

Usefulness of offline fractional exhaled nitric oxide measurements in the elderly asthmatic patients

Jeng-Yuan Hsu, M.D.,^{1,2} Wei-Chang Huang, M.D.,² Pey-Ling Huang, M.S.,² Ya-Wen Cheng, Ph.D.,³ and Ming-Chih Chou, M.D., Ph.D.¹

ABSTRACT

Fractional exhaled nitric oxide (FeNO) measurement is an important noninvasive tool for evaluating eosinophilic inflammation of the airways. However, measurement of online FeNO in elderly people may not be possible if they are unable to perform the expiratory maneuver. The offline FeNO test is an alternative method that obviates the need for the expiratory maneuver. Adult asthma patients were enrolled and assigned to one of two age groups: group 1, aged >65 years old ($n = 29$), and group 2, control group, aged 20–65 years old ($n = 27$). Online and offline FeNO measurements were performed for all patients. There were no significant differences in pulmonary function parameters (forced expiratory volume in 1 second [FEV₁], percentage of predicted FEV₁, and FEV₁/forced vital capacity [FVC]) between the two groups but group 1 had significantly lower Asthma Control Test scores. There was a high correlation between online and offline FeNO levels ($r = 0.908$; $p < 0.001$). There was significantly higher mean FeNO level, regardless of online or offline measurements, in patients with poorly controlled asthma. Offline FeNO measurement was found to be a simple and convenient method of measuring FeNO in elderly patients and can be used as an objective guide for further management of eosinophilic inflammatory airway disease in this population.

(Allergy Asthma Proc 34:434–438, 2013; doi: 10.2500/aap.2013.34.3692)

Bronchial asthma often occurs in childhood, but may affect all age groups.¹ Careful differential diagnosis is recommended in elderly people with asthma-like symptoms.^{2–4} Among the methods for diagnosing and monitoring bronchial asthma, fractional exhaled nitric oxide (FeNO) is a sensitive, noninvasive tool that can detect airway inflammation.⁵ FeNO can also be used to monitor treatment response in asthmatic patients.^{6,7} The American Thoracic Society (ATS) and European Respiratory Society have published recommendations for standardizing procedures of measuring eNO.⁸ The online measurement method, adapted by many as the standard tool, requires patients to be present in the laboratory room and to exhale directly into a mouthpiece that is connected to the NO analyzer at a constant expiratory flow of 50 mL/s ($\pm 10\%$) for a given time. Many factors may limit the application of online FeNO measurement, such as difficulty maintaining the required constant expiratory flow rate in elderly patients.

The offline method of measuring FeNO has been shown to be a useful alternative technique. The online

method involves directly blowing into a mouthpiece that is connected to an NO analyzer, but in the offline method, the exhaled gas is first collected in a bag that is then connected to the NO analyzer. The offline FeNO measurement method is more flexible than the online method and avoids the influence of poor exhalation skills.⁹

Online and offline FeNO have been shown to have a good intercorrelation in a large epidemiological survey of schoolchildren.¹⁰ However, there are few studies of FeNO in elderly asthmatic patients.¹¹ In our previous study, we determined FeNO concentrations in 248 normal Taiwanese patients and obtained a reference value of 27.9 ppb,¹² but the reference value of offline FeNO is unknown. We therefore conducted the present study to investigate the correlation between online and offline FeNO measurements. In addition, we compared the success rates of online and offline FeNO measurement in asthmatic patients in different age groups.

METHODS

Asthma patients aged ≥ 20 years old, who were nonsmokers or former smokers with a smoking history < 10 pack-years and had quit smoking for at least 10 years, were enrolled. The patients were assigned to one of two age groups: group 1, aged >65 years old, and group 2 (control group), age 20–65 years old. The diagnosis of asthma was based on the criteria of the Global Initiative for Asthma (GINA).¹³ Those with upper or lower respiratory tract infection, asthma exacerbation within 1 month, or evidence of active pulmonary tuberculosis were excluded. The study was approved by the Institutional Review Board of Taic-

From the ¹Institute of Medicine, Chung-Shan Medical University, Taichung, Taiwan, ²Division of Chest Medicine, Department of Internal Medicine, Taichung Veterans General Hospital, Taichung, Taiwan, and ³Graduate Institute of Cancer Biology and Drug Discovery, College of Medical Science and Technology, Taipei Medical University, Taiwan

The authors have no conflicts of interest to declare pertaining to this article. Address correspondence to Ming-Chih Chou, M.D., Institute of Medicine, Chung-Shan Medical University, Taichung, Taiwan, Number 110, Section 1, Jiaanguo N. Road, Taichung 40201, Taiwan

E-mail address: gruadechou@hotmail.com

Copyright © 2013, OceanSide Publications, Inc., U.S.A.

