

Original article

Nitrogen washout slope in poorly controlled asthma

Short-term control of asthma is often lacking even though forced expiratory volume in 1 s (FEV₁) remains above normal value. Small airways are a potential key site of persistent inflammation and structural modification. Noninvasive assessment of small airways was found to be difficult, but the computerized single breath nitrogen washout test (SBNT) has been recently successfully reintroduced with this aim. Twenty-four asthmatics (13 females) of various severity but with normal FEV₁ were compared with 24 healthy volunteers (13 females) and studied at steady-state after bronchodilatation (400 µg salbutamol). Spirometric values, plethysmographic data, phase III (slope of phase III of the SBNT, dN2) and IV [closing volume (CV), with closing capacity (CC) = CV + residual volume (RV)] of the SBNT were checked. Asthma severity, recent control, exacerbation rate, and therapy requirements were assessed on the basis of validated questionnaires (ACQ) and international guidelines. Patients were prospectively pooled into two equal groups according to their exacerbation rate. The reproducibility of the measurements obtained on 2 following days was assessed. All plethysmographic values, except total lung capacity (TLC), differentiated asthmatic patients from controls. The CC/TLC [124 (117–148) *vs* 117 (112–123), *P* = 0.04] and dN2 [110 (99–190) *vs* 94 (75–111), *P* = 0.02] were increased in asthma. The dN2 was significantly increased in patients with frequent exacerbations [100 (83–105) *vs* 195 (141–212), *P* = 0.0005]. A correlation was obtained between dN2 and recent asthma control (*ρ*: 0.62; *P* = 0.003), number of exacerbations (*ρ*: 0.71, *P* = 0.0008), and RV/TLC (*ρ*: 0.49, *P* = 0.026). This study demonstrated that ventilation inequalities assessed by dN2 represent an important indicator of poor asthma control and high exacerbation rate in high symptom perceivers. New therapies focused on small airways should now be developed.

A. Bourdin¹, F. Paganin¹, C. Préfaut², D. Kieseler², P. Godard¹, P. Chanez¹

¹Service des Maladies Respiratoires, Hôpital Arnaud de Villeneuve, CHU de Montpellier, Montpellier; ²Service de Physiologie Clinique, Hôpital Arnaud de Villeneuve, CHU de Montpellier, Montpellier, France

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Pascal Chanez MD, PhD
Service des Maladies Respiratoires
Hôpital Arnaud de Villeneuve
CHU de Montpellier
34295-Montpellier-Cedex 5
France

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The physiopathologic mechanisms that underlie asthma control are not clearly understood. A recent large-scale European multinational survey revealed that patients with asthma were poorly controlled. Interestingly, many patients presented with uncontrolled asthma despite regular use of prescribed therapies, whereas forced expiratory volume in 1 s (FEV₁) remained normal at 80% above the predicted values (1).

Many patients with asymptomatic asthma can present ventilation distribution abnormalities with abnormal lung volumes and airway closure (2, 3). Routine

measurement of airflow obstruction is unable to detect peripheral airway obstruction (4). These abnormalities can be associated with initial airway structural changes, leading to persistent airflow obstruction, as described in severe asthma (5). Expiratory computed tomography (CT)-scanning has been successfully used to document this gas trapping in asthmatic patients (6). Pathologic findings from autopsy specimens and more recently using transbronchial biopsies have clearly shown inflammatory processes at the parenchyma level (small airways and alveoli) (7–9). Persistent functional abnormalities assessed by the single breath nitrogen washout test (SBNT) have been described after resolution of asthma exacerbation, whereas other functional indices [FEV₁ or peak expiratory flow (PEF) rate] became normal, thus indicating persistent small airway inflammation. Difficult to manage asthma with frequent exacerbations has been explained by documented evidence of air trapping, persistent ventilation inequalities (10), and early airway closure, although FEV₁ stay

Abbreviations: FEV₁, forced expiratory volume in 1 s; SBNT, single breath nitrogen washout test; dN2, slope of phase III of the SBNT; TLC, total lung capacity; RV, residual volume; CV, closing volume or phase IV of the SBNT; CC, closing capacity; PEF, peak expiratory flow; FRC, functional residual capacity; VC, vital capacity; ACQ, asthma control questionnaire; MMEF25–75, maximum mid-expiratory flow between 25% and 75% of the VC; V50, expiratory flow at 50% of the FVC.

above normal values (11). Such patients might have ongoing inflammation, despite all prescribed therapies, and even during stable episodes.

