

ORIGINAL ARTICLE

Measurement of Fractional Exhaled Nitric Oxide by a New Portable Device: Comparison with the Standard Technique

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Background. Fractional exhaled nitric oxide (FeNO) measurement is a reliable, noninvasive marker of airway inflammation. The use of portable FeNO analyzers may enable the assessment of airway inflammation in primary care. **Objective.** The authors compared FeNO values obtained by a new portable device (NObreath, Bedfont, UK) to those of the standard stationary analyzer (NIOX, Aerocrine, Sweden) in a large cohort of asthmatic patients. **Methods.** One hundred and fifty-four (age range: 14–83 years, forced expiratory volume in one second [FEV₁] range: 48–134% predicted, asthma control test [ACT] range: 7–25) out of 168 recruited patients completed the study. Each patient performed at least two valid FeNO measurements at a constant flow rate of 50 ml/s on each of the two analyzers. **Results.** A significant relationship between the FeNO values obtained by the two devices ($r = .95, p < .001$) was found. Altman-Bland plot confirmed this agreement. Within-patient repeatability was excellent in both devices. Intraclass correlation coefficients for NIOX and NObreath values were .925 and .967, respectively. By means of receiver operating characteristic curve analysis, the FeNO cutoff points that better identified patients with ACT ≥ 20 were 15 ppb (0.84 sensitivity and 0.42 specificity) by NIOX and 25 ppb (0.53 sensitivity and 0.69 specificity) by NObreath. Ease of use of both devices, assessed by visual analogue scale was not different. **Conclusion.** FeNO measurements obtained by the new portable FeNO analyzer are reliable because they are directly comparable with those obtained by the stationary standard device. The use of portable instruments may facilitate the FeNO measurement in primary care.

Keywords airway inflammation, asthma, exhaled nitric oxide

INTRODUCTION

The measurement of fractional exhaled nitric oxide (FeNO) is a reliable, noninvasive marker of airway inflammation (1). FeNO has been shown to be increased in some airway diseases, such as asthma (2), allergic rhinitis (3), chronic rhinosinusitis (4), and chronic cough (5). Notably, FeNO measurement has been proved to play a role in asthma diagnosis (6) and management (7) and has become a clinical routine in asthmatic patients.

The chemiluminescence analyzer is currently considered as the standard technique for measuring FeNO and a FeNO stationary chemiluminescence analyzer (NIOX; Aerocrine AB, Solna, Sweden) has been approved by the U.S. Food and Drug Administration for use in asthma management (8). However, limitations of the stationary device, such as cost, size, and frequent calibration requirement, may preclude its wide-scale introduction into clinical practice. Furthermore, a portable FeNO analyzer has shown to be extremely useful in primary care as an additional tool to improve care of asthmatic patients (9).

A new portable device using electrochemical sensors (NObreath; Bedfont, Rochester, UK) has recently been developed but no published study has evaluated its reliability. The aim of the study was, therefore, to compare FeNO values obtained by the new portable device to those of the standard

stationary chemiluminescence FeNO analyzer and to calculate a conversion equation, in a large cohort of asthmatic patients. Additionally, we evaluated the within-subject reproducibility of the FeNO values obtained by both devices, as well as their capability to discriminate asthmatic patients with controlled disease from those with uncontrolled disease. Lastly we assessed the ease of use of the two devices.

METHODS

Subjects

Patients (14 years of age and older) with asthma diagnosis according to the international guidelines (10) were eligible to take part in the study and were prospectively recruited over a 6-month period from our Asthma Outpatient Clinic. In each patient, duration of disease and smoking habit were recorded. Atopy was assessed by skin prick tests with a battery of 10 common inhalant allergens. No patient has undergone any FeNO measurement before the study. We ensured that the patients were not affected by any acute respiratory infection and had followed the pre-test instructions, i.e., no nitrate-rich foods and beverages, including alcoholic ones, no tobacco smoking, and no exercise within 1 h preceding the test, as these factors can affect the test results. Moreover, all patients underwent FeNO measurement before lung function test. Only patients able to perform at least two acceptable FeNO measurements both with NIOX and with NObreath (up to six attempts per device) were included in the analysis. The study was approved by local ethics committee and all patients gave their informed consent.

