Influence of Lung Parenchymal Destruction on the Different Indexes of the Methacholine Dose-Response Curve in COPD Patients

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Influence of Lung Parenchymal Destruction on the Different Indexes of the Methacholine Dose-Response Curve in COPD Patients*

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Study objectives: **The interpretation of nonspecific bronchial provocation dose-response curves in COPD is still a matter of debate. Bronchial hyperresponsiveness (BHR) in patients with COPD could be influenced by the destruction of the parenchyma and the augmented mechanical behavior of the lung. Therefore, we studied the interrelationships between indexes of BHR, on the one hand, and markers of lung parenchymal destruction, on the other.**

Patients and methods: **COPD patients were selected by clinical symptoms, evidence of chronic, nonreversible airways obstruction, and BHR, which was defined as a provocative dose of a substance (histamine) causing a 20% fall in FEV_1 (PC_{20}) of ≤ 8 mg/mL. BHR was subsequently studied by methacholine dose-response curves to which a sigmoid model was fitted for the estimation of plateau values and reactivity. Model fits of quasi-static lung pressure-volume (PV) curves yielded static lung compliance (Cstat), the exponential factor (KE) and elastic recoil at 90% of total lung capacity (P90TLC). Carbon monoxide (CO) transfer was measured with the standard single-breath method.**

Results: **Twenty-four patients were included in the study, and reliable PV data could be obtained from 19. The following mean values (± SD) were taken: FEV_1, 65 ± 12% of predicted; reversibility, 5.6 ± 3.1% of predicted; the PC_{20} for methacholine, 4.3 ± 5.2 mg/mL; reactivity, 11.0 ± 5.6% FEV_1/doubling dose; plateau, 48.8 ± 17.4% FEV_1; transfer factor, 76.7 ± 17.9% of predicted; transfer coefficient for carbon monoxide (KCO), 85.9 ± 22.6% of predicted; Cstat, 4.28 ± 2.8 kPa; shape factor (KE), 1.9 ± 1.5 kPa; and P90TLC, 1.1 ± 0.8 kPa. We confirmed earlier reported relationships between Cstat, on the one hand, and KE ($p < 0.0001$), P90TLC ($p = 0.0012$), and KCO percent predicted ($p = 0.006$), on the other hand. The indexes of the methacholine provocation test were not related to any parameter of lung elasticity and CO transfer.**

Conclusion: **BHR in COPD patients who smoke most probably is determined by airways pathology rather than by the augmented mechanical behavior caused by lung parenchymal destruction.**

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Key words: bronchial provocation tests; COPD; dose-response relationship; forced expiratory flow rates; human; lung compliance; lung volume measurements; methacholine bromide/diagnostic use; pulmonary diffusing capacity

Abbreviations: BHR = bronchial hyperresponsiveness; CO = carbon monoxide; Cstat = static lung compliance; FRC = functional residual capacity; IVC = inspiratory vital capacity; KCO = transfer coefficient for carbon monoxide; KE = shape factor; LE = linear-exponential; PC_{20} = provocative concentration of a substance causing a 20% fall in FEV_1; P90TLC = elastic recoil pressure at 90% of total lung capacity; PV = pressure-volume; TLC = total lung capacity; TLCO = transfer factor for carbon monoxide.

Bronchial hyperresponsiveness (BHR) is present in patients with asthma and COPD.[1] Approximately half of the subjects with COPD in a general population have BHR.[2] In the Lung Health Study,[3] BHR was noted in 85.1% of the women and 58.9% of the men with mild-to-moderate airflow limitation. The estimation of BHR is important for the diagnosis of asthma and for determining asthma severity, whereas the meaning of BHR for the clinical management of COPD is still unclear.[4] COPD patients with BHR appear to be prone to a more rapid decline of their FEV_1.[5]

Clinical studies suggest that BHR in patients with COPD differs from BHR in patients with asthma.[6–9] For example, in patients with COPD, BHR for

