



Evaluation of exhaled nitric oxide's ability to predict methacholine challenge in adults with nonobstructive spirometry

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ABSTRACT

Background: Compared with methacholine challenge, exhaled nitric oxide is less expensive, is performed as a simpler point-of-care maneuver, and may provide treatment guiding information.

Objective: To determine whether exhaled nitric oxide levels can predict the outcome of methacholine challenge in patients without obvious baseline obstruction.

Methods: This retrospective database and medical record review included adults presenting to a tertiary care center from November 1, 2009, through August 31, 2013, who underwent methacholine challenge and exhaled nitric oxide measurement within 2 weeks of each other. Database extraction identified age, sex, body mass index, baseline corticosteroid use, testing indication, forced expiratory volume in 1 second (FEV₁), and testing results. Individuals taking corticosteroid-containing medication or who had an FEV₁ less than the lower limit of normal at baseline were excluded.

Results: A total of 1,322 individuals were identified, with 774 meeting the eligibility criteria. A total of 123 methacholine challenges (16%) had positive results. Fractional exhaled nitric oxide was statistically higher in those with positive methacholine challenge results (mean [SD], 41.9 [54.5] ppb vs 25.2 [19.7] ppb; $P < .01$). Receiver operating characteristic curve revealed an area under the curve of 0.572 ($P < .01$), suggesting a poor correlation between exhaled nitric oxide level and methacholine challenge outcome. Fractional exhaled nitric oxide of greater than 50 ppb has a sensitivity, specificity, positive predictive value, and negative predictive value of 12%, 89%, 17%, and 84%, respectively, for a positive methacholine challenge result.

Conclusion: In this large, retrospective cohort of corticosteroid-naïve pulmonary patients with normal FEV₁ on baseline spirometry, exhaled nitric oxide poorly predicts the outcome of a methacholine challenge.

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Introduction

Patients commonly present with symptoms of dyspnea, cough, wheeze, and chest tightness concerning for airway obstruction and hyperresponsiveness. Detection and severity assessment of airway obstruction require spirometry.^{1,2} If the spirometry reveals no obvious airway obstruction, an assessment for airway hyperresponsiveness with methacholine is often performed.³ Inhaled methacholine is a direct stimulator of smooth muscle and has a high negative predictive value (ie, a lack of response to methacholine practically excludes asthma in symptomatic patients).⁴ Although safe and commonly performed, methacholine challenges are time-consuming; require serial drug administrations,

serial spirometric measurements, and a skilled respiratory technician; and can be technically challenging for patients.³

Exhaled nitric oxide is elevated in some asthma phenotypes and eosinophilic bronchitis.⁵ Nitric oxide synthase is up-regulated in bronchial epithelial damage because of eosinophilic bronchitis, whether asthma or non-asthma related.^{6,7} Exhaled nitric oxide is being used in the clinical setting, and several organizations have supported its use in the setting of diagnosis and managing asthma.^{5,8,9} Exhaled nitric oxide is inexpensive, requires no medication administration, does not require a respiratory technician, and is easy for the patients to perform.¹⁰ In addition, an elevated fractional exhaled nitric oxide may be predictive of response to inhaled corticosteroids (ICSs).^{5,11}

Previous work has suggested a correlation between exhaled nitric oxide levels and bronchial hyperresponsiveness, particularly in the setting of obstructive spirometry.^{12,13} Patients presenting with pulmonary symptoms without obvious obstruction on

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spirometry present a clinical challenge. Given exhaled nitric oxide's time-efficient (point of care, simple to perform) and cost-effective profile compared with methacholine challenge, we are interested in exploring whether exhaled nitric oxide can be leveraged in a diagnostic algorithm in patients presenting with pulmonary symptoms without obvious obstruction on spirometry. We had an exploratory hypothesis that exhaled nitric oxide outcomes would predict the presence or absence of airway hyperresponsiveness by methacholine challenge. To explore this hypothesis, we performed a retrospective study of adults presenting with pulmonary symptoms with a forced expiratory volume at 1 second (FEV₁) greater than the lower limit of normal (LLN), who were tested with measurement of exhaled nitric oxide and methacholine challenge.

