Airway occlusion assessed by single breath N2 test and lung P-V curve in healthy subjects and COPD patients

Matteo Pecchiari[a], Dejan Radovanovic[b], Pierachille Santus[b] and Edgardo D'Angelo[a,*]

[a]Dipartimento di Fisiopatologia e dei Trapianti, Università degli Studi di Milano, Milan, Italy.

[b]Dipartimento di Scienze Biomediche, Università degli Studi di Milano, Milan, Italy.

E-mail addresses: matteo.pecchiari@unimi.it; danko86@hotmail.com; pierachille.santus@unimi.it

DOI: http://dx.doi.org/10.1016/j.resp.2016.09.006

Corresponding author: Edgardo d'Angelo, Università degli Studi di Milano, Dipartimento di Fisiopatologia e dei Trapianti, via Mangiagalli 32, 20133 Milan, Italy

E-mail address: edgardo.dangelo@unimi.it

Abstract

Purpose: To determine whether the analysis of the slow expiratory transpulmonary pressure–volume (P_L-V) curve provides an alternative to the single-breath nitrogen test (SBN) for the assessment of the closing volume (CV).

Methods: SBN test and slow deflation P_L-V curve were simultaneously recorded in 40 healthy subjects and 43 COPD patients. Onset of phase IV identified CV in SBN test (CV_{SBN}), whereas in the P_L-V curve CV was identified by: a) deviation from the exponential fit (CV_{exp}), and b) inflection point of the interpolating sigmoid function (CV_{sig}).

Results: In the absence of phase IV, COPD patients exhibited a clearly discernible inflection in the P_L-V curve. In the presence of phase IV, CV_{SBN} and CV_{exp} coincided (CV_{SBN}/CV_{exp} =1.04±0.04 SD), whereas CV_{sig} was systematically larger (CV_{sig}/CV_{exp} =2.1±0.86).

Conclusion:, The coincidence between CV_{SBN} and CV_{exp}, and the presence of the inflection in the absence of phase IV indicate that the deviation of the P_L-V curve from the exponential fit reliably assesses CV.

Keywords: airway resistance, closing volume, lung pressure-volume curve, phase IV, single breath nitrogen test, single breath oxygen test.

1. Introduction

The closing volume (CV) identifies the point at which some regions of the lung stop contributing to lung deflation and trap air beyond functionally closed airways (Milic-Emili et al., 2007). An increased CV is responsible for two major abnormalities: maldistribution of ventilation with impaired gas exchange and peripheral lung injury (Milic-Emili et al., 2007). Experiments in normal animals have shown that mechanical ventilation at low lung volume induces peripheral airway injury with concurrent increase in airway resistance (D'Angelo et al., 2002, 2004). Further damage and substantial inflammatory reaction occur when small airway collapse is enhanced by increasing surface tension (D'Angelo et al., 2007), thus supporting the suggestion that also in man opening and closing of small airways during tidal breathing causes lung injury (Robertson, 1984). This should occur whenever CV exceeds the expiratory reserve volume (ERV) or the closing capacity (CC), the sum of CV and residual volume (RV), exceeds the functional residual capacity (FRC).

In humans, CV and CC are currently operationally assessed from the exhaled volume at the transition from phase III to IV of the single breath nitrogen (SBN) test, but absence of phase IV has been described in healthy youngsters (Knudson et al., 1977) and, despite an enhanced small airways collapse and gas trapping, also in COPD patients (Demedts et al., 1975, 1976; George et al., 1976; Gennimata et al., 2010).

Another proposed operational indicator of the onset of airway closure is the inflection of the transpulmonary pressure-volume (P_L-V) curve obtained during slow exhalation from TLC. This is supported by the strong correlation between the volume at the onset of phase IV of the SBN test and that at the inflection point of the deflation P_L-V curve of excised animal lungs (Glaister et al., 1973b; Warner et al., 1988). Technical difficulties to obtain reliable P_L-V curves and controversies concerning the criteria for the identification of the inflection point have limited the use of this indicator in humans (Ingram et al., 1974; Demedts et al., 1975).

In view of the relevance of assessing CV, more exhaustively outlined in the most recent review on the topic (Milic-Emili et al., 2007), we have attempted a) to validate a method for the identification of the inflection point on the deflation P_L-V curve that could reliably substitute the SBN test; and b) to verify if the absence of phase IV is still associated with peripheral airway closure.