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physiologic stimuli (*eg*, cold air) usually is not found in the presence of BHR for pharmacologic agents (*eg*, histamine). In patients with asthma, both types of stimuli cause bronchoconstriction.[6,7] The explanation for these differences might be found in the different pathologic changes in the airways and in the lung parenchyma of asthma and COPD patients.[1] The main and clearest difference between asthma and COPD is destruction of the lung parenchyma in COPD, leading to emphysema and loss of lung elasticity. In patients with COPD, compared to those with asthma, there is a relationship between baseline FEV_1 and the level of BHR.[8,9] The FEV_1 is, however, not a good predictor of the amount of parenchymal destruction and loss of elastic recoil.[10–13] The most reliable test for lung elasticity is the direct estimation of quasi-static esophageal pressure-volume (PV) curves.[10,11,14] The destruction of parenchymal tissue also is shown by the impairment of carbon monoxide (CO) transfer.[15–18] We performed these lung function tests in COPD patients who smoked, who fulfilled the established clinical and functional criteria for COPD, and who also had a provocative concentration of a substance (histamine) causing a 20% fall in FEV_1 (PC_{20}) of ≤ 8 mg/mL. In subsequent methacholine dose-response curves, not only PC_{20} (sensitivity) but also maximal bronchoconstriction (plateau) and the slope of the curve (reactivity) were estimated, because these factors should yield additional information on the causative mechanisms of BHR.[19]

The aim of our study was to investigate the influence of the impairment of lung parenchymal structure on BHR by the estimation of the interrelationships between indexes related to lung parenchymal destruction (lung elasticity and CO transfer) and indexes from methacholine log-dose response curves.

Materials and Methods

COPD patients were recruited according to generally accepted clinical and functional criteria.[20] The inclusion criteria were the following: chronic productive cough; age between 40 and 70 years; current smokers; negative skin tests for standard inhalation allergens; FEV_1 or FEV_1/inspiratory vital capacity (IVC) ratio $\leq 70\%$ of the predicted normal value; reversibility of FEV_1 of $< 10\%$ predicted after 750 μg terbutaline administered by metered-dose inhalation; and nonspecific BHR, defined by a PC_{20} for histamine of ≤ 8 mg/mL. Exclusion criteria were the following: a history of asthma; complaints of wheezing; radiographic signs of bullous emphysema; recent respiratory tract infection; and recent or concurrent usage of anti-inflammatory drugs. Eligible patients refrained from oral anti-inflammatory medication at least 3 months and from inhaled glucocorticoids for at least 6 weeks before the start of the study.

The study was approved by the Medical Ethics Committee of the University Hospital Dijkzigt, and written informed consent was obtained from all participants.

Functional tests included the following: spirometry (total lung capacity [TLC], functional residual capacity [FRC], RV, IVC, FEV_1); reversibility test (FEV_1 percent predicted); single-breath CO transfer (transfer factor of the lung for CO [TLCO] and transfer coefficient for CO [KCO]); and PV curves. Spirometry was measured with the multiple-breath, closed Helium wash-in method, using a water-sealed spirometer (model D53R; Lode; Groningen, The Netherlands) and with the patient in sitting position. The lung volumes were corrected to body temperature and ambient pressure, saturated with water vapor. All reference values were derived from the standards of the European Community for Steel and Coal.[21,22]

Histamine and methacholine provocation tests were performed according to the 2-min tidal breathing method that was first described by Cockcroft et al.[23,24] The PC_{20} for histamine was used as an inclusion criterion. Because of lesser side effects, methacholine was used for obtaining as complete as possible dose-response curves.[25] After inhalation of an isotonic saline solution, doubling concentrations of histamine-sulfate or acetyl-β-methylcholine-bromide were administered, starting with doses of 0.03 mg/mL. Methacholine was prepared by our hospital pharmacy department. Solutions were stored at 4°C and were used at room temperature. Aerosols were generated by a nebulizer (model 646; DeVilbiss Co; Somerset, PA) (measured output, 0.13 mL/min) and were inhaled by tidal breathing over a 2-min period at 5-min intervals. The response to methacholine or histamine was measured as the change in FEV_1 expressed as a percentage of the initial value. The histamine provocation test was interrupted if a 20% fall in FEV_1 occurred before or at a dose of 8 mg/mL. The methacholine tests were continued up to a concentration of 256 mg/mL but were interrupted if the FEV_1 fell by $> 60\%$ or if unpleasant side effects occurred.

From the methacholine provocation tests, the log2 concentration and the measured percentage of changes in FEV_1 were imported to a computer program that fitted a sigmoid function (cumulative Gaussian distribution) to the data.[26] Reactivity (the slope of the curve), and plateau values (maximal bronchoconstriction) were taken from this model fit, whereas the PC_{20} was calculated from the measured data by linear interpolation of adjacent data points.