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FeNO Measurement

FeNO was measured according to the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines (11) using a hand-held device NObreath (FeNO_{NObreath}) and a stationary NIOX (FeNO_{NIOX}). The detection principle of NO for the novel hand-held FeNO analyzer is based on the electrochemical sensor technology. Any gas that can be electrochemically oxidized or reduced can also be detected by means of an electrochemical sensor. An important characteristic of the newly developed sensor is its high sensitivity down to the level of a few ppb (<5 ppb). The sensitivity drift is less than 5% per annum and the sensor operating life is 1 to 2 years.

All tests were performed at the same time of day (± 2 h) to allow a possible circadian rhythm effect. The order of the measurements was random. For both types of measurements, patients were seated in the upright position without a nose clip. In a subgroup of patients, the within-subject reproducibility of FeNO_{NObreath} and FeNO_{NIOX} was assessed by repeating measurements on two separate days by 1 week. To participate in repeatability study, patients had to be clinically stable, maintain their existing therapeutic regimen and demonstrate a change in forced expiratory volume in one second (FEV₁) less than 10% during the two study visits.

In detail, FeNO_{NObreath} was obtained by asking subjects to inhale as deeply as possible and after 3 s, guided by an auditory cue, to exhale through the mouthpiece, keeping the ball in the flow indicator in the middle of the black band in the center of the tube, at a constant flow rate of 50 ml/s. The required exhalation time is approximately 16 s. To ensure a breath sample was taken at the correct flow rate, the monitor was held upright at all times during the test. FeNO_{NIOX} was performed asking the subjects to inhale nitric oxide-free air deeply to total lung capacity through a filter connected to the device and then to exhale for 10 s at a constant pressure guided by a visual cue to stabilize flow rate. All tests were performed at an exhalation pressure of 10 to 20 cm H₂O, to maintain a fixed flow rate of 50 ml/s. Measurements were repeated after a brief rest period until two acceptable values (± 2.5 ppb for measurements <50 ppb and $\pm 5\%$ for measurements ≥ 50 ppb) were performed (maximum six attempts). The mean of two adequate values for each subject was recorded for analysis. For NIOX, the system calibration was performed every 14 days, whereas NObreath was set to zero every month.

After FeNO_{NObreath} and FeNO_{NIOX} patients rated the easiness to use of both devices on an interval scale, which was a 100-mm horizontal visual analogue scale (VAS). The VAS consisted of a horizontal ruler without any mark on the patient's side with the words "easy to use" and "not easy to use" on the left and right end, respectively. Easiness to use ratings were expressed in mm from 0 to 100 and corresponded to the distance of the marker from the left end of the visual analogue scale.

Lung Function Testing

Lung function was measured by a flow-sensing spirometer connected to a computer for data analysis (CPFS/D Spirometer; MedGraphics, St Paul, MN, USA) which met American Thoracic Society (ATS) standards. Forced vital

capacity (FVC), forced expiratory volume in one second (FEV₁), and FEV₁/FVC ratio were recorded. FVC and FEV₁ are expressed as percent of predicted value (12), FEV₁/FVC as percent.

Asthma Control Assessment

Asthma control was assessed using the Italian version of the Asthma Control Test (ACT) (13). Patients subjectively evaluated the degree of impairment caused by their disease during the preceding 4 weeks by responding to five questions using a 5-point scale. The ACT is reliable, valid, and responsive to changes in asthma control over time (13, 14). The sum of the scores of the five questions gave the total ACT score (range: 5–25). A cutoff score of 20 or more identifies patients with well-controlled asthma.

Statistical Analysis

The distribution of variables was assessed by means of Kolmogorov-Smirnov goodness-of-fit test. To normalize the distribution, FeNO data were log-transformed for analysis and reported as geometric mean \pm GSEM. Other numerical variables were expressed as mean \pm SD, unless otherwise specified. Paired *t* test and unpaired *t* test were used for comparisons, when appropriate. The relationship between measures was estimated by Pearson's correlation coefficient (*r*) and linear regression analysis. The agreement between measures was assessed by the method of differences against the means according to Bland and Altman (15) and the Spearman correlation coefficient (*r_s*) was used to identify any potential tendency for the separation of agreement at higher or lower values. The repeatability of measures was expressed as intra-class correlation coefficient (*r_I*) (16). The receiver operating characteristic (ROC) curve method (17) was used to plot the true positive rate (sensitivity) in function of the false-positive rate (100 – specificity) for discriminating patients with asthma under control from those with poorly controlled asthma for each device. A *p* value $\leq .05$ was considered as significant.