Table 1 Demographic data of the participants

	All	Group 1, Aged >65 yr	Group 2, Aged 20–65 yr	<i>p</i> Value
<i>n</i>	56	29	27	
Age (yr)	62.3 ± 16.3	75.0 ± 6.7	48.7 ± 11.7	0.013
Male/female	35/21	20/9	15 /12	
FEV ₁ (L/min)	2.1 ± 0.8	1.6 ± 0.5	2.6 ± 0.8	0.052
FEV ₁ predicted (%)	80.7 ± 20.4	76.3 ± 21.9	85.6 ± 17.8	0.327
FEV ₁ /FVC (%)	66.9 ± 10.2	62.8 ± 9.9	71.4 ± 8.6	0.479
ACT score	20.7 ± 4.1	19.8 ± 4.8	21.8 ± 2.8	0.040

ACT = *Asthmas Control Test*; FEV₁ = *forced expiratory volume in 1 s*; FVC = *forced vital capacity*.

hung Veterans General Hospital and all patients provided written informed consent.

Patients enrolled in this study were provided with medication for asthma control according to the recommendations proposed in the GINA guidelines¹³ and remained stable for at least 1 month before the day of the study. No food or medication was allowed for at least 8 hours after midnight before FeNO measurements were taken. FeNO measurements were performed using a chemiluminescence NO analyzer (NOA 280i; Sievers, Boulder, CO) according to the ATS/European Respiratory Society recommendations.⁸ For online measurements, the patient was instructed to blow into a mouthpiece connected to the NO analyzer. The patient had to maintain an expiratory flow rate of 50 mL/s for at least 6 seconds and to keep the flow at the plateau for at least 3 seconds. At least three measurements with <10% of variation were taken to calculate the mean FeNO level. After 3 minutes of rest, an offline FeNO measurement was taken.

The Deadspace Discard Bag Collection and Sampling Kit (Sievers) was used to collect the expired gas. The kit was composed of a 1.5-L collection bag (Mylar bag; Sievers), a pressure gauge to ensure the recommended exhalation pressure, a bag filler with a divert valve to connect to the bag, and an NO filter to ensure the NO level in the inspired ambient air was <5 ppb. The patient was asked to place the mouthpiece in his/her mouth and inhale orally to total lung capacity and then blow into the mouthpiece with the divert valve pulled out to discard the gas in the physiological dead space. The patient was instructed to watch the pressure gauge and adjust the force of exhalation to keep a pressure ~12 cmH₂O. The exhaling pressure (P_{exh}) of 12 cmH₂O resulted in a gas flow rate of 80 mL/s ($\text{flow} = 21.1 \times P_{\text{exh}}^{0.53306}$). (This formula was presented in the *Operation and Maintenance Manual* of NOA 280i, no reference is available). At this moment the patient was asked to push the divert valve to the “in” position to start collecting gas in the bag. The patient must continue exhaling for 4–6 seconds until 320–480 mL of gas was collected. The bag was then sealed with a Luer cap

(Sievers). Three bags were collected per patient and these bags were stored in an air-conditioned room at around 25°C for analysis of the NO level, which was done within 4 hours. By using a three-way connector to connect the bag to the NOA 280i NO analyzer, the NOA sampled the gas from the bag automatically. Each measurement lasted for at least 15 seconds. The mean of three measurements was used as the final result of the test.

The status of asthma control was assessed according to the criteria of the GINA.¹³ Uncontrolled asthma was defined as having at least three factors that did not meet the asthma control criteria (*i.e.*, daytime or nighttime symptoms, activity limitation, forced expiratory volume in 1 second [FEV₁] or peak expiratory flow rate of <80% of predicted value, and frequent use of rescue medication). The Asthma Control Test¹⁴ (ACT) score was also used to assess the level of asthma control, whereby an ACT score of ≤19 was defined as poorly controlled asthma.