The lung elasticity measurements were performed immediately after the reversibility test. This sequence was chosen for reasons of standardization and in order to minimize the potentially disturbing effect of airway closure on lung elasticity. Quasi-static deflation exercises were performed according to the method that has been described before.[27] In short, the transpulmonary pressure was measured via a transducer (model P45; Validyne Engineering Corp; Northridge, CA) coupled to a balloon that was positioned in the lower third of the esophagus. The simultaneous recording of volume changes was obtained during a slow expiration (*ie*, expiration not exceeding 0.3 L/s). Selected curves were smoothed by drawing a line by hand through catacrotic points of the cardiac pulsations on the curve. Volume data were obtained at equal transpulmonary pressure intervals, yielding an average of 10 to 30 data points up to the TLC level. A linear-exponential (LE) and an exponential model were fitted to the measured data. The LE model gave the most accurate fit to experimental curves and was, therefore, used for the estimation of static lung compliance (Cstat) and volume-dependent recoil pressures.[27,28] For this fit, the curve was considered to be composed of a linear part, from the first data points on starting at the FRC level, and an exponential part, starting at the higher volume level. Cstat was obtained from the linear part if four or more data points contributed to that part. In a minority of cases, we calculated Cstat by hand as the slope between FRC and FRC + 0.5 L. The elastic recoil pressure at 90% of TLC (P90TLC) also was derived from the LE model fit. This pressure

index was considered to be the elastic recoil index with the lowest variation coefficient.[29] Additionally, we prefer to use the P90TLC values above the recoil pressure at TLC because P90TLC is less dependent on inspiratory muscle force. The shape factor (KE) was determined from the following generally used exponential equation:

$$V = Vmax\{1 - \exp[KE(P - P0)]\}$$

where Vmax is the asymptotic value (in liters) and P0 is the intercept with the P axis at V = 0 kPa.

KE can be considered as an elasticity index, independent of lung size.[28] For the fit with the exponential model, we used the same (measured) input data as for the LE model fit. KE was considered as an additional elasticity index.

Linear regression analysis between variables, pairwise multivariate correlation, and statistical significance were calculated with the use of a package of statistical software (Statistical Graphics Corp; Rockville, MD). Test results were considered statistically significant at $p < 0.05$.

Results

Twenty-four patients were included in the study. From 19 patients, we obtained reliable PV curves; the remaining patients did not tolerate the esophagus balloon long enough or showed effects of swallowing that hampered an accurate interpretation of the data. The mean age of the patients was 56 years (Table 1). The mean FEV_1 was 65% of predicted. One patient had an $FEV_1 > 70\%$ of predicted but was included because his FEV_1/IVC ratio was 0.51. The mean reversibility of FEV_1 after terbutaline inhalation was 5.6%. Four patients showed no reversibility at all.

The patients had moderate or severe BHR (Table 1). The mean PC_{20} for methacholine was higher than that for histamine (4.3 vs 1.7 mg/mL, respectively). This difference also was reported in an earlier study of smokers with mild chronic airflow limitation.[30] After correction for the difference in molecular-weight (1 mg of the bromide compound is equivalent to 0.82 mg of the chloride compound), the corrected mean bromide value of the PC_{20} became 3.5 mg/mL.

The mean plateau value was 48.8% of the FEV_1. In Figure 1, we present a curve in which the fitted plateau is almost equal to the measured data (Fig 1, *top*) and a curve in which the plateau value is derived from extrapolation (Fig 1, *bottom*). If the experimental plateau estimate was defined by the mean value of the last two provocative concentrations with a variation of $< 5\%$, we observed that the fitted plateau was almost equal to the experimental plateau estimate in 13 of the 24 dose-response curves.

Cstat ranged from 1.06 to 10.52 kPa, which indicates a range from moderately low to clearly increased if a normal range of 1.5 to 2.5 kPa is taken into account.[21] Mean Cstat was 4.6 kPa (Table 1). TLCO was between 34% of predicted and 106% of predicted, and KCO ranged from low (43% of predicted) to higher than normal (139% of predicted).