2. Methods

2.1 Subjects

Twenty young and twenty elderly healthy subjects were studied together with forty three patients with COPD. Patient inclusion criteria were: >50 years of age; FEV_1/FVC <0.7 and FEV_1 <80% of predicted value; current or former smokers (>20 pack years); stable clinical conditions. Exclusion criteria were: known unstable or moderate-severe heart disease; history of asthma or active pulmonary disease other than COPD; neuromuscular or disabling cognitive problems; BMI≥30 kg/m^2 and restrictive-obstructive pattern; drug abuse. Staging of the disease was according to GOLD criteria (2013). The study was approved by the local Ethics Committee (Fondazione Salvatore Maugeri–654 CEC), and all subjects gave written informed consent. The trial has been registered at www.clinicaltrials.gov (NCT01377051).

2.2 Basic lung function tests and dyspnea

Lung volumes and total specific airway resistance (sRaw) were assessed by means of a constant-volume body plethysmograph (Master Screen Body, Erich Jaeger GmbH, Würzburg, Germany). Intra-thoracic gas volume (ITGV) was measured close to the end-expiratory lung volume during quiet breathing. Chronic dyspnoea was rated according to the modified MRC scale (Eltayara et al., 1996).

2.3 SBN test and P_L-V curve

Experiments consisted of slow expiratory vital capacities during which N_2-washout and P_L-V curves were simultaneously recorded. The SBN test was performed with the VMAX Encore (Viasys Health Care. Yorba Linda, USA). While sitting comfortably, the subjects were asked to exhale to residual volume (RV) and immediately perform a slow maximal inspiration of oxygen followed by a slow expiration to RV. To minimize any leftward shift of the deflation P_L-V curve due to higher airway resistance occurring at mid and low lung volumes especially in COPD patients, the subjects, aided by visual feedback, exhaled at flows 0.2-0.3 $L \cdot s^{-1}$, instead of the recommended 0.4-0.5 $L \cdot s^{-1}$ (Robinson, 2013). Note, however, that the flow dependence of CV is minimal in the range 0.5 -0.2 $L \cdot s^{-1}$ (Rodarte et al., 1975); moreover, most of the patients and some elderly subjects would have been unable to exhale at 0.4-0.5 $L \cdot s^{-1}$ in the mid and low volume range, as anticipated by current guidelines (Robinson, 2013). The subjects were instructed not to move neck and trunk during the manoeuvre. A balloon-tipped catheter (inner diameter 1 mm, length 70 cm) and pressure transducer system (Celesco LCVR-0100,

Raytech Instruments, Vancouver, Canada) allowed to measure oesophageal pressure (Pes). The balloon (Mocrotek Medical B.V., Zutphen, NL; circumference: 2.1 cm; length: 10 cm; filled with 0.8-1 ml of air) was advanced through the nose and placed in the lower third of the oesophagus. Correct positioning was checked as suggested by Baydur et al. (1982). Pressure measured in the balloon kept suspended in air immediately after its extraction from the oesophagus at the end of the experiment, was subsequently subtracted to pressures measured with the balloon *in situ*. Pressure at the mouth was measured with a Celesco LCVR-0100 transducer. P_L was computed as mouth pressure minus Pes.

