabbri LM, et al. Management of chronic refractory cough in adults. Thorax. 2006;61(Suppl 1):i1–i24.

[54] Gao J, Wu F. Association between fractional exhaled nitric oxide, sputum induction and peripheral blood eosinophil in uncontrolled asthma. Allergy Asthma Clin Immunol. 2018;14:21.

[55] Sabatelli L, Seppala U, Sastre J, et al. Cost-effectiveness and budget impact of routine use of fractional exhaled nitric oxide monitoring for the management of adult asthma patients in Spain. J Investig Allergol Clin Immunol. 2017;27(2):89–97.

[56] Buendia JA, Acuna-Cordero R, Rodriguez-Martinez CE. Cost utility of fractional exhaled nitric oxide
monitoring for the management of children asthma. Cost Eff Resour Alloc. 2021;19(1):33.

[57] Leeflang MM, Deeks JJ, Gatsonis C, et al.; Cochrane Diagnostic Test Accuracy Working Group. Cochrane diagnostic test accuracy working G: Systematic reviews of diagnostic test accuracy. Ann Intern Med. 2008;149(12):889–897.

[58] Brenner H, Gefeller O. Variation of sensitivity, specificity, likelihood ratios and predictive values with disease prevalence. Statist Med. 1997;16(9):981–991.

[59] Soter S, Barta I, Antus B. Predicting sputum eosinophilia in exacerbations of COPD using exhaled nitric oxide. Inflammation. 2013;36(5):1178–1185.

[60] Rio Ramirez MT, Juretschke Moragues MA, Fernandez Gonzalez R, et al. Value of exhaled nitric oxide (FeNO) and eosinophilia during the exacerbations of chronic obstructive pulmonary disease requiring hospital admission. Copd. 2018;15(4):369–376.

[61] Bersuch E, Graf F, Renner ED, et al. Lung function improvement and airways inflammation reduction in asthmatic children after a rehabilitation program at moderate altitude. Pediatr Allergy Immunol. 2017;28(8):768–775.

[62] Clini E, Bianchi L, Foglio K, et al. Effect of pulmonary rehabilitation on exhaled nitric oxide in patients with chronic obstructive pulmonary disease. Thorax. 2001;56(7):519–523.