Peer Review File

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Reviewer A

Comment 1: Thank you for work. Relevant to lung function testing. Limitations of study, which are clearly stated, mainly related to lack of definitive highly accurate diagnostic test for small airway disease and inherent properties of routine PFTs.

Reply 1: Thank you for your encouragement. As spirometry is a widely accepted and established method for assessing lung function and the results are relatively straightforward to interpret for clinicians, we aimed to provide the reference data and enable its use in clinical practice. Differences or collaboration between spirometry and more advanced tests for identifying small airway disease are worth discussed in future studies. Once again, we are very grateful for your valuable insights.

Reviewer B

Comment 1: Thank you for the opportunity to review this well-written manuscript. The authors used well-defined statistical methods to establish reference equations for FEF50 and FEF70 in the Chinese population. Although reference equations for FEF50 and FEF75 carry academic merit, their clinical use is controversial and often limited. The limited clinical relevance for FEF50 and FEF75 significantly decreases the impact of this manuscript.

Reply 1: We appreciate your encouragement and valuable comments on our study. We also agree that the clinical relevance of FEF_{50} and FEF_{75} is indeed controversial and often limited in practice. However, we believe that the academic value of defining these reference equations should not be underestimated, as they provide a more comprehensive understanding of lung function and can serve as a basis for further research.

Comment 2: The definition of positive bronchodilator response has changed in the 2021 ATS guideline. Does using the new guideline change the sample of possible asthma patients?

Reply 2: Thank you for pointing out this. The result of bronchodilator responsiveness (BDR) test is one of the important criteria for the diagnosis of asthma. In our study, patients with asthma were

included by referring to the medical history, symptoms and standards in Global Initiative for Asthma (GINA) guidelines as assessed by physicians. In clinical practice, a positive result of BDR is more commonly observed in patients who have not previously received treatment for asthma. We neglected the fact that many patients with asthma may have a negative response to the bronchodilator in the previous version of our manuscript. In our study, a total of 2,459 (2,459/4,356) and 1,206 (1,206/4,356) cases had positive results of BDR according to the 2005 criteria and 2021 criteria. However, after careful review of the manuscript, we thought that it was more appropriate to address this issue by no longer limiting the inclusion criteria for patients with suspected asthma based on the results of BDR test. Therefore, results related to this issue have changed. Hope our response and revisions can meet your satisfaction.

Changes in the text:

Abstract: A total of 4,356 patients with suspected asthma (51.1% female; a mean age of 45.4 years) and 6,558 patients with suspected COPD (10.1% female; a mean age of 65.0 years) were included. The present equations defined 95.7% and 99.9% of SAD in these patients. After bronchodilator inhalation, greater mean improvement rates in small airway indices were observed both in patients with suspected asthma (mean, SD = 48%, 47%) and in patients with suspected COPD (mean, SD = 20%, 30%) ($p < 0.05$). **(see Page 3 - 4, line 58 - 63)**

Methods- Population: Data from patients who underwent bronchodilator responsiveness (BDR) test between January 1st, 2017 to April 12th, 2022, in the center of PFT of First Affiliated Hospital of Guangzhou Medical University were extracted. Only the first report was selected for subjects who completed a BDR test more than once. The data was primarily reviewed using the standard terminology of the International Classification of Diseases Volume 10 (ICD-10) and the Systematised Nomenclature of Medicine Clinical Terms (SNOMED). In the review of electronic medical records, asthma was diagnosed based on the Global Initiative for Asthma (GINA) guidelines and COPD was defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines (1, 2). Patients with interstitial lung disease, pulmonary tuberculosis, obliterative bronchiolitis, lung tumors, pneumoconiosis, bronchiectasis, and asthma-COPD overlap were excluded. **(see Page 8, line 132 - 141)**

Results- Information on the bronchodilator responsiveness test dataset: A total of 4,356 patients with clinical suspicion of asthma and 6,558 patients with suspected COPD and a FEV_1/FVC ratio of less than 0.7 after bronchodilation were included. The suspected asthma group included 2,224 (51.1%) female patients, with the mean age of 45.4 years (range 6-80 years) and the height of 107.7cm to 190.5cm. The COPD group included 665 (10.1%) female patients, with the mean age of 65.0 years (range 36-80 years) and the height of 132.5cm to 188.5cm. **Table 3** shows detailed information. **(see Page 15, line 227 - 232)**