Signals were sampled at 200 Hz, stored on a computer, and for each SBN test and P_L-V curve, values within each 0.5% of the expired VC were averaged, thus resulting in smoother tracings. SBN test and P_L-V curve were separately analyzed using a custom-made program (LabVIEW Software; National Instruments, Austin, USA). For SBN test, it allowed: a) measure of the expired vital capacity (VC), nitrogen concentration at VC, mean expiratory flow, and flow at CV; b) trace subdivision to compute phase III and IV slopes, phase III elevation, and anatomic dead space (aDS); and c) location of phase III to IV transition to obtain the closing volume (CV_{SBN}). For the P_L-V curve, the program allowed: a) measure of P_L at TLC (P_{LTLC}) and residual volume (P_{LRV}); b) fitting the entire curve with the sigmoid function $V/VC=a+b\cdot EXP(-e^{-(P_L-P_{Lcvsig})/c})$; and c) selection of the part of the curve to be fitted with the mono-exponential function $V/VC=1-e^{-K\cdot(P_L-Po)}$. Indeed, both the exponential and sigmoid functions have been used to describe the deflation limb of the P_L-V curve in man (Salazar and Knowles, 1964; Murphy and Engel, 1978). The inflection point was chosen according to two criteria: a) point where the first derivative of the sigmoid fits is maximal, i.e. P_{LCVsig}; and b) point of departure from the exponential fit, i.e. the lowest point of the interpolated curve (P_{LCVexp}). The volumes of the P_L-V curve corresponding to P_{LCVsig} and P_{LCVexp} were indicated as CV_{sig} and CV_{exp}. Absence of inflection in the volume range fitted with the exponential function was ensured by negative values of P_{LCVsig} and $CV_{sig}=(a+b\cdot 0.368)$ obtained when the same data used for the exponential fit were fitted with the sigmoid function. Tests were repeated three times, and to ensure the attainment of TLC, the analysis was performed on the proof with the highest end-inspiratory P_L and a VC not smaller than 50 ml relative to the largest one measured in that subject. Variables were assessed by two observers in a blind fashion on two occasions several weeks apart; inter-observer difference did not exceed 0.13 L, 0.15 L, and 0.04 kPa for CV_{SBN}, CV_{exp}, and P_{LCVexp}, respectively. Fig. 1 provides examples of how the program was working.

2.4 Statistics

Data, presented as mean±SD, were analyzed using SPSS 19.0 (SPSS Inc., Chicago, USA). Normal distribution of data was assessed by means of the Kolmogorov-Smirnow test. Predicted normal values of spirographic, plethysmographic, and P_L indices are from Quanjer (1993), those for SBN tests from Buist and Ross (1973a; 1973b). Comparisons were performed with a two-way mixed between-within groups ANOVA; when significant differences were found, values of each variable were compared using paired or unpaired Student *t*-test with Bonferroni correction. GOLD stage distribution was evaluated by means of χ^2 test. Relationships between variables were assessed by means of linear regression analysis. Statistical significance was taken at $p \leq 0.05$.

3. Results

Young and elderly subjects exhibited normal lung function tests (Table 1). Patients were stratified according to the presence (COPD*ys*, n= 22) or absence (COPD*no*, n=21) of phase IV. COPD*no* had higher mMRC and GOLD score, and worse functional variables than COPD*ys* patients (Table 1), but stepwise regression analysis could not identify any functional parameter preferentially linked to the absence of phase IV, both sR_{AW} and FEV_1 being equally good predictors of this occurrence.

3.1 SBN test

Variables of SBN tests are summarized in Table 2. The slope of phase III and phase IV increased progressively from young subjects to COPD patients. Phase IV was present in all healthy subjects, except in three youngsters. CV and CV/VC %pred did not differ among groups. Because of a progressive increase in RV and comparable TLC, both CC and CC/TLC increased progressively from young subjects to COPD*ys* patients. No differences occurred for aDS and expiratory flows.

3.2 P_L-V curve

Except in three youngsters in whom phase IV was absent, an inflection was invariably and clearly present in the slow deflation P_L-V curve (Fig. 1). Both P_{LTLC} and P_{LRV} decreased progressively from youngsters to COPD patients (Table 3), and P_{LRV} was correlated with the relative volume at the inflection point (CV_{exp}/VC) (Fig. 2). No differences in P_L-V curve variables occurred between COPD*ys* and COPD*no* patients (Table 3). Overall compliance (K) increased from youngsters to COPD patients.

P_o was essentially zero, indicating that in the volume range encompassed by the exponential fit the contribution to P_L of airway resistive pressure was trivial and no abnormal stresses were affecting Pes.