Methods

Study Participants

A retrospective cohort study was designed enrolling adults (≥ 18 years old) who underwent methacholine challenge and exhaled nitric oxide measurement within 2 weeks of each other. The patients were identified from the Mayo Clinic pulmonary function laboratory database for tests performed from November 1, 2009, through August 31, 2013. Medical record abstraction was performed to obtain age, sex, body mass index, exhaled nitric oxide level, and methacholine challenge result. The *International Classification of Diseases, Ninth Revision (ICD-9)* code was used to determine indication: asthma, code 493; cough, code 786.2; dyspnea, code 786.0; and shortness of breath, code 786.05. Nonrespiratory disease test indications were classified as other (which represented $<5\%$ of the cohort). Patients with FEV₁ less than LLN were excluded. Patients taking ICSs or systemic glucocorticosteroids were excluded because corticosteroids can decrease the exhaled nitric oxide level and affect methacholine challenge responses.^{4,14–16} The study was conducted in accordance with Mayo Clinic Institutional Review Board policy and was approved with an exempt status under study 14-006851. Patients without documented consent for research participation were excluded ($<5\%$ of eligible candidates).

Diagnostic Testing Protocols

Exhaled nitric oxide was measured by chemiluminescence (Niox MINO, Aerocrine Inc, Morrisville, North Carolina) as recommended by the manufacturer. This protocol was concordant with the American Thoracic Society guideline.¹⁷ Exhaled nitric oxide levels were reported in parts per billion.

Methacholine challenge was performed with a dosimeter after an abbreviated methacholine challenge protocol.^{3,18} A single breath of 25 mg/mL of methacholine was administered by dosimeter (KoKo, Ferraris Respiratory, Louisville, Colorado) set to 0.8 seconds and resulted in a delivered dose of 9 μ L per actuation (225 μ g of methacholine per dose). Forced vital capacity maneuver was performed 3 minutes after methacholine dosing. If the FEV₁ did not decrease by 20% or more with the first dose, 4 additional breaths of 25 mg/mL of methacholine were administered. A positive methacholine challenge result was defined by a decrease in FEV₁ of 20% or more.

Outcome Measures

The primary outcome of this study was the correlation between exhaled nitric oxide level and methacholine challenge response at cut points of 25, 39, and 50 ppb (sensitivity, specificity, predictive values, and likelihood ratios). A receiver operating curve (ROC) was constructed to determine the overall performance profile of exhaled nitric oxide in predicting methacholine challenge outcome and to determine the optimal cut point for exhaled nitric oxide in doing so.

Statistical Analysis

JMP, version 10.0 (SAS Institute Inc, Cary, North Carolina), and MedCalc, version 13.1 (MedCalc Software, Mariakerke, Belgium), were used for statistical analysis. Methacholine challenge response was analyzed dichotomously. The ROC was used to compare the continuous variable of the exhaled nitric oxide with a binary outcome of the methacholine challenge (positive or negative) by plotting false-positive results against sensitivity. The area under the curve (AUC) was calculated to estimate the predictive value to exhaled nitric oxide, with an AUC of 1 reflecting a perfect test and 0.5 suggesting the test results reflect random chance. Two-tailed Fisher exact testing was used when comparing categorical outcomes of exhaled nitric oxide levels at current published cut-off points (≥ 25 , ≥ 39 , and ≥ 50 ppb) and methacholine challenge outcome. $P < .05$ was used to determine statistical significance.

Results

The consort diagram shown in Figure 1 outlined the study cohort selection process. Of the 1322 patients identified as having an exhaled nitric oxide and methacholine challenge within 2 weeks, 774 ICS-naïve patients met inclusion criteria. Table 1 gives the demographic characteristics of the study cohort, which was predominately white (97.8%) and female (64.9%). Mean (SD) age was 54.4 (15.9) years. Most patients had testing indications of cough, dyspnea, or asthma. Fifty-eight patients had testing indications for each test that differed.

Of the 774 patients, 123 (15.9%) had a positive methacholine challenge result. Of this group, the arithmetic mean (SD) of the exhaled nitric oxide level was 41.9 (54.5) ppb, with a median of 25 ppb. Those with a negative methacholine challenge result (652 [84.1%]) had a statistically significant lower mean (SD) exhaled nitric oxide level of 25.2 (19.7) ppb ($P < .01$), with a median of 20 ppb. There was no statistical difference based on testing indication in methacholine challenge outcome based on Pearson χ^2 test (6.558, $P = .26$) or mean exhaled nitric oxide based on pairwise testing. See eTable 1 and eTable 2 in the supplementary material for full details.