Table 3. Characteristics of patients with suspected asthma and COPD

Characteristics	**Suspected Asthma group (n=4,356) ***	**Suspected COPD group (n=6,558) ****
Sex		
female	2,224 (51.1)	665 (10.1)
male	2,132 (48.9)	5,893 (89.9)
Age (years)	45.4 (17.7)	65.0 (7.9)
Weight (kg)	58.3 (14.4)	59.1 (10.8)
Height (cm)	159.0 (11.6)	163.7 (7.0)
BMI (kg/m^2)	22.7 (4.1)	22.0 (3.5)
FEV_1 (L)		
baseline	1.6 (0.6)	1.2 (0.6)
after bronchodilator	1.8 (0.7)	1.3 (0.6)
FVC (L)		
baseline	2.7 (0.9)	2.6 (0.8)
after bronchodilator	2.9 (1.0)	2.8 (0.8)
FEV_1/FVC (%)		
baseline	58.5 (12.9)	45.7 (12.1)
after bronchodilator	59.0 (22.1)	45.0 (16.9)
FEF_{75} (L/s)		
baseline	0.4 (0.3)	0.2 (0.1)
after bronchodilator	0.5 (0.4)	0.2 (0.1)
FEF_{50} (L/s)		

baseline	1.1 (0.7)	0.6 (0.4)
after bronchodilator	1.3 (1.0)	0.7 (0.5)
MMEF (L/s)		
baseline	0.8 (0.5)	0.5 (0.3)
after bronchodilator	1.0 (0.8)	0.5 (0.4)

Continuous data are described as mean (standard deviation); Categorical data are presented as frequency (percentage). Sample size: FEV_1/FVC (%), FEF_{75} (L/s), FEF_{50} (L/s) and MMEF (L/s) were available in 4,002, 3,778, 3,837 and 3,714 cases with suspected asthma and 6,151, 5,413, 5,945 and 5,456 cases with suspected COPD; *patients with clinical consideration for asthma, age ranges from 6 to 80 years old. **patients with suspected COPD and a FEV_1/FVC ratio of less than 0.7 after bronchodilation, age ranges from 36 to 80 years old. Abbreviations: FEF_{50}, forced expiratory flow at 50% of FVC; FEF_{75}, forced expiratory flow at 75% of FVC; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; FEV_1/FVC, the ratio of forced expiratory volume in 1 second to forced vital capacity; MMEF, maximal mid-expiratory flow.

Results- Patients with suspected asthma: In the BDR dataset, a total of 3,360 patients with suspected asthma had all small airway indices available at baseline. According to the present equations, the measured values were below 65% of the predicted value (94.6%, 95.8% and 95.7% of 3,360 cases had FEF_{50}, FEF_{75} and MMEF < 65% of the predicted value) or LLN (93.3%, 90.0% and 94.2% of 3,360 cases had FEF_{50}, FEF_{75} and MMEF < LLN) in the majority of cases. SAD was defined in 3,214 cases (95.7%) when 65% of the predicted value was used as the threshold **(Figure 2 & 3)**, and a total of 3,321 asthma patients (98.8%) were classified as SAD when at least two of the FEF_{50}, FEF_{75}, and MMEF parameters dropped to 80% below the predicted value ($p < 0.05$). Compared to the LLN classification as a gold standard, both 80% (sensitivity = 100.0%, accuracy = 95.0%) and 65% (sensitivity = 99.9%, accuracy = 98.1%) of the predicted value as a threshold had high sensitivity and accuracy. However, the false positive rate reached 81.2% when 80% of the predicted value was used as the threshold, while it was 30.4% when 65% of the predicted value was used as the threshold **(Table 4)**. **(see Page 17, line 235 - 247)**