The PASW (SPSS, Inc., Chicago, IL) statistical software Version 18 was used to analyze the data. The *t*-test was used to compare differences between means and data are presented as mean ± SD. Correlation and regression analyses were used to compare online and offline FeNO. Repeat-measure ANOVA was used to check the within-subject variation of the off line FeNO values. Statistical significance was set at *p* < 0.05.

RESULTS

From December 1, 2011, to November 30, 2012, a total of 56 eligible asthma patients were enrolled for both online and offline FeNO measurements. In total, 29 patients aged >65 years were assigned to group 1, and the other 27 patients, aged 20–65 years, were assigned to group 2 (control group). Group 1 (only 24 patients completed online measurement) had a significantly higher mean age than that of group 2 (75.5 ± 6.7 years versus 48.7 ± 11.7 years; *p* = 0.013). There were no significant differences in prebronchodilator FEV₁ (*p* = 0.052), percentage predicted value of FEV₁ (*p* = 0.327), and FEV₁/forced vital capacity (*p* = 0.479) between the two groups. Group 1 also had

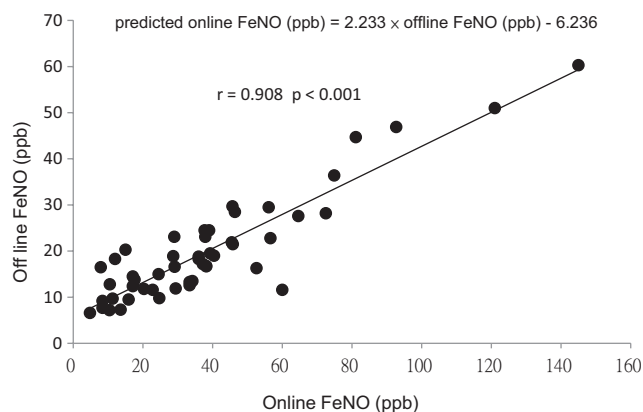


Figure 1. Plot of online vs. offline FeNO data. A formula was obtained for predicting the online FeNO data from offline measurements. (A) Value of online FeNO. (B) Value of offline FeNO.

significantly lower mean ACT than that of group 2 (19.8 ± 4.8 versus 21.8 ± 2.8 ; $p = 0.040$; Table 1).

All patients in group 2 completed both online and offline FeNO measurements and five patients in group 1 failed to provide valid data because they could not maintain the required expiratory flow at plateau for at least 3 seconds. Repeat-measure ANOVA showed no significant difference of the FeNO values obtained from three bags, ($F = 1.235$; $p = 0.295$). There was only one patient in group 2 that was deemed to have “variable data” (FeNO, 11.1 ppb, 6.4 ppb, and 18.9 ppb) from the three collected bags, and the offline FeNO measurement could not be obtained. There was no difference in the mean group 1 (37.2 ± 19.9) versus mean group 2 online (39.8 ± 33.8) or group 1 (19.2 ± 9.0) versus group 2 (20.5 ± 13.5) offline FeNO levels.

The correlation between online and offline FeNO measurements in all participants was 0.908 ($p < 0.001$). A correlation formula (predicted online FeNO [ppb] = $2.233 \times$ offline FeNO [ppb] - 6.236) was obtained from the result of regression analysis performed the PASW statistical software (Fig. 1). There was no correlation between online or offline FeNO value and FEV₁ or percentage predicted FEV₁. In terms of asthma control, the correlation between GINA control criteria and ACT score was 0.753 ($p < 0.001$). Patients with uncontrolled asthma, as assessed by the GINA criteria, or with poorly controlled asthma, as assessed by ACT score, had significantly higher FeNO levels using both online and offline measurements (Table 2).

DISCUSSION

This study compared the results of online and offline FeNO measurements in asthmatic adults and elderly patients. For each patient, the online and offline FeNO measurements were performed sequentially

and were completed on the same day to eliminate factors such as weather, patient’s health status, and medication usage, which may have confounded the results.

In current practice, FeNO is extensively used as a tool for evaluating and managing eosinophilic inflammatory airway disease. Based on our experience, patients with advanced age frequently have difficulty complying with the procedure for online FeNO measurement. Therefore, one of the objectives of the present study was to confirm the value of offline FeNO measurement in elderly patients. As expected, a number of patients in group 1 ($n = 5$; 17.2%), but none in group 2, failed to provide valid online FeNO data. However, the results showed that, overall, both groups provided acceptable results for both online and offline measurements.