RESULTS

One hundred sixty-eight asthmatic patients were enrolled. Fourteen patients were excluded after failing to perform acceptable FeNO measurements (six for NIOX, five for NObreath, one for both NIOX and NObreath, two unapproved values). A total of 154 consecutive patients (age range: 14–83 years, 54 males, 18 current smokers, 112 atopics) completed the study (Table 1). One hundred and seven patients (69%) were receiving therapy. In all patients, spirometry ranged from a severe ventilatory defect to normal value (FEV₁ range: 48–134%) (Table 1).

FeNO_{NIOX} and FeNO_{NObreath} values were significantly different (24.6 ± 1.073 ppb versus 22.6 ± 1.075 ppb; *p* = .0002) (Table 2) and were significantly related (*r* = .95, *p* < .001) with the following regression equation: $\log \text{FeNO}_{\text{NIOX}} = 0.287 (SE = 0.078) + 0.935 (SE = 0.024) \times \log \text{FeNO}_{\text{NObreath}}$ (*r*² = .91, *p* < .001) (Figure 1). The Bland-Altman plot showed a high degree of agreement between the devices (Figure 1) and Spearman correlation coefficient confirmed the lack of bias at either end of the range of values (*r_s* = -.088, *p* = .275).

TABLE 1.—Characteristics of 154 patients with asthma.

Age (years)	43 ± 16
Sex (M/F)	0.54
BMI (kg/m ²)	25 ± 5
Atopy (Yes/No)	2.67
Disease duration (years)	11 ± 11
Smoking habit (Yes/No)	0.13
ACT (0–25)	21 (17–23)
FVC (% of predicted)	105 ± 17
FEV ₁ (% of predicted)	94 ± 17
FEV ₁ /FVC (% of predicted)	75 ± 10

Note. Values are expressed as mean ± SD, ratio, or median (25th–75th percentile).

ACT = Asthma Control Test; BMI = body mass index; FEV₁ = forced expiratory volume in one second; FEV₁/FVC = forced expiratory volume in one second/forced vital capacity ratio; FVC = forced vital capacity.

In the subgroup of 20 patients (age range: 19–70 years; 6 males), who performed the FeNO_{NOBreath} and FeNO_{NIOX} reproducibility tests, spirometry did not significantly change in the two occasions (FVC = 111% ± 21% predicted versus 106% ± 16% predicted; FEV₁ = 100% ± 19% predicted versus 96% ± 17% predicted; FEV₁/FVC = 76% ± 10%