The sigmoid function $V/VC=a+b\cdot EXP(-e^{-(P_L-P_{LCVsig})/c})$ consistently represented the entire P_L-V curve ($r\geq0.985$), except in the young subjects in whom phase IV was absent (Fig. 1). The section of the P_L-V curve above the inflection was always accurately described by the function $V/VC=1-e^{-K\cdot(P_L-P_o)}$ ($r\geq0.989$), but the fraction fitted with this function (V_{exp}/VC) decreased progressively from youngsters to COPD patients (Table 3). P_{LCVexp}, P_{LCVsig}, CV_{exp} and CV_{sig} were similar among groups, averaging 0.17±0.12 and 0.37±0.11 kPa, 0.65±0.29 and 1.35±0.33 L, respectively. P_{LCVsig} and CV_{sig} were significantly higher than P_{LCVexp} and CV_{exp} (ΔP_L=0.20±0.11 kPa, ΔCV=0.70±0.37 L; $p<0.001$) (Table 3).

P_{LCVexp} and CV_{exp} correlated with P_{LCVsig}, and CV_{sig} ($r=0.640$ and 0.568), the elevation of these relationships being significant (0.25±0.02 kPa and 0.76±0.10 L; $p<0.001$). A better correlation occurred between CV_{exp} and CV_{SBN}, and between P_{LCVexp} and P_L measured at CV_{SBN} (P_{LCVSBN}) (Fig. 3). The slope of these relationships did not differ significantly from unity, whereas the elevation did not differ from zero. A synoptic view of the measurements made on the deflation P_L-V curve is provided in Fig. 4.

3.3 Closing volume vs end-expiratory volume

ERV largely exceeded CV in all young subjects, thus ensuring a considerable safety margin (1.41±0.41 L) against the risk of tidal airway opening and closure (Fig. 5). This occurred also in elderly subjects (75%) and in both COPD*ys* and COPD*no* patients (~50%), but the safety margin was progressively reduced (0.64±0.43, 0.33±0.29, and 0.25±0.12 L, respectively). In the remaining subjects, especially COPD patients, airway opening and closure took place over a considerable fraction of the resting tidal volume.

4. Discussion

This study shows that COPD patients with no phase IV in SBN tests, exhibit a clearly discernible inflection in the simultaneously recorded slow deflation P_L-V curve, whereas healthy subjects without phase IV do not present any inflection in this curve (Fig. 1). In all other instances, the onset of phase IV occurs at essentially the same lung volume of the inflection point assessed according to the exponential criterion (Fig. 1 and 3).

It is currently believed that the main mechanism that causes phase IV consists of peripheral airway closure, which takes place when a critical pressure (P_{Lcrit}) is attained (Milic-Emili et al., 2007). A direct demonstration of the dependent gas trapping has been provided both in healthy subjects and COPD patients (Davis et al., 1970; Forkert et al., 1979; Susskind et al., 1981), and a link between basal airway closure and onset of phase IV has been established using the N_2O technique (Forkert et al., 1979). Extensive gas trapping should have occurred in the present elderly subjects and COPD patients, as shown by the markedly negative values of P_{LRV} (Table 3) and the inverse relationship between P_{LRV} and CV (Fig. 2). In contrast, gas trapping should have been modest in youngsters, in whom mean P_{LRV} was only slightly negative (Table 3), and practically absent in the three youngsters in whom P_{LRV} was still positive or nil (Fig. 1) and phase IV absent. In a mechanically homogeneous lung, the vertical gradient of P_L ensures that P_{Lcrit} is reached first in the lowermost zones with low regional RV/TLC and nitrogen concentration, involving more elevated regions with higher regional RV/TLC and nitrogen concentration as expiration proceeds. While giving rise to phase IV, the progressive increase of regions that stop emptying implies a leftward shift of the deflation P_L-V curve relative to that pertaining to a homogeneous deflation. Hence, the volumes at the inflection of the P_L-V curve and at the onset of phase IV should coincide. This is consistent with the findings in healthy subjects, including the three youngsters in whom both the inflection in the P_L-V curve and phase IV were absent, and in COPD patients in whom phase IV was present (Fig. 3).