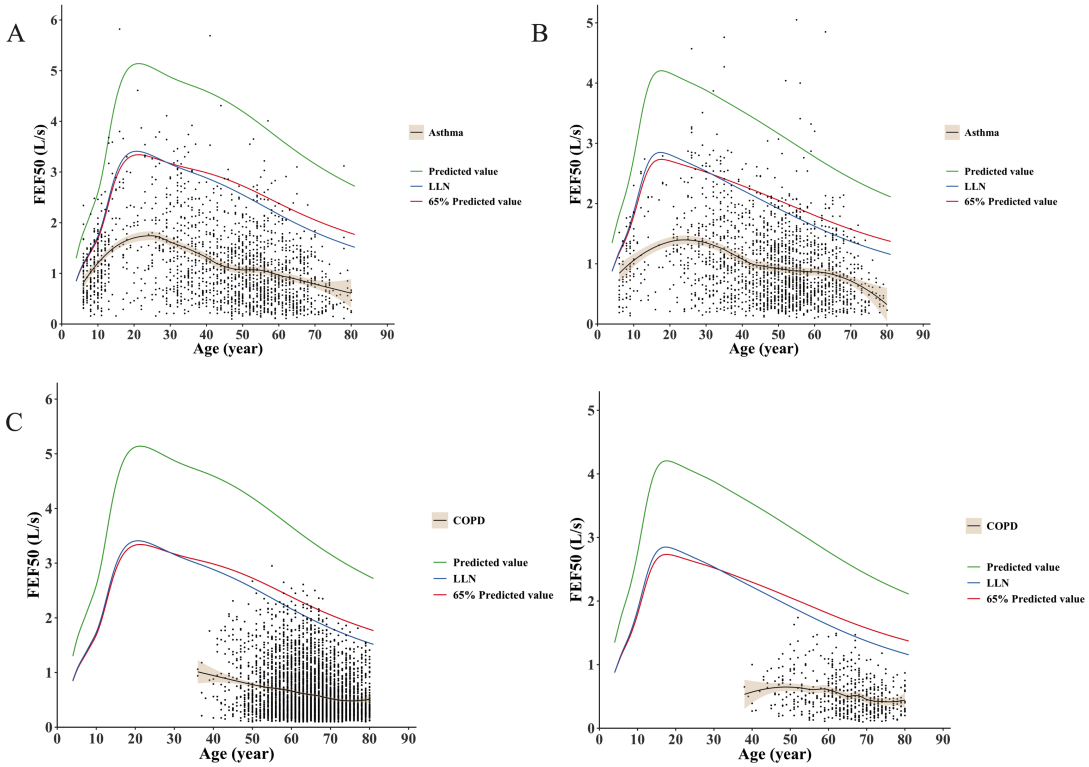

Figure 2. The measured values of FEF_{50} in patients compared with the predicted values, 65% of predicted values and LLN; Black dots represent the measured values of FEF_{50} in patients; black lines are generated by the smoothing splines models; green lines, blue lines, and red lines represent the predicted values, LLN, and 65% of predicted values, respectively, generated by the present reference equations. A: male patients with suspected asthma, B: female patients with suspected asthma, C: male patients with suspected COPD, D: female patients with suspected COPD

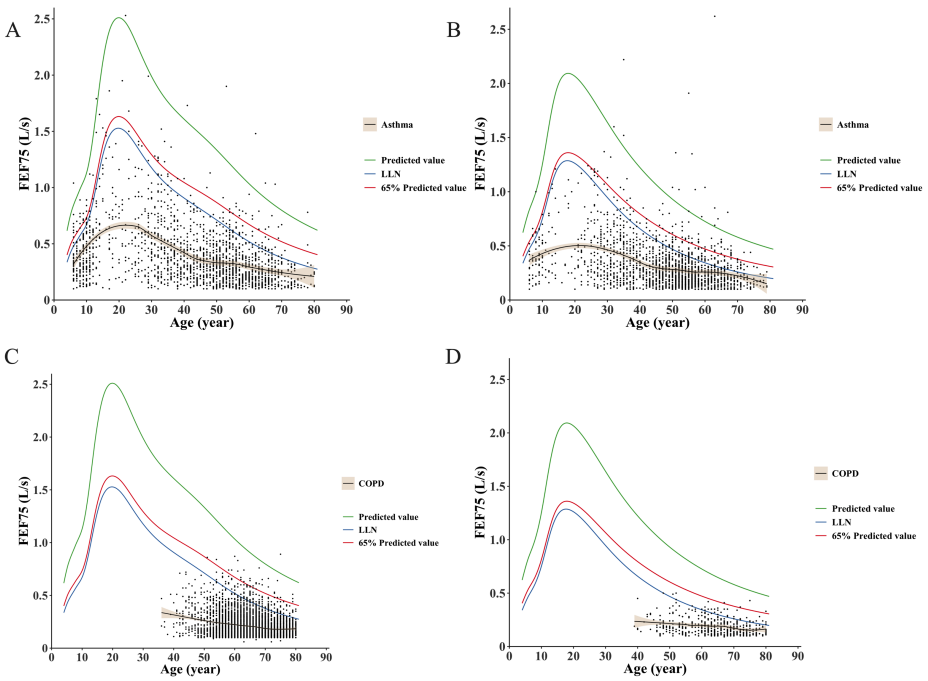

Figure 3. The measured values of FEF_{75} in patients compared with the predicted values, 65% of predicted values and LLN; Black dots represent the measured values of FEF_{75} in patients; black lines are generated by the smoothing splines models; green lines, blue lines, and red lines represent the predicted values, LLN, and 65% of predicted values, respectively, generated by the present reference equations. A: male patients with suspected asthma, B: female patients with suspected asthma, C: male patients with suspected COPD, D: female patients with suspected COPD