It has been shown that computerized SBNT can efficiently detect small airway dysfunction (12). The aim of the present study was to evaluate the potential role of small airway abnormalities in asthma control using the SBNT, in patients of various severity and control but normal FEV₁ values.

Materials and methods

Subjects

Twenty-four asthmatics (18–53 years, 13 females) were enrolled and participated in the study. They were separated in two groups according to their exacerbation rate the year before (<2 or ≥ 2 /year). All patients during the preceding 12 months had filled in the asthma control questionnaire (ACQ) (13) at least once, and were demonstrated to have clear airflow obstruction reversibility after inhalation of 400 µg of salbutamol, as recommended in the standards for asthma diagnosis.

Asthma severity was classified on a 1–4 scale and based on history, symptoms, clinical features, and medication requirement according to international guidelines (14) and the validated *asthm-expert* (15). The FEV₁ in both subject groups was within the normal range ($>80\%$ predicted) after inhalation of 400 µg salbutamol using a metered dose inhaler (MDI) connected to an aerosol chamber. All patients had a significant reversibility in their airflow impairments as shown by an increase in FEV₁ superior to 15% of the initial value after inhaling 400 µg salbutamol. The atopic status was assessed by skin prick tests to the common allergens of our region. None of the patients had a history of a respiratory disease other than asthma, or did they use any other pulmonary medications than inhaled corticosteroids and short-acting β_2 -agonists or ipratropium bromide on-demand for their asthma during the study. All patients were nonsmokers or ex-smokers (for more than 12 months, with a smoking history of <5 pack-years). The patients were included for at least 2 months after an exacerbation (defined as an unscheduled visit to a physician or emergency room, requirement for a systemic corticosteroid course, or a modification in the long-term treatment) and had not had symptoms of a respiratory tract infection for at least 4 weeks before the study.

Twenty-four normal nonsmoking volunteers (21–45 years, 13 females), matched with the asthmatic patients for age and sex, were enrolled. None of them had any history of respiratory (including cigarette smoking) or allergic disease (negative clinical history and skin prick test). The study was approved by the Hospital Medical Ethics Committee of the Montpellier University Medical Center, and informed consent was given by all patients.

Design

Patients were enrolled after consultation in our department, including the first visit during which they filled in the ACQ. Long-acting β_2 -agonists and theophylline preparations, if used, were discontinued at least 72 h before this visit. Prebronchodilator and postbronchodilator FEV₁ were measured. The inhaled corticosteroid medication of all patients was recorded and not modified for at least 4 weeks in order to eliminate any exacerbation. At the second visit, the patients inhaled salbutamol (400 µg via MDI connected to a spacer device) at least 20 min before body plethysmography and

subsequent SBNTs were performed. The reproducibility of the flow–volume curve and SBNT were assessed the day after.

Lung function and inhalation challenge tests

Spirometry was performed in the laboratory to measure the slow inspiratory vital capacity (VC) and postbronchodilator FEV₁ with a dry rolling seal spirometer (Morgan Spiroflow, Kent, UK). Distal airflow indices, classically associated with small airways obstruction, derived from the spirometry curves were recorded (maximum mid-expiratory flow between 25% and 75% of the VC, MMEF25–75, and V50, the expiratory flow at 50% of the VC). The total lung capacity (TLC), functional residual capacity (FRC), and residual volume (RV) were assessed with a standardized body plethysmography (Masterscreen FRC; Jaeger, Breda, the Netherlands). The SBNT was conducted with a dry rolling-seal spirometer (Morgan Spiroflow) filled with 100% oxygen and equipped with an N2 meter (Morgan) connected to the mouthpiece, thus allowing continuous sampling. During this test, patients performed a slow, full inspiratory and expiratory VC maneuver at inspiratory and expiratory flow rates of approximately 0.5 l/s. The expiratory N2 concentration was plotted against volume changes between TLC and RV, and the slope (dN2) of the N2 alveolar plateau was calculated by computer analysis of best-fit line through phase III of the expiratory volume–concentration curve. The first departure from this straight line, assessed by a computer analyser, which calculated the best rupture-point between slopes, was considered as indicative of airway closure, and closing volume (CV; phase IV) and closing capacity (CC; RV + CV) were calculated. The measurements were accepted only if the VC during the single breath N2 test was within 10% of the VC measured at spirometry. All volumes were corrected for body temperature pressure saturation (BTPS) with water vapor.