Statistically significant correlations existed among all the parameters of the PV curve (Table 2). The strongest correlation was between Cstat and KE ($R = 0.81$; $p < 0.0001$). The KCO percent predicted correlated strongly with Cstat ($R = -0.60$; $p = 0.006$; Table 2) but not with KE and P90TLC. The TLCO percent predicted showed no significant correlation with Cstat, KE, or P90TLC.

The indexes of BHR (PC_{20}, reactivity, and plateau value) were tested for correlation with the indexes of the PV curve (Cstat, KE, and P90TLC), CO transfer (TLCO and KCO), and FEV_1. In Table 3, we present the correlations between Cstat and the BHR in-

Table 1—*Smoking Habits, FEV_1, Reversibility, BHR, CO Transfer, and Lung Elasticity Data*

Data	No. of Patients	Mean ± SD	Median	Range
Age, yr	24	55.5 ± 8.5	54	42–69
Actual smoking, cigarettes/d	24	15.6 ± 6.8	13	6–30
Pack-years	24	23 ± 10.5	23	5–50
FEV_1, % predicted	24	64.5 ± 11.9	65	34–93
Reversibility, % predicted	24	5.6 ± 3.1	5.5	0–9.8
PC_{20}				
Histamine	24	1.66 ± 2.00	0.87	0.11–8
Methacholine	24	4.27 ± 5.2	1.46	0.4–17.4
log2 PC_{20} methacholine	24	1.07 ± 1.74	0.53	−1.3–4.1
Reactivity, % FEV_1/doubling dose	24	11.0 ± 5.6	8.98	3.9–26.8
Plateau, %FEV_1	24	48.8 ± 17.4	48.3	20.8–95.7
Cstat, kPa	19	4.6 ± 2.8	4.1	1.1–10.5
KE, kPa	19	2.5 ± 1.5	2.2	0.7–6.3
P90TLC, kPa	19	1.1 ± 0.8	0.8	0.4–2.7
TLCO, % predicted	24	76.7 ± 17.9	75	34–106
KCO, % predicted	24	85.9 ± 22.6	86	43–139

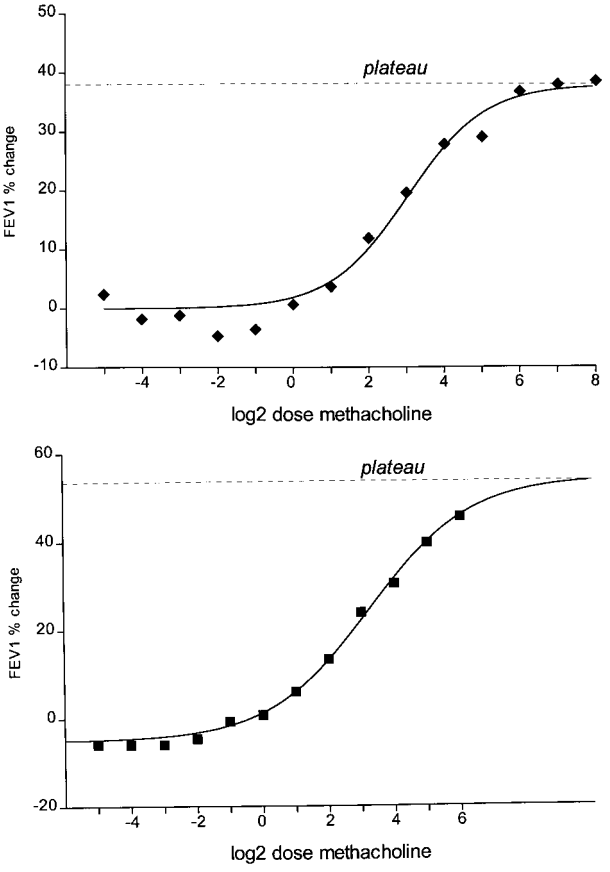

FIGURE 1. Two examples of sigmoid fitting of the methacholine provocation tests. *Top*: the measured data (◆) are almost equal to the fitted plateau. *Bottom*: the measured data (■) show that the plateau was not reached during the test.

dexes. No significance was found and no significance was found for the additional elasticity indexes and diffusion parameters.

There was a significant correlation between the FEV_1 percent predicted, on the one hand, and log2 PC_{20} for histamine and log2 PC_{20} for methacholine, on the other hand ($R = 0.44$, $p = 0.024$; and $R = 0.46$, $p = 0.023$, respectively; Table 3). There was also a significant (negative) correlation between the FEV_1 percent predicted and reactivity ($R = -0.52$; $p = 0.008$; Table 3). The correlation between the FEV_1 percent predicted and the plateau value was not significant (Table 3).