Table 4. The performance of using 80% and 65% of the predicted value as the threshold to calculate the proportion of small airway dysfunction compared to the LLN classification as the gold standard

Variables	**Suspected Asthma group***		**Suspected COPD group****	
	80% of the predicted value	65% of the predicted value	80% of the predicted value	65% of the predicted value
Calculating the proportion of	98.8%	95.7%	100.0%	99.9%

small airway dysfunction					*: All
Sensitivity	100.0%	99.9%	100.0%	100.0%	
Specificity	18.8%	69.6%	0.0%	15.4%	
False Negative Rate	0.0%	0.0%	0.0%	0.0%	
False Positive Rate	81.2%	30.4%	100.0%	84.6%	
Accuracy	95.0%	98.1%	99.2%	99.3%	

small airway indices were available at baseline in a total of 3,360 patients with suspected asthma. **: All small airway indices were available at baseline in a total of 4,674 patients with suspected

Characteristics	Full sample	Male	Female	Aged < 14 years	Aged ≥ 14 years
large airway	13 (13)	13 (13)	12 (13)	15 (14)	13 (13)
small airway	48 (47)*	49 (47)*	48 (46)*	68 (52)*	46 (46)*
FEV_1	19 (16)	20 (16)	18 (16)	22 (17)	18 (16)
FEV_1/FVC	11 (10)	11 (10)	10 (10)	16 (10)	10 (10)
FVC	9 (11)	10 (10)	9 (11)	8 (10)	9 (11)
FEF_{75}	54 (59)	55 (60)	54 (58)	75 (60)	52 (58)
FEF_{50}	45 (38)	46 (38)	44 (38)	64 (47)	43 (37)
MMEF	46 (40)	47 (40)	46 (39)	65 (47)	44 (38)

COPD and a FEV_1/FVC ratio of less than 0.7 after bronchodilation.

Results- Comparison of the improvement rates between small and large airway indices: After bronchodilator inhalation, the mean improvement rate in small airway indices (mean, SD = 48%, 47%) was greater than that in large airway indices (mean, SD = 13%, 13%) in patients with suspected asthma ($p < 0.05$). For large airway indices, the greatest mean improvement rate was observed in FEV_1 (mean, SD = 19%, 16%), while it reached about 50% for all small airway indices **(Table S7)**. **(see Page 19, line 261 - 266)**

Table S7. Comparison of the improvement rates between small and large airway indices in patients with suspected asthma

*$p < 0.05$, small airway parameters compared with large airway parameters.

Improvement rate = [(value$_{(post\text{-}BD)}$ - best value$_{(pre\text{-}BD)}$) / best value$_{(pre\text{-}BD)}$] × 100%.

References:

1. Global strategy for the diagnosis，management and prevention of chronic obstructive pulmonary disease 2023 report. Available from https://goldcopd.org/2023-gold-report/.
2. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2023. Available from: www.ginasthma.org.

Comment 3: In table 1, it will be more useful to list BMI rather than weight.

Reply 3: Thank you for your suggestion. We have added BMI in table 1 and 3.

Changes in the text:

Table 1. Characteristics of healthy individuals

Characteristics	**n=7,115**
Center	
north	2,956 (41.5)
south	4,159 (58.5)
Sex	
female	3,622 (50.9)
male	3,493 (49.1)
Age (years)	24.1 (17.9)
range	4 to 80
Height (cm)	151.4 (19.1)
range	95.0 to 190.0
BMI (kg/m^2)	20.1 (4.0)
MMEF (L/s), n = 6874	2.9 (1.2)
FEF_{50} (L/s), n = 7050	3.5 (1.4)
FEF_{75} (L/s), n = 7034	1.5 (0.7)

Continuous data are described as mean (standard deviation); Categorical data are presented as frequency (percentage); n: sample size. Abbreviations: FEF_{50}, forced expiratory flow at 50% of FVC; FEF_{75}, forced expiratory flow at 75% of FVC; MMEF, maximal mid-expiratory flow.