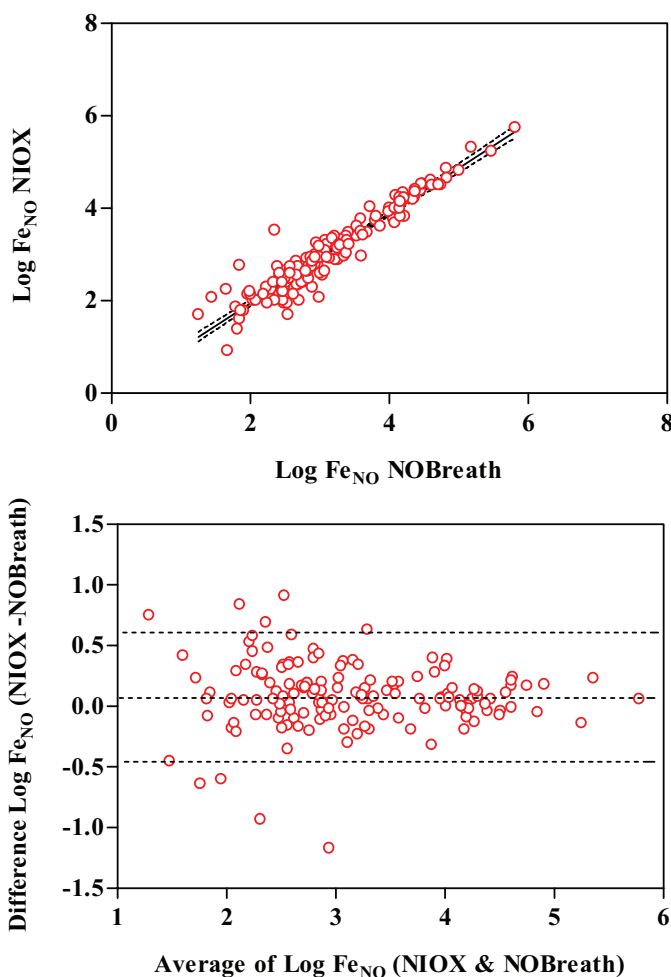


FIGURE 1.—Linear regression with 95% confidence interval (upper panel) and Altman-Bland plot (lower panel) of NOBreath versus NIOX values in 154 asthmatic patients.

TABLE 2.—FeNO values obtained by NIOX and NOBreath devices, number of attempts to obtain at least two acceptable FeNO values by the devices, and easiness to use of the devices.

	NIOX	NOBreath	<i>p</i>
FeNO (ppb)	24.6 ± 1.073	22.6 ± 1.075	.002
Attempts (no.)	3 (2–4)	2 (2–3)	.01
Easiness to use (VAS, mm)	10 (0–22)	10 (0–22)	.93

Note. Values are expressed as geometric mean ± GSEM or median (25th–75th percentile). VAS = visual analogue scale.

predicted versus 76% ± 10% predicted). In these patients, FeNO_{NIOX} and FeNO_{NOBreath} values obtained in the two occasions were respectively 21.8 ± 1.18 ppb and 21.0 ± 1.20 ppb (*r*₁ = .925) and 19.1 ± 1.20 ppb and 19.9 ± 1.19 ppb (*r*₁ = .967) (Figure 2).

Ninety-six patients out of 154 had well-controlled asthma (ACT ≥ 20) and lower FeNO_{NIOX} and FeNO_{NOBreath} values, as compared to those with poorly controlled asthma (20.9 ±

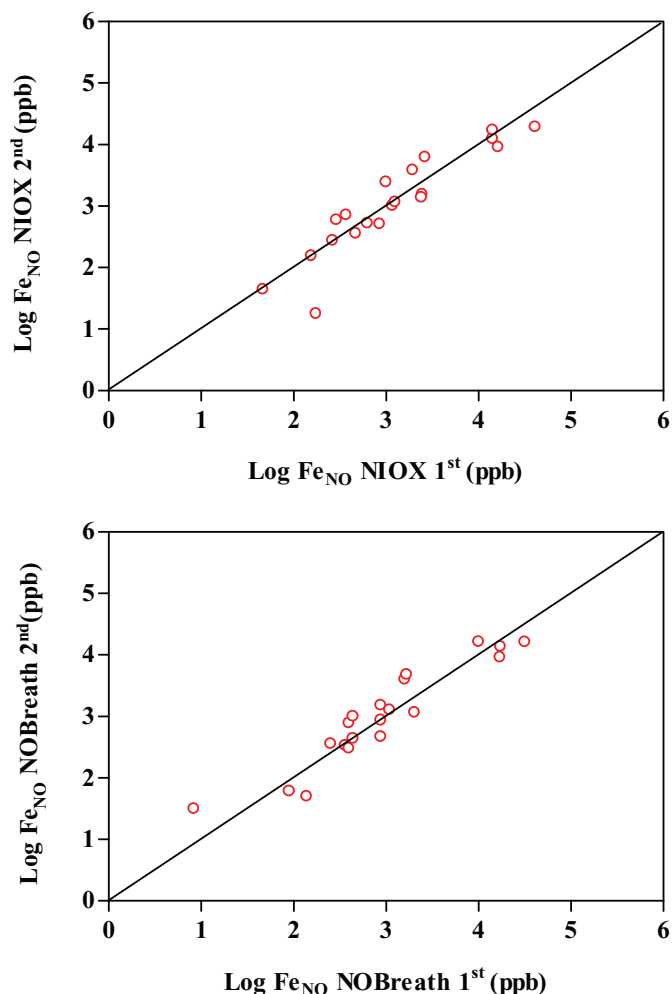


FIGURE 2.—Within-subject repeatability of NIOX values (upper panel) and NOBreath (lower panel) in 20 asthmatic patients. The intraclass correlation coefficients were .925 and .967, respectively. The continuous line is the line of identity.