Using the different published recommended exhaled nitric oxide cutoff points (≥ 25 , ≥ 39 , and ≥ 50 ppb), contingency tables are shown in Table 2. Using 2-tailed Fisher exact testing, the only cutoff

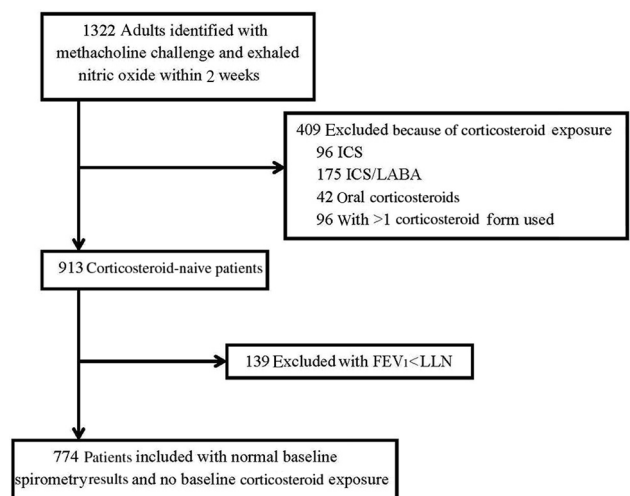


Figure 1. Consort diagram showing protocol to identify 774 steroid-naïve patients with normal baseline spirometry results who received exhaled nitric oxide and methacholine challenge within a 2-week time frame. ICS indicates inhaled corticosteroid; ICS/LABA, combination inhaled corticosteroid and long-acting β -agonist; FEV₁, forced expiratory volume in 1 second; LLN, lower limit of normal.

Table 1

Demographic Characteristics for 774 Corticosteroid-Naïve Adults With Normal Baseline Spirometry Results

Characteristic	Finding
Age, mean (SD), y	54.4 (15.9)
Sex, M/F, No. (%)	272/502 (35.1/64.9)
Race, No. (%)	
White	757 (97.8)
Black	11 (1.4)
Asian	6 (0.8)
BMI, mean (SD)	28.9 (6.1)
MCH, No. (%)	
Asthma	81 (10.5)
Cough	431 (55.7)
Dyspnea	113 (14.6)
Shortness of breath	43 (5.6)
Other	106 (13.7)
FeNO test, No. (%)	
Asthma	85 (11.0)
Cough	422 (54.5)
Dyspnea	111 (14.3)
Shortness of breath	39 (5.0)
Other	117 (15.1)

Abbreviations: BMI, body mass index (calculated as the weight in kilograms divided by square of height in meters); FeNO, exhaled nitric oxide; MCH, methacholine challenge.

point that had a statistically significant association was the 25-ppb cutoff point.

The diagnostic performance characteristics of each of the exhaled nitric oxide cutoffs are given in Table 3. As expected, the sensitivity of exhaled nitric oxide decreases as the cutoff points increases and, inversely, the specificity increases with increased cutoff points. The only cutoff point that has statistically significance likelihood ratios is the 25-ppb cutoff point, with a positive likelihood ratio of 1.38 (95% CI, 1.13–1.69) and a negative likelihood ratio of 0.77 (95% CI, 0.64–0.94), although the clinical relevance of these modest likelihood ratio is low.¹⁹

A ROC, plotting false-positive results against sensitivity, is shown in Figure 2. The AUC is 0.572 with $P < .01$. The optimal intercept point for exhaled nitric oxide compared with methacholine challenge is 38 ppb.

Discussion

This study evaluates the ability of exhaled nitric oxide to predict the outcome of a methacholine challenge in a real-world setting of corticosteroid-naïve adults presenting with pulmonary symptoms without a significant decrease in FEV₁ on baseline spirometry. Mean exhaled nitric oxide level is elevated in patients with positive methacholine challenge results. Despite this correlation, the level of exhaled nitric oxide only weakly predicts the outcome of a

Table 2Contingency Tables for Different Cutoff Points for FeNO Testing Compared With Methacholine Challenge in 774 Corticosteroid-Naïve Adults With Normal Baseline FEV₁ on Spirometry

FeNO, ppb	Methacholine challenge result		
	No. of positive results	No. of negative results	<i>P</i> value ^a
≥25	63	241	<.01
<25	60	410	
≥39	22	135	.54
<39	101	516	
≥50	15	71	.64
<50	108	580	

Abbreviations: FeNO, exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second.