Table 3. Characteristics of patients with suspected asthma and COPD

Characteristics	**Suspected Asthma group (n=4,356) ***	**Suspected COPD group (n=6,558) ****
Sex		
female	2,224 (51.1)	665 (10.1)
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BMI (kg/m^2)	22.7 (4.1)	22.0 (3.5)
FEV_1 (L)		
baseline	1.6 (0.6)	1.2 (0.6)
after bronchodilator	1.8 (0.7)	1.3 (0.6)
FVC (L)		
baseline	2.7 (0.9)	2.6 (0.8)
after bronchodilator	2.9 (1.0)	2.8 (0.8)
FEV_1/FVC (%)		
baseline	58.5 (12.9)	45.7 (12.1)
after bronchodilator	59.0 (22.1)	45.0 (16.9)
FEF_{75} (L/s)		
baseline	0.4 (0.3)	0.2 (0.1)
after bronchodilator	0.5 (0.4)	0.2 (0.1)
FEF_{50} (L/s)		
baseline	1.1 (0.7)	0.6 (0.4)
after bronchodilator	1.3 (1.0)	0.7 (0.5)
MMEF (L/s)		

baseline	0.8 (0.5)	0.5 (0.3)
after bronchodilator	1.0 (0.8)	0.5 (0.4)

Continuous data are described as mean (standard deviation); Categorical data are presented as frequency (percentage). Sample size: FEV_1/FVC (%), FEF_{75} (L/s), FEF_{50} (L/s) and MMEF (L/s) were available in 4,002, 3,778, 3,837 and 3,714 cases with suspected asthma and 6,151, 5,413, 5,945 and 5,456 cases with suspected COPD; *patients with clinical consideration for asthma, age ranges from 6 to 80 years old. **patients with suspected COPD and a FEV_1/FVC ratio of less than 0.7 after bronchodilation, age ranges from 36 to 80 years old. Abbreviations: FEF_{50}, forced expiratory flow at 50% of FVC; FEF_{75}, forced expiratory flow at 75% of FVC; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; FEV_1/FVC, the ratio of forced expiratory volume in 1 second to forced vital capacity; MMEF, maximal mid-expiratory flow.

Comment 4: Are there any data regarding different ethnic groups within China and if this impacts their reference equations?

Reply 4: The dataset used to establish the reference equations for the small airway indices in this study is an extension of the dataset previously reported for other lung function indices. In the previous data collection period, paper records were manually entered into the computer system. Although a small number of non-Han individuals were included during data collection, this subgroup was excluded from the original analysis. And this data is now lost, which limited our ability for further analyses on different ethnic groups within China. This is indeed another limitation of our study. Although it is challenging to collect data from healthy individuals from multiple regions, it is important to update our data to improve the accuracy and application of the reference equations. Therefore, we added this as another limitation in our revised manuscript.

Changes in the text: Fourth, only the Han population in China was analyzed. Whether there are differences in the reference values for other ethnic groups remains unclear. **(see Page 20, line 294 - 295)**

Reviewer C

The establishment of the reference equations for FEF50 and FEF75 is very important issue. This manuscript is well described. However, I have a few questions as follows. I want that authors will describe these points in more detail.

Comment 1: How did authors exclude patients with ACO?

Reply 1: We thank the reviewer for the valuable comment. We apologize if that the details to define patient populations apart from the spirometry technical standards was unclear. In our study, patients with asthma-COPD overlap (ACO) were defined as patients having features of both asthma and COPD, following the commonly used definition from guidelines of the Global Initiative for Chronic Obstructive Lung Disease (GOLD). If the FEV_1/FVC ratio after bronchodilator inhalation is less than 0.7 and is associated with persistent airflow limitation that is either reversible or partially reversible, an ACO should also be considered. However, due to a lack of data to indicate the utility of the criteria for ACO, the diagnostic criteria are yet to be established. In the revised manuscript, we have added more details to understand the selection process for the patient population.