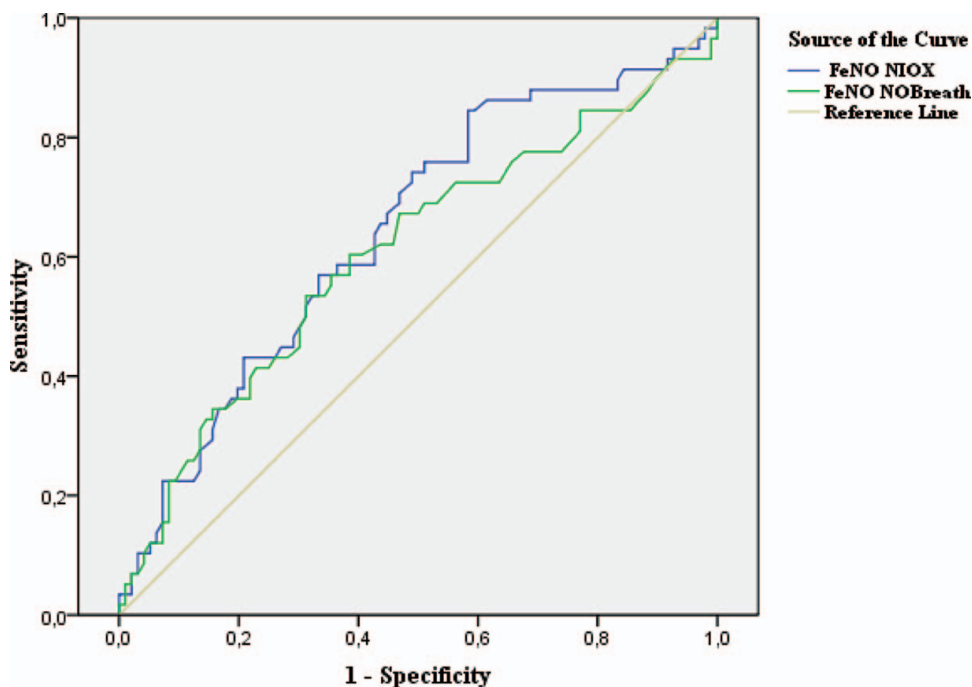


FIGURE 3.—Receiver operating characteristic (ROC) curve analysis for different cutoff points of FeNO measurements obtained by NIOX (—) and by NObreath (· · · · ·) with respect to an Asthma Control Test score ≥ 20 , as threshold value. Area under curve (AUC) values of NIOX and NObreath measurements were 0.644 ($p = .002$) and 0.607 ($p = .0251$), respectively. Pairwise comparisons of the ROC curves revealed a difference in the AUC of 0.0369 ($p = .028$).

1.08 ppb versus 31.8 ± 1.13 ppb, $p = .004$, and 19.9 ± 1.08 ppb versus 27.6 ± 1.13 ppb, $p = .041$). According to the ROC curve method, the plot of the true-positive rate in function of the false-positive rate for different cutoff points of FeNO_{NIOX} and FeNO_{NObreath} values with respect to a ACT ≥ 20 , as threshold value, showed respectively 0.644 (95% confidence interval [CI]: 0.562–0.719; $p = .002$) and 0.607 (95% CI: 0.525–0.684; $p = .0251$) area under curve (AUC) values. Pairwise comparisons of the ROC curves revealed a difference in the AUC of 0.0369 (95% CI: 0.004–0.0697; $p = .028$) between the FeNO_{NIOX} and FeNO_{NObreath} values. The FeNO_{NIOX} and FeNO_{NObreath} cutoff points, which maximized sensitivity and specificity, were 15 ppb (0.84 sensitivity and 0.42 specificity) and 25 ppb (0.53 sensitivity and 0.69 specificity), respectively (Figure 3). Well-controlled and poorly controlled patients did not differ in FEV₁ values ($95\% \pm 15\%$ versus $93\% \pm 20\%$, $p = .477$).