Statistical analysis

Asthmatics and healthy control subjects were compared first in order to eliminate nonspecific functional findings. The CV and CC were expressed as ratios to VC (CV/VC) and TLC (CC/TLC), respectively. The ACQ scores were expressed as the sum of the 7-items, as previously recommended. Previous year exacerbations were recorded according to our outpatient clinic database while respecting the definition mentioned above. The body mass index (BMI) was calculated according to Harris and Benedict (16). Daily doses of inhaled steroid were recorded on the basis of the last 4 weeks. All data are expressed as mean $\pm$ SD unless otherwise indicated. Between-group comparisons were performed first with nonparametric tests, as appropriate. Differences were considered to be statistically significant at a *P*-value of 0.05. Sample size estimations made with published data revealed that at least 11 patients in each group would be sufficient to detect a difference between groups of at least 25% in SBNT (two-sided α : 0.05, one-sided β : 0.80) (11).

Results

Subject characteristics and functional measurements

All patients were life-long nonsmokers and all asthmatics were allergic. The first asthma group consisted of 12 patients with less than two exacerbations the previous year, which was compared with the group of 12 patients who had experienced at least two exacerbations during the previous year. The asthma duration was

14 ± 9 years, and 9 ± 8 years without anti-inflammatory treatment for the whole group.

The day-to-day reproducibility of the SBNT results assessed according to Bland and Altman (17) was satisfactory [intraclass coefficient of correlation for dN₂: 0.86 (0.81–0.91); Fig. 1].

The healthy control volunteer characteristics are presented in Table 1. They had significantly higher airflows and lower lung volumes than the asthma patients (postbronchodilator values at visit 2 are presented). Moreover, the SBNT was significantly different for dN₂ and CC/TLC in healthy vs asthmatics patients (Table 1).

There were no significant differences in anthropometrical characteristics between the two groups of asthma patients (sex, age, weight, height, and BMI). Severity in both groups was not significantly different (2.35 ± 1.21 vs 2.55 ± 0.93).

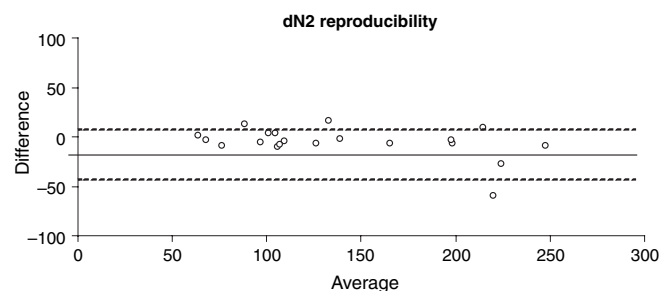


Figure 1. Day-to-day reproducibility of dN₂ values according to Bland and Altman (17).

Table 1. Functional characteristics of asthma patients compared with healthy control subjects

	Asthmatic patients	Control subjects	P-value
FEV ₁	3030 (2740–3510)	4000 (3670–4160)	
%predicted	92 (89–98)	105 (97.25–113.25)	0.04
MMF25–75	2520 (2015–2775)	4160 (3753–4820)	
%predicted	57 (52–64)	96 (82.5–110)	0.001
TLC	6210 (5420–7470)	6210 (5350–6730)	ns
%predicted	112 (107–119)	105 (96–113)	
FRC	3510 (2900–4180)	3490 (2900–3880)	
%predicted	132 (111–138)	121 (113–138)	0.05
RV	2100 (1690–2330)	1730 (1560–1840)	
%predicted	134 (116–140)	108 (97–122)	0.003
dN ₂	1.4 (1.0–2.5)	1.1 (0.9–1.3)	
%predicted	110 (99–190)	94 (75–111)	0.02
RV/TLC (%predicted)	134 (116–140)	105 (95–111)	0.03
CV/VC (%predicted)	81 (65–111)	79 (68–116)	ns
CC/TLC (%predicted)	124 (117–148)	117 (112–123)	0.04

Values are given as absolute volumes and as a percentage of predicted values. The data are mean values with 95% confidence interval. P-values were calculated with a Mann–Whitney U-test.

FEV₁, forced expiratory volume in 1 s; MMF25–75, maximum mid-expiratory flow between 25% and 75%; TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume; CV, closing volume; VC, vital capacity; dN₂, slope of phase III of the single breath nitrogen washout test; CC, closing capacity.

Other functional indices did not differentiate the asthma groups: no differences were observed for flows at visits 1 and 2 (MMF25–75, V50, FEV₁), volumes (RV, TLC and RV–TLC ratio, FRC). The CV/VC and CC/TLC did not significantly differ between groups (Table 2).

Clinical outcomes

The two groups differed significantly only with respect to: (i) daily dose of inhaled steroids, (ii) dN₂ and (iii) ACQ scores (Table 2).