Changes in the text: Data from patients who underwent bronchodilator responsiveness (BDR) test between January 1st, 2017 to April 12th, 2022, in the center of PFT of First Affiliated Hospital of Guangzhou Medical University were extracted. Only the first report was selected for subjects who completed a BDR test more than once. The data was primarily reviewed using the standard terminology of the International Classification of Diseases Volume 10 (ICD-10) and the Systematised Nomenclature of Medicine Clinical Terms (SNOMED). In the review of electronic medical records, asthma was diagnosed based on the Global Initiative for Asthma (GINA) guidelines and COPD was defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines (1, 2). Patients with interstitial lung disease, pulmonary tuberculosis, obliterative bronchiolitis, lung tumors, pneumoconiosis, bronchiectasis, and asthma-COPD overlap were excluded. Cases with missing key values such as sex, age, height and baseline lung function were also excluded from some analyses. For patients with suspected COPD, only those with a FEV_1/FVC ratio of less than 0.7 after bronchodilation were included (1). **(see Page 8, line 132 - 143)**

References:

1. Global strategy for the diagnosis，management and prevention of chronic obstructive pulmonary disease 2023 report. Available from https://goldcopd.org/2023-gold-report/.
2. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention, 2023. Available from: www.ginasthma.org.

Comment 2: Patients with asthma had low pulmonary function data. Were these patients COPD or ACO rather than asthma?

Reply 2: We thank the reviewer for pointing this out. After careful review of the manuscript, we found that the reason for the lower pulmonary function data in asthma patients compared to those with COPD was related to the inclusion criteria in our study. Only patients with clinical consideration for asthma and a positive response to bronchodilators were included in the previous version, suggesting poorer baseline pulmonary function. We neglected the fact that many patients with asthma may have a negative response to the bronchodilator. Therefore, in this revision, we addressed this issue by no longer limiting the inclusion criteria for patients with suspected asthma based on the results of BDR test. Results related to this issue have also changed and higher pulmonary function data were observed in patients with suspected asthma. Once again, we are very grateful for your valuable insights.

Changes in the text: A total of 4,356 patients with clinical suspicion of asthma and 6,558 patients with suspected COPD and a FEV_1/FVC ratio of less than 0.7 after bronchodilation were included. The suspected asthma group included 2,224 (51.1%) female patients, with the mean age of 45.4 years (range 6-80 years) and the height of 107.7cm to 190.5cm. The COPD group included 665 (10.1%) female patients, with the mean age of 65.0 years (range 36-80 years) and the height of 132.5cm to 188.5cm. **Table 3** shows detailed information. **(see Page 15, line 226 - 232)**

Table 3. Characteristics of patients with suspected asthma and COPD

Characteristics	**Suspected Asthma group (n=4,356) ***	**Suspected COPD group (n=6,558) ****
Sex		
female	2,224 (51.1)	665 (10.1)
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BMI (kg/m^2)	22.7 (4.1)	22.0 (3.5)
FEV_1 (L)		

baseline	1.6 (0.6)	1.2 (0.6)
after bronchodilator	1.8 (0.7)	1.3 (0.6)
FVC (L)		
baseline	2.7 (0.9)	2.6 (0.8)
after bronchodilator	2.9 (1.0)	2.8 (0.8)
FEV_1/FVC (%)		
baseline	58.5 (12.9)	45.7 (12.1)
after bronchodilator	59.0 (22.1)	45.0 (16.9)
FEF_{75} (L/s)		
baseline	0.4 (0.3)	0.2 (0.1)
after bronchodilator	0.5 (0.4)	0.2 (0.1)
FEF_{50} (L/s)		
baseline	1.1 (0.7)	0.6 (0.4)
after bronchodilator	1.3 (1.0)	0.7 (0.5)
MMEF (L/s)		
baseline	0.8 (0.5)	0.5 (0.3)
after bronchodilator	1.0 (0.8)	0.5 (0.4)

Continuous data are described as mean (standard deviation); Categorical data are presented as frequency (percentage). Sample size: FEV_1/FVC (%), FEF_{75} (L/s), FEF_{50} (L/s) and MMEF (L/s) were available in 4,002, 3,778, 3,837 and 3,714 cases with suspected asthma and 6,151, 5,413, 5,945 and 5,456 cases with suspected COPD; *patients with clinical consideration for asthma, age ranges from 6 to 80 years old. **patients with suspected COPD and a FEV_1/FVC ratio of less than 0.7 after bronchodilation, age ranges from 36 to 80 years old. Abbreviations: FEF_{50}, forced expiratory flow at 50% of FVC; FEF_{75}, forced expiratory flow at 75% of FVC; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; FEV_1/FVC, the ratio of forced expiratory volume in 1 second to forced vital capacity; MMEF, maximal mid-expiratory flow.