^aTwo-tailed Fisher exact test.

Table 3Diagnostic Performance of Literature Proposed Cutoff Values for FeNO Compared With Methacholine Challenge in 774 Corticosteroid-Naïve Adults With Normal Baseline FEV₁ on Spirometry

Variable	FeNO threshold (95% CI), ppb		
	<25	>39	>50
Sensitivity, %	51.2 (42.1–60.3)	17.9 (11.6–25.8)	12.2 (7.0–19.3)
Specificity, %	63.0 (59.1–66.7)	79.3 (75.9–82.3)	89.1 (86.4–91.4)
Positive predictive value, %	20.7 (16.3–25.7)	14.0 (9.0–20.4)	17.4 (10.1–27.1)
Negative predictive value, %	87.2 (83.9–90.1)	83.6 (80.5–86.5)	84.3 (81.4–86.9)
Positive likelihood ratio	1.38 (1.13–1.69)	0.86 (0.57–1.30)	1.12 (0.66–1.89)
Negative likelihood ratio	0.77 (0.64–0.94)	1.04 (0.95–1.14)	0.99 (0.92–1.06)

Abbreviations: CI, confidence interval; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second.

methacholine challenge. The results of this exploratory study informs our understanding of how the point-of-care, office-based measurement of exhaled nitric oxide may be leveraged in the diagnostic algorithm for patients with pulmonary symptoms.

In clinical practice, exhaled nitric oxide is more frequently being used.^{1,5,8} In 2011, the American Thoracic Society released guidelines that recommend measurement of exhaled nitric oxide to diagnose and guide treatment in patients with suspected eosinophilic inflammation of the airways.⁵ In 2014, the National Institute for Health and Care Excellence recommended the use of fractional exhaled nitric oxide in the diagnosis of asthma.⁸

Exhaled nitric oxide measures a different aspect of airway physiology compared with methacholine challenge and measures different underlying pathophysiologic mechanisms. Methacholine is a direct stimulator of airway smooth muscle receptors.³ The exact mechanism for pathologic bronchial hyperresponsiveness is not known, and it is not clearly correlated with eosinophilic airway inflammation.²⁰ Elevated levels of nitric oxide come from injured bronchial epithelial cells associated with eosinophilic inflammation.²¹ Inducible nitric oxide synthase expression is increased in patients with asthma and decreases with treatment with corticosteroids. Our findings underscore that the pathophysiologic processes associated with an elevated exhaled nitric oxide are different from those governing hyperresponsiveness. Our study suggests

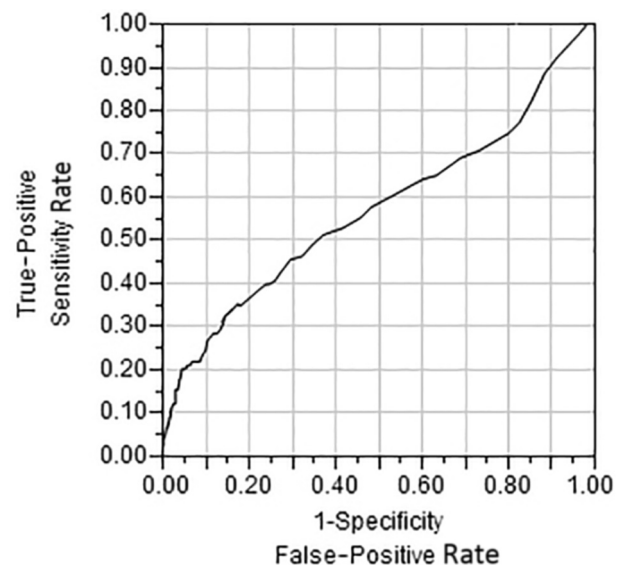


Figure 2. Receiver operating curve plotting true-positive rates vs false-positive rate in a cohort of 774 adult steroid-naïve patients with normal baseline spirometry results who underwent exhaled nitric oxide and methacholine challenge within 2 weeks of each other.

these tests provide complementary information during the evaluation of a patient with undifferentiated pulmonary symptoms.