Table 3—*Correlations of the Indexes of BHR With Quasi-Static Compliance and FEV_1 by Pairwise Multivariate Analysis

Variable 1	Variable 2	No. of Patients	*R* Value	p Value
PC_{20} hist	Cstat	19	0.282	0.24
PC_{20} meth	Cstat	19	0.061	0.82
Reactivity	Cstat	19	0.291	0.24
Plateau	Cstat	19	0.321	0.19
PC_{20} hist	FEV_1	24	0.483	0.01
PC_{20} meth	FEV_1	24	0.470	0.02
Reactivity	FEV_1	24	−0.522	0.0075
Plateau	FEV_1	24	−0.194	0.35

*hist = histamine; meth = methacholine.

Because smoking is the most important risk factor for emphysema, we looked at paired correlations among smoking data, CO transfer, and indexes of the PV curve. There was a significant correlation only between KE and the actual number of cigarettes smoked per day ($R = 0.53$; $p = 0.019$; Table 2).

DISCUSSION

The aim of our investigation was to study the interrelationships among indexes describing BHR, lung elasticity, and CO transfer in patients with COPD. For the estimation of BHR and the degree of impairment of lung mechanics, detailed information was obtained by fitting models of methacholine dose-response curves and quasi-static PV curves.

Several mechanisms have been proposed for explaining enhanced bronchoconstriction as a reaction to inhaled stimuli.[1,19,31] Detailed analysis of methacholine log-dose response curves is supposed to offer additional information on the causative mechanisms

Table 2—*Correlations of the Indexes of the PV Curves, CO Transfer, and Smoking by Pairwise Multivariate Analysis

Variable 1	Variable 2	No. of Patients	*R* Value	p Value
Cstat	KE	19	0.821	< 0.0001
Cstat	P90TLC	19	−0.687	0.0012
TLCO, % predicted	Cstat	19	−0.290	0.23
TLCO, % predicted	KE	19	−0.254	0.29
TLCO, % predicted	P90TLC	19	0.123	0.62
KCO, % predicted	Cstat	19	−0.604	0.006
KCO, % predicted	KE	19	−0.414	0.077
KCO, % predicted	P90TLC	19	0.438	0.061
KE	Act smoking	19	0.534	0.019

*Act smoking = actual smoking (cigarettes/day).

of BHR.[19] PC_{20} and reactivity are considered to be determined by prejunctional mechanisms, and the plateau value is more dependent on postjunctional mechanisms.[19] In patients with COPD, both prejunctional mechanisms (*ie*, epithelial damage, neural control, and inflammation) and postjunctional mechanisms (*ie*, loss of lung elasticity, swelling of airway wall, and intraluminal secretions) can be responsible for the occurrence of BHR. Because lung elasticity in stable patients with asthma is not appreciably disturbed, this would be an attractive explanation for the occurrence of BHR in patients with COPD and to relate it to the loss of elastic recoil. Theoretically, a decrease in lung elasticity can facilitate an amplified bronchomuscular response.[31]

First, we have studied the functional indexes of lung parenchymal destruction.

Some degree of emphysema, which is present in patients with mild COPD, already influences the PV relationships.[10,11,14] A PV curve can be obtained with relatively simple techniques but has the disadvantage of being an invasive test. The reproducibility of estimates, especially of KE, was reported to be good, at least for healthy adults.[29,32,33] KE was found to be a good indicator for the presence of mild emphysema.[10,15,32] We found that Cstat and P90TLC from the LE model fit and KE from the exponential model fit correlated well with each other, indicating that these indexes were linked to elastic properties of the lung (Table 2).

An additional index of lung parenchymal destruction is CO diffusion. Berend et al[16] were the first to report a correlation between CO transfer and severity of emphysema. Others have confirmed the relationship between emphysema and KCO.[15,17,18] We found also a significant correlation of Cstat with KCO (Table 2). KCO can be considered as an index, related to structural aspects of the lung parenchyma, whereas TLCO is a measure of overall gas transport.