FeNO_{NIOX} required a significant greater number of attempts to obtain two acceptable values, as compared to FeNO_{NObreath} ($p = .01$). However, easiness to use of both devices, assessed by means of VAS, was not different (Table 2).

DISCUSSION

The results of the present study show that FeNO measurements obtained by NObreath, a new portable FeNO analyzer, are reliable because they are directly comparable with those obtained by NIOX, the stationary device currently considered as the standard for measuring FeNO. Furthermore, because of its manageability and easiness to use, the portable device could be suitable for home-monitoring of asthma as well as for epidemiological studies.

It is important to appreciate that FeNO measurements in both healthy subjects and asthmatic patients are right-skewed (18, 19) and require appropriate transformation prior to statistical analysis. This may imply some difficulties for interpreting measurements obtained in the clinical setting. In this regard, we found that there was a significant difference between FeNO values obtained by the two devices and that the regression line in Figure 1 did not dissect the origin of the axis, thereby confirming a consistently lower value from the NObreath device compared with that from the NIOX device. We provided the regression equation to convert FeNO values measured by NObreath into those obtained by NIOX. However, it is of note that the difference between the values measured by the two devices amounts to approximately 2 ppb, which would not be clinically significant.

In the present study, Bland-Altman plot demonstrated agreement between both devices and, importantly, the difference between values obtained by the NObreath and NIOX did not change with increasing FeNO values. This finding is of clinical relevance because measurements with NObreath can be reliably performed in any asthmatic patient regardless of the degree of airway inflammation. Moreover, the reliability of NObreath device is further supported by its excellent degree of within-subject repeatability over time (intraclass correlation coefficient $>.9$). Furthermore, the repeatability of NObreath was similar to that of the stationary standard analyzer. Our data are in line with previous reports comparing agreement (18, 19) and repeatability (19) between the stationary standard analyzer and the MINO, a portable device of FeNO measurement.

In this study, we also assessed the capability to discriminate poorly controlled asthmatic patients from well

controlled asthmatics, by using the two FeNO measurement devices. As expected, we found that well-controlled patients had significantly lower FeNO values, as compared to poorly controlled patients. Importantly, this difference was found with both devices, even if the magnitude of the difference was greater with the standard device. Additionally, in our cohort of patients a FeNO value greater than 15 ppb obtained by NIOX or greater than 25 ppb obtained by NObreath had a high likelihood to be associated to poorly controlled asthma. According to the ROC curve analysis, NIOX sensitivity was higher than that of NObreath, which conversely showed higher specificity. The ROC AUC value generated using the NIOX device was higher than that of NObreath. Taken together, our findings suggest that the portable device has a slightly lower discriminating power than the standard device, at least in terms of controlled versus uncontrolled asthma. Interestingly, our results are similar to those of a previous paper (18), which reported a slightly lower discriminating power of MINO, as compared to NIOX, to separate asthmatic patients from healthy subjects.

We found that the number of attempts to achieve the required acceptable measurements was slightly, but significantly, lower for NObreath than that for NIOX. This result is consistent with a previous report (20), which compared NIOX and MINO and reported a significantly less number of attempts needed to achieve the acceptable measurements by using MINO. This finding could at least partly be explained by the fact that some measurements in the NIOX may be discarded after a linear regression analysis of the NO plateau has been performed, even though the number of regression failures was not recorded in the present study. The linearized plateau must not deviate more than 10% from the horizontal axis according to current guidelines (11). Notably, the difference in the number of attempts between the devices was not perceived by the patients in terms of different easiness to use of NIOX in comparison with NObreath. Easiness to use ratings assessed by the visual analogue scale were not different between NIOX and NObreath.

In conclusion, this study shows that there is clinically acceptable agreement between the new portable NObreath and the stationary standard device NIOX. The repeatability of measurements obtained by NObreath was similar to those obtained by NIOX. In addition, FeNO measurements with both devices was a reliable method of differentiating well-controlled from poorly controlled asthmatic patients. Lastly, more than 90% of the subjects of our large cohort of asthmatics recruited, with a wide spectrum of ages, were able to successfully use both devices. The use of portable instruments will enable the introduction of FeNO measurements into the primary health care setting.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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