In addition, the clinical decision making based on these 2 tests is different. Methacholine challenge is most useful when it provides a negative data point (ie, helpful in ruling out asthma), whereas exhaled nitric oxide appears to be most helpful as a positive data point. When the pretest probability for asthma is between 30% and 70%, a negative methacholine challenge result has a high negative predictive value (>90%), suggesting that the patient very likely does not have asthma (>90%).^{3,22,23} The positive predictive value of a positive methacholine challenge result is significantly lower. In regard to predicting response to ICSs, in chronic cough, for example, a positive methacholine challenge result has a positive predictive value of responding to corticosteroids of only 64% (95% CI, 48%–77%).²⁴ In contrast, an elevated exhaled nitric oxide (≥ 35 ppb) has an 88% (95% CI, 75%–95%) positive predictive value and a likelihood ratio of 4.9 (95% CI, 2.2–10.9) in predicting whether chronic cough will respond to ICSs in the same study.

In another study of 52 patients with undiagnosed respiratory symptoms, an exhaled nitric oxide level of 47 ppb or higher had a greater response (based on change in symptoms, peak flows, spirometry, or airway hyperresponsiveness to adenosine) to ICSs compared with those below this cutoff.²⁵ An elevated exhaled nitric oxide may prove to be overall a more efficient point-of-care diagnostic intervention compared with methacholine challenge. By drawing this distinction, we suggest that exhaled nitric oxide may be a more appropriate initial test after normal spirometry results because it potentially could be more useful in clinical decision making. A prospective trial to test this hypothesis is needed.

Elevations in exhaled nitric oxide and positive methacholine challenge results are likely driven by different underlying mechanisms and hence are poorly correlated. The ROC shows that exhaled nitric oxide is a relatively poor test in predicting the outcome of a methacholine challenge. Despite the overall poor performance, the AUC was statistically significant, suggesting that although an overall poor predictor of methacholine challenge outcome, there is some predictive value of an exhaled nitric oxide, perhaps in a yet-to-be-defined subset of the population. It is possible that for a certain phenotype of asthma or another pulmonary disease, exhaled nitric oxide may predict methacholine challenge results. Also of interest, ROC analysis yielded an optimal cut point for exhaled nitric oxide at 38 ppb. This value is very similar to the population-based cut point that has been suggested of 39 ppb.²⁶

Beyond the differences in clinical information provided by these 2 tests, cost profile and practice efficiency differ significantly. Methacholine challenges are typically billed via the *Common Procedural Terminology* as group: 94010 (baseline spirometry), 95070 (inhalation bronchial challenge testing), 95070 (bronchospasm provocation evaluation), and J7674 (drug reimbursement code).²⁷ Using the Medicare Physician Fee Schedule search tool, the national average reimbursed by Medicare for these codes is \$127.71 per evaluation.²⁸ Exhaled nitric oxide is billed using *Common Procedural Terminology* code 95012 at a national average of \$19.65 per evaluation by Medicare based on the same open access tool. Methacholine challenge also takes significantly longer to complete. According to the *Uniform Reporting Manual for Respiratory Care*, a methacholine challenge takes an average of 76.56 minutes to complete compared with 14.89 minutes for an exhaled nitric oxide.²⁹ On the basis of these resources, methacholine challenge costs 6.5 times more and takes 5 times as long to perform than exhaled nitric oxide.

This study has several limitations. As a retrospective study enrolling patients based on previous testing, the cohort represents a heterogeneous group of patients and a variety of underlying disease states. The broad inclusion of symptoms and suspected disease states is an accepted condition of this analysis. Although

this makes it difficult to apply these results to a specific disease state (ie, chronic cough, asthma), this study was a pragmatic, real-world assessment of the clinical use of 2 commonly used tests in respiratory medicine. We excluded all patients with an FEV₁ less than the lower limit of normal in an attempt to capture those who did not have baseline obstruction or restriction. This was selected as a practical marker that could be collected from the large pulmonary database. There may be other useful parameters that were not captured in our data extraction, such as the forced expiratory flow between 25% and 75%.^{30,31}

Retrospective studies are subject to observer bias. In this study, the objective nature of the outcomes of interest (levels of exhaled nitric oxide and >20% decrease with methacholine challenge) limits this bias. Information on baseline demographics, such as medication use (a key exclusion criteria), although deemed reliable by the investigators, was not confirmed with independently verifiable sources, such as pharmacy records.