Correlations between results

In the two asthma groups, there were no significant correlations between dN₂ and FEV₁ (ρ : –0.04). The MMF25–75% and V50 were not correlated with dN₂ (ρ : –0.03, P = 0.89, and ρ : –0.07, P = 0.75, respectively) but with FEV₁ (P = 0.002 and 0.02, respectively). In addition, a significant correlation was found between dN₂ and the ACQ score (ρ : 0.62, P = 0.003; Fig. 2) and with the number of exacerbations (ρ : 0.711, P = 0.0008; Fig. 3). The dN₂ and RV/TLC presented a weak but significant correlation (ρ : 0.49, P = 0.026). Severity was not correlated with any pulmonary function tests. We then observed that patients with high dN₂ values were much more likely to have a high exacerbation rate (Fig. 4).

Table 2. Clinical and functional characteristics of the two asthma groups with their exacerbation rate

	Rare exacerbations (<2/year)	Frequent exacerbations (≥2/year)	P-value
Number of subjects	12	12	
Sex	6M/6F	5M/7F	
Age	28 (24–36)	30 (25–47)	ns
BMI	23 (21–24)	22 (21–25)	ns
Severity	2.34 (1.13–3.55)	2.55 (1.83–3.48)	ns
Duration of asthma (years)	14 (2.5–17)	13 (8.5–23)	ns
Duration without ICS (years)	9 (2–14)	8 (6–18)	ns
Dose of ICS (µg/d)	550 (200–1100)	1200 (750–1525)	0.05
FEV ₁ %predicted	92 (90–98)	92.5 (88.5–98.5)	ns
MMF25–75 %predicted	57 (53–65)	57 (55–65)	ns
TLC %predicted	110 (107–124)	112.5 (105–119)	ns
FRC %predicted	121 (111–133)	134.5 (114–176)	ns
RV %predicted	119 (107.5–138.5)	137 (129.5–150.5)	ns
dN ₂ %predicted	100 (83–105)	195 (141–212)	0.0005
RV/TLC	113 (102–130)	126 (111–137)	ns
CV/VC %predicted	91 (63–111)	70 (64–129)	ns
CC/TLC %predicted	118 (110–143)	129 (124–148)	ns
Control (ACQ Juniper value)	5.2 (2.39–7.94)	12 (7.21–16.78)	0.02

Data are mean values with 95% confidence interval. P-values were calculated with Mann–Whitney U-test.

BMI, body mass index; ICS, inhaled corticosteroid; FEV₁, forced expiratory volume in 1 s; MMF25–75, maximum mid-expiratory flow between 25% and 75%; TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume; dN₂, slope of phase III of the single breath nitrogen washout test; CV, closing volume; VC, vital capacity; CC, closing capacity; ACQ, asthma control questionnaire.

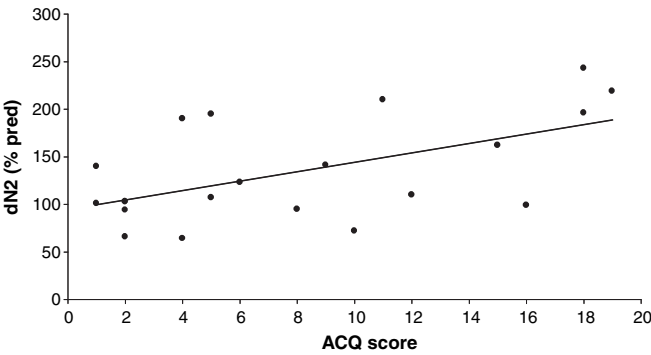


Figure 2. Spearman's correlations between the dN2 (percentage of predicted value) and asthma control questionnaire (ACQ) score.

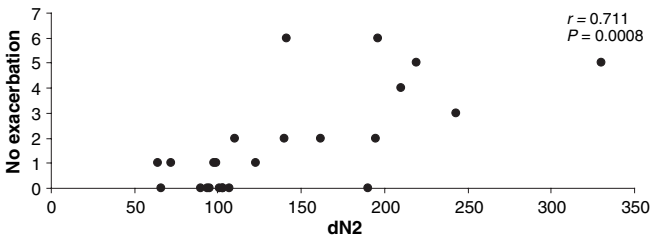


Figure 3. Spearman's correlation between dN2 values (percentage of predicted value) and exacerbation numbers.