In this study, we also tested the indexes of the impairment of lung parenchymal structure for correlation with cigarette smoke exposure, pack-years and actual smoking. There was a significant correlation of KE only with actual smoking (Table 2). There are few data about the correlation of smoking with parenchyma impairment. In one study, there was no detectable effect of smoking on lung elastic recoil in healthy men.[34] Other investigators have reported a quantitative relationship between the total exposure to cigarette smoke and both alveolar and airway pathologic features in a necropsy study.[35] So, although the assumption is plausible that there is a relationship between cigarette smoke exposure and loss of elastic recoil, it is not yet clear how this influences the derivatives of the PV curve. We have assumed that differences in vulnerability of the lung parenchyma to cigarette smoke influence the measured loss of elastic recoil more than the amount of cigarette smoke exposure.

In patients with α_1-antitrypsin deficiency, Cheung et al[36] found a relationship between the loss of elastic recoil and maximal airway narrowing (plateau). It should be noted that their patient group was selective; five of eight patients had an $FEV_1 > 80\%$ of predicted, patients were clinically stable, and patients were ex-smokers or nonsmokers. These patients seemingly had only parenchymal disease. The effect of the involvement of airways disease was shown in a study by Eidelman et al.[13] They described different patterns of mechanical abnormalities between smoking and nonsmoking patients with α_1-antitrypsin deficiency. In COPD patients, especially in those who smoke, it is likely that there are both parenchymal and airway changes. In our study and in the study by Koyama et al[37], no significant correlations were found among indexes of the PV curve, on the one hand, and BHR, on the other hand. This means either that PV curves do not represent elastic recoil changes or that BHR is also influenced by airway pathology. There are several arguments that support the last mechanism. First, as discussed above, indexes of PV curves have been found to correlate with pathologic assessment of lung parenchyma. Second, the significant correlations among the different indexes of the PV curve, and between elasticity and KCO, indicate that our results are a good reflection of the loss of elastic recoil of the lung. Third, there were significant relationships between the determinant of airways obstruction (FEV_1) and PC_{20} (Table 3).

In the present study, not only were the PC_{20} for histamine and the PC_{20} for methacholine correlated with the FEV_1 percent predicted, but also with reactivity. The first correlation was reported elsewhere[2,8,9] and was found also by Cheung et al[36] and Koyama et al.[37] This indicates that the definition of PC_{20} as a 20% fall of the starting FEV_1 makes the outcome highly dependent on measurement of FEV_1 in patients with a low FEV_1. Our finding that reactivity (the slope of the dose-response curve) is steeper at a lower FEV_1 percent predicted, indicates that reactivity also was hampered by the way in which the response is expressed. This appeared to be distinct for the plateau value, which was not correlated with the starting FEV_1 (Table 3). The clinical significance of the level of a plateau value is that it is a measure of the maximal acute bronchoconstriction that can be provoked in an individual. The application of the plateau value in combination with the PC_{20} for methacholine has been suggested for the distinction between asthma and COPD.[1] While BHR can be found both in patients with asthma and

patients with COPD when considering PC_{20}, the plateau value usually is not reached in patients with moderately severe or severe asthma. In our study of patients with COPD who have moderately severe BHR, the plateau was reached in the majority of the patients, but not in all. It appears, therefore, that the estimation of the plateau does not always provide a clear distinction between asthma and COPD.

Because none of the indexes of BHR is related to any of the functional data of lung elasticity or CO transfer in COPD patients who smoke, airway pathology determines the response to methacholine at least to such an extent that it overrules a possible correlation with parenchymal destruction. The nature and extent of airways disease seem to be more important for the occurrence of BHR in patients with COPD than does parenchymal pathology. Taylor et al[38] have compared PC_{20} for methacholine *in vivo* with the function of bronchial smooth muscle strips from surgical specimens. No correlation was found, which led to their conclusion that smooth muscle pathophysiologic changes were not responsible for BHR in COPD. One other study provided evidence that BHR in patients with emphysema is related to differences among types of emphysema and to the cell infiltrate in the airway walls.[39]

In conclusion, we found no relationship between the impairment of lung parenchymal structure, either from PV curves or CO diffusion, and indexes of BHR. Nonspecific BHR in COPD patients who smoke is determined by small airway pathology to such an extent that it overrules a possible correlation with parenchymal impairment. The combination of our findings with those from clinicopathologic studies suggests that the plateau value (maximal airway constriction) is a better indicator of small airways pathologic changes than are PC_{20} and reactivity.

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