Finally, this cohort was identified at a tertiary care center, whose patient population may not be generalizable to other patient populations.^{32,33} Similarly, the standard methacholine challenge performed at the Mayo Clinic is an abbreviated dosing protocol as described in the Methods section. Although this method delivers an adequate overall dose of methacholine and is validated for safety and reproducibility, it differs from the more commonly used prolonged dosing protocol.^{3,18,34,35} A prospective study that more completely documents baseline disease characteristics and medication use and recruits study participants from a diverse patient population is needed to address these potential confounders and confirm our findings. Specific phenotypes (ie, those with identified allergic sensitivities) may affect an exhaled nitric oxide ability to predict the outcome of a methacholine challenge.

This study was designed to determine the ability of exhaled nitric oxides to predict the outcome of a methacholine challenge in corticosteroid-naïve pulmonary patients with normal FEV₁ on baseline spirometry. In this large retrospective cohort, exhaled nitric oxide does not strongly predict of the outcome of a methacholine challenge, either positive or negative. Although our hypothesis was disproved, we believe this finding is informative for clinicians and future research directions. When faced with a patient with respiratory symptoms and normal baseline spirometry, it appears that the information gleaned from exhaled nitric oxide and methacholine challenge testing is not redundant but can be distinct data points for the clinician. Placing this study's findings in the current evidence base and economic context, we suggest that evaluating exhaled nitric oxide provides a potential treatment guiding data point (eg, an elevated level suggests responsiveness to ICSs) and can be performed more quickly and at lower cost. If confirmed in prospective studies, this overall profile may make exhaled nitric oxide testing an attractive first-line test to consider in this clinical context. Future prospective studies are needed to validate this hypothesis and would benefit from the addition of phenotypic features (such as presence of atopy), patient outcomes, and cost-effectiveness analyses.

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Supplementary Data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anaai.2016.07.032>.

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eTable 1

Fractional Exhaled Nitric Oxide and Methacholine Challenge Outcome by Indication for 774 Corticosteroid-Naive Adults With Normal Baseline Spirometry Results

Indication	No. of patients	FeNO, mean (SE) [95% CI], ppb	Positive methacholine challenge result, No. (%) ^a
Cough	416	28.68 (1.42) [25.9–31.46]	67 (16.1)
Dyspnea	98	27.85 (2.92) [22.12–33.57]	14 (14.3)
Other	96	28.78 (2.95) [23–34.56]	21 (21.9)
Asthma	69	23.77 (3.47) [16.95–30.59]	12 (17.4)
Different indications	58	24.59 (3.79) [17.15–32.03]	4 (6.9)
Shortness of breath	37	28.62 (4.74) [19.31–37.94]	5 (13.5)

^aPearson χ^2 - 6.558 ($P = .26$).**eTable 2**

Pairwise Table Comparing Exhaled Nitric Oxide Level Based on Indication for 774 Corticosteroid-Naive Adults With Normal Baseline Spirometry Results

Indication	Difference, mean (SE) [95% CI]	P value ^a
Asthma		
Cough	4.912173 (3.751457) [–2.4522 to 12.2765]	.19
Different	0.818091 (5.14119) [–9.2744 to 10.91054]	.87
Dyspnea	4.078823 (4.535456) [–4.8245 to 12.98218]	.37
Shortness of breath	4.853506 (5.880684) [–6.6906 to 16.39763]	.41
Other	5.013134 (4.554934) [–3.9285 to 13.95473]	.27
Cough		
Different	4.094082 (4.045096) [–3.8467 to 12.03484]	.31
Dyspnea	0.83335 (3.240576) [–5.5281 to 7.19479]	.80
Shortness of breath	0.058667 (4.951102) [–9.6606 to 9.77797]	.99
Other	0.100962 (3.267782) [–6.3139 to 6.51581]	.98
Different indication		
Other	4.195043 (4.799667) [–5.227 to 13.61707]	.38
Dyspnea		
Different	3.260732 (4.781185) [–6.125 to 12.64647]	.495
Shortness of breath	0.774683 (5.568693) [–10.157 to 11.70635]	.89
other	0.934311 (4.144313) [–7.2012 to 9.06984]	.82
Shortness of breath		
Different	4.035415 (6.072217) [–7.8847 to 15.95553]	.51
Other	0.159628 (5.584569) [–10.8032 to 11.12246]	.98

^a P value based on pairwise t test.