The method of offline FeNO measurement is a whole set of standard procedures listed in the NOA 280i *Operation and Maintenance Manual* supplied by Sievers Instruments, Inc. It is not possible to control the variation between three samples at the time of gas collection. However, repeat-measure ANOVA showed the agreement of the three measurements in the same patient is acceptable. Only one patient was observed to have marked variable data from the three collected bags.

This study revealed an excellent correlation ($r = 0.908$; $p < 0.001$) between values obtained using the online and offline FeNO tests, but there was an approximately twofold higher concentration of FeNO obtained by the online method compared with that obtained by the offline method. Regarding the factors that contribute to this variation, the difference of the expiratory flow rate (online, 50 mL/s, and offline, 80 mL/s) plays the major role.^{15,16} Other variables, such as ambient NO, dead space volume, and pulmonary function data are not relevant in this study.¹⁰ Using the formula developed in this study, online the FeNO level could be predicted by offline FeNO data. A previous study on 249 nonsmoking healthy adults in Taiwan showed that the mean online FeNO level was 27.9 ppb (range, 12.5–58 ppb),¹² so it can be postulated that the normal offline FeNO level would be 15.3 ppb. This result suggests that the offline FeNO method is not only a reliable and practical technique for assessing FeNO levels in elderly patients, but it also provides an objective guide for further management of eosinophilic inflammatory airway disease in these patients.

The stability of the exhaled gas sample is a critical issue for the offline FeNO measurement. The exhaled gas can remain stable for 24 hours in a new Mylar bag or for at least 8–12 hours in a reused bag.^{17,18} Storage temperature is another issue that may influence the measurement value. A study by Shimizu *et al.* has shown that the gas sample remains stable at

Table 2 Comparison of patients' asthma control status and FeNO levels obtained by online and offline FeNO methods

	GINA			ACT Score		
	Controlled/Partly Controlled	Uncontrolled	<i>p</i> Value	≥20	<19	<i>p</i> Value
Online FeNO	35.1 ± 20.4	45.5 ± 38.8	0.018	31.7 ± 15.2	44.5 ± 38.0	0.004
Offline FeNO	18.1 ± 9.0	23.2 ± 14.4	0.022	16.7 ± 6.3	22.3 ± 15.2	0.001

ACT = Asthma Control Test; FeNO = fractional exhaled nitric oxide; GINA = Global Initiative for Asthma.

4°C after 96 hours of storage. However, at 25°C, a significant change is observed within 24 hours.¹⁹ In the present study, the collected bags were stored at room temperature, but the analysis was made as soon as possible to minimize the adverse effects of high temperature.

The results of this study are consistent with those of a previous report showing no correlation between FEV₁ and FeNO.²⁰ However, significantly higher mean FeNO levels (both online and offline) were observed in patients with uncontrolled asthma (by GINA criteria, *p* = 0.004) or poorly controlled asthma (ACT, <19; *p* = 0.001). Previous studies have shown a good correlation of FeNO and inflammatory markers obtained from bronchial biopsy or induced sputum eosinophil count.^{21,22} The ATS guidelines published in 2011 recommends FeNO measurement as a good tool for assessing and monitoring eosinophilic inflammation-related airway diseases.²³ Furthermore, FeNO is suggested to be used in difficult-to-treat asthma for monitoring response to inhaled corticosteroids or nonadherence to inhaled corticosteroids treatment.^{24–26} All of these suggest that FeNO measurement can not be omitted in elderly patients if they have difficulty performing online FeNO measurements.

CONCLUSIONS

In conclusion, elevated FeNO levels were seen in patients with uncontrolled or poorly controlled asthma. The offline FeNO measurement was shown to be a viable and practical alternative method for obtaining valuable data that may be useful in guiding the management of patients with advanced age.