In spite of its incidence in COPD patients (40-50%) (Demedts et al., 1975, 1976; George et al., 1976) and youngsters (~20%) (Knudson et al., 1977), absence of phase IV has been practically ignored. Owing to the larger lung recoil (Fig. 4 and Table 3), in some youngsters P_{Lcrit} is reached only at volumes close to RV, thus explaining the absence of both phase IV and the inflection in the deflation P_L-V curve (Fig. 1). Indeed slow deflation P_L-V curves without inflections and relatively high P_L values have been observed in healthy subjects (Butler et al. 1957, Milic-Emili et al., 1964; Murphy and Engel, 1978), although the shape of this curve was usually sigmoid (Butler et al. 1957, Milic-Emili et al., 1964; Murphy and Engel, 1978) (Fig. 1 and 3). In line with the physiological age-related decrease of lung recoil (Table 3), all elderly subjects exhibited a phase IV, and because of the further fall of lung recoil, P_{Lcrit} in COPD*ys* patients was reached at higher volumes (CV_{SBN}/VC,% and CV_{exp} /VC,%; Table 2 and 3). On the other hand, P_{LCVexp} and CV_{exp} /VC,% were similar in COPD*ys* and COPD*no* patients (Table 3 and Fig. 4), suggesting that absence of phase IV might be related to the distribution of local P_{Lcrit} rather than its mean value. COPD*no* patients had in fact enhanced heterogeneity of regional RV/TLC distribution, and, presumably, regional mechanical properties, as indicated by unchanged

elevation of phase III and higher nitrogen concentration at RV (Table 2), besides worse functional parameters (Table 1), in line with previous observations (George et al., 1976; Gennimata et al., 2010).

It has been hypothesized that absence of phase IV could be related either to early sequential closure of lung regions (George et al., 1976; Gennimata et al., 2010) or regional flow limitation occurring at high lung volumes (Gennimata et al., 2010). However, neither hypothesis has received experimental evidence or support from model analysis. Furthermore, flow limitation has been shown to be a potential cause of phase IV (Hyatt and Rodarte, 1975). It can be shown that a model made of two two-compartment units, a smaller one with a moderate RV/TLC difference and a larger one with a greater RV/TLC difference, that empties at a constant rate, is capable to reproduce a markedly sloping phase III, no phase IV, but an inflection on the deflation P_L-V curve as the smaller unit stops deflating. One can devise other multi-compartmental, mechanically heterogeneous models that can be made to empty in order to reproduce the features above, but the required patterns of unit emptying are generated arbitrarily, thus confining these models to pure speculation. As mentioned above, another possibility is that lungs with enhanced mechanical heterogeneity exhibit a wider range of P_{Lcrit}, whereby the normal gravity dependent sequence of airway closure that originates phase IV is totally disrupted. Indeed, a simulation study has shown that a wide distribution of maximal tissue densities, the equivalent of P_{Lcrit}, suppresses phase IV (Kitaoka and Kawase, 2007). Whichever the mechanisms leading to the absence of phase IV, a leftward shift in the P_L-V curve should take place during expiration to RV whenever an adequate amount of lung tissue ceases to deflate and resists further compression.

Essential for the assessment of the inflection of the P_L-V curve is the knowledge of the shape of this curve in the absence of peripheral airway occlusion. Despite the shape of the *in situ* deflation P_L-V curve is most commonly sigmoid (Fig. 1), two lines of evidence indicate that the sigmoid criterion provides an inappropriate assessment of P_{LCV} and, hence, CV. Firstly, the mono-exponential function adequately fits the entire quasi-static deflation P_L-V curve of excised lungs, both normal and emphysematous (Table 4), which, therefore, does not show any inflection. Secondly, CV_{sig} was larger than ERV in nearly all elderly subjects (Table 1 and 3), and even in some youngsters, thus leading to the unlikely conclusion that tidal airway closure is commonplace in healthy subjects. Slow dynamic deflation P_L-V curves of isolated normal lungs exhibit, however, an inflection that systematically occurs at positive values of P_L, about 0.3 kPa at 33% VC when assessed with the sigmoid function applied to the entire curve, but only 0.1 kPa at 12% VC when assessed with the mono-exponential approach (Table 4). The gross similarity of these differences and those between P_{LCVsig} and P_{LCVexp} or CV_{sig}/VC and CV_{exp}/VC (Table 3) further indicates that the exponential approach is a better procedure

for the assessment of the inflection point of the deflation P_L-V curve *in situ*.

In excised, normal human lungs, P_L at the onset of phase IV has been estimated to be about 0.15 kPa (Berend et al., 1961; Silvers et al., 1977). Although these values are comparable with P_{LCVexp} (Table 3 and Fig. 4), the similarity should be regarded with caution, because *in vitro* P_{