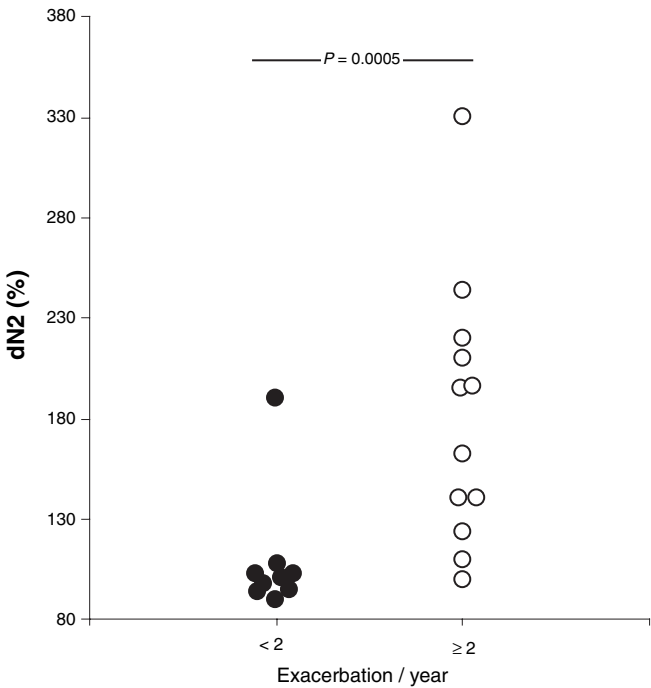


Figure 4. Significant differences between patients with respect to their dN2 values and exacerbation rates.

Discussion

This study provides new insight into the abnormal dN2 values noted in asthma patients. The present results indicate that an abnormal dN2 value can be associated with an increased ACQ score, a high exacerbation rate, and the need for a high daily dose of inhaled steroids, but this is not influenced by the severity of the disease or its duration.

It appears that these functional abnormalities have some specificity because they differentiate healthy volunteers and patients. We would like to stress that the methodological aspects of our study were rigorous. Patients were well known in our department and carefully checked before inclusion. The ACQ scores were not known by the investigator who performed the SBNT. It could be argued that whole body plethysmography we used could have overestimated the lung volumes, especially in the patients with a predisposition for early airway closure. This could be an additional explanation for the differences between our results and those of In't Veen et al. (11). In difficult to control *vs* stable severe asthmatics, all normalized at 1000 µg/day of fluticasone propionate, they observed increased CV/VC and CC/TLC ratios, without any differences in dN2 values. In the present study, patients kept their usual treatment dose, had different severities, so the conditions were close to our everyday practice. It is known that anti-inflammatory treatment can affect the CV, which could further explain the differences between In't Veen et al.'s results and ours. The SBNT tests were carefully explained and repeated as recommended, and fulfilled the quality and reproducibility criteria for all patients.

Insufficient reproducibility of SBNT has been reported (18), but computerized plotting of the curves led to satisfactory results, according to a previously published method (12). This good reproducibility enhance the significance of the message we provide suggesting that small airways could maintain this abnormality at least on 2 following days.

The correlation obtained between ACQ and dN2 (and not with other functional tests) provides evidence that dN2 is actually a sensitive test for detecting 'silent' abnormalities at the small airway level. Finally, it was found that dN2 did not assess the same level as that measured via specific conductance, FEV₁, and especially V50 or MMEF25–75, but may have some relationships with alveolar hyperinflation (i.e. RV/TLC). The indices derived from the flow–volume curves were clearly not sufficiently discriminative in the assessment of small airway abnormalities, as previously mentioned (4). Then, an isolate abnormality of V50 or MMEF25–75 should not be considered to reflect small airways obstruction. Monitoring these indices in asthma might clearly be of no additional value in patients with normal FEV₁ values. The dN2 has previously been associated with regional heterogeneity of ventilation, induced by small airways

abnormalities (2). We did not measure elastic recoil forces, which precludes more important extrapolation on the structure–function relationships explored in this study. The absence of comorbidity or smoking history in all patients, without difference for BMI, made us confident in our results.

Our results indicate that a sensitive test can detect functional abnormalities in patients who highly perceive symptoms and require high medication levels. This study highlights the importance of routinely assessing asthma control because uncontrolled patients actually present with functional abnormalities undetected by FEV₁ alone.

Strong efforts should be made in these difficult cases to focus on the small airways, rather than considering that a normal FEV₁ measurement is a sufficient basis to consider that a treatment is effective enough (19).

In conclusion, the dN₂ value can be a good functional indicator related to asthma control and exacerbation rate in global asthma population regardless of the severity and this factor should be more carefully checked in symptomatic patients with normal FEV₁ values.

The present results indicate that asthma therapies focused on the distal part of the lung should be of interest.

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