REFERENCES

- Hsu JY, King SL, Kuo BI, and Chiang CD. Age of onset and the characteristics of asthma. *Respirology* 9:369–372, 2004.
- Pierson DJ. Asthma in the elderly: Special challenge. *Geriatrics* 37:87–88, 93–95, 1982.
- Subramanian D, and Guntupalli KK. Diagnosing obstructive lung disease. Why is differentiating COPD from asthma important? *Postgrad Med* 95:69–70, 75–78, 83–85, 1994.
- Löwhagen O. Asthma and asthma-like disorders. *Respir Med* 93:851–855, 1999.
- Kharitonov SA, Rajakulasingam K, O'Connor B, et al. Nasal nitric oxide is increased in patients with asthma and allergic rhinitis and may be modulated by nasal glucocorticoids. *J Allergy Clin Immunol* 99:58–64, 1997.
- Hahn PY, Morgenthaler TY, and Lim KG. Use of exhaled nitric oxide in predicting response to inhaled corticosteroids for chronic cough. *Mayo Clin Proc* 82:1350–1355, 2007.
- Smith AD, Cowan JO, Brassett KP, et al. Exhaled nitric oxide: A predictor of steroid response. *Am J Respir Crit Care Med* 172:453–459, 2005.
- ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *American J Respir Crit Care Med* 171:912–930, 2005.
- Kharitonov SA, and Barnes PJ. Clinical aspects of exhaled nitric oxide. *Eur Respir J* 16:781–792, 2000.
- Linn WS, Berhane KT, Rappaport EB, et al. Relationships of online exhaled, offline exhaled, and ambient nitric oxide in an epidemiologic survey of schoolchildren. *J Expo Sci Environ Epidemiol* 19:674–681, 2009.
- Columbo M, Wong B, Panettieri RA Jr., et al. Asthma in the elderly: The role of exhaled nitric oxide measurements. *Respir Med* 107:785–787, 2013.
- Liu HC, Hsu JY, Cheng YW, and Chou MC. Exhaled nitric oxide in a Taiwanese population: Age and lung function as predicting factors. *J Formos Med Assoc* 108:772–777, 2009.
- Bateman ED, Hurd SS, Barnes PJ, et al. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 31:143–178, 2008.
- Nathan RA, Sorkness CA, Kosinski M, et al. Development of the asthma control test: a survey for assessing asthma control. *J Allergy Clin Immunol* 113:59–65, 2004.
- Silkoff PE, Stevens A, Pak J, et al. A method for the standardized offline collection of exhaled nitric oxide. *Chest* 116:754–759, 1999.
- Deykin A, Massaro AF, Drazen JM, and Israel E. Exhaled nitric oxide as a diagnostic test for asthma: Online versus offline techniques and effect of flow rate. *Am J Respir Crit Care Med* 165:1597–1601, 2002.
- Jöbsis Q, Schellekens SL, Kroesbergen A, et al. Off-line sampling of exhaled air for nitric oxide measurement in children: Methodological aspects. *Eur Respir J* 17:898–903, 2001.
- Bodini A, Pijnenburg MW, Boner AL, and de Jongste JC. Exhaled nitric oxide in mylar balloons: Influence of storage time, humidity and temperature. *Mediators Inflamm* 12:47–49, 2003.
- Shimizu H, Obase Y, Ikeda M, et al. Stability of sealed-bag samples for off-line measurement of fractional exhaled nitric oxide. *Ann Allergy Asthma Immunol* 106:378–380, 2011.

20. Spergel JM, Fogg MI, and Boksztzanin-Knosala A. Correlation of exhaled nitric oxide, spirometry and asthma symptoms. *J Asthma* 42:879–883, 2005.
21. Jatakanon A, Lim S, Kharitonov SA, et al. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. *Thorax* 53:91–95, 1998.
22. Payne DN, Adcock IM, Wilson NM, et al. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in children with difficult asthma, after treatment with oral prednisolone. *Am J Respir Crit Care Med* 164:1376–1381, 2001.
23. Dweik RA, Boggs PB, Erzurum SC, et al. An official ATS clinical practice guideline: Interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med* 184:602–615, 2011.
24. Pérez-de-Llano LA, Carballada F, Castro Añón O, et al. Exhaled nitric oxide predicts control in patients with difficult-to-treat asthma. *Eur Respir J* 35:1221–1227, 2010.
25. Bukstein D, Luskin AT, and Brooks EA. Exhaled nitric oxide as a tool in managing and monitoring difficult-to-treat asthma. *Allergy Asthma Proc* 32:185–192, 2011.
26. Zeiger RS, Schatz M, Zhang F, et al. Association of exhaled nitric oxide to asthma burden in asthmatics on inhaled corticosteroids. *J Asthma* 48:8–17, 2011. □

Copyright of Allergy & Asthma Proceedings is the property of OceanSide Publications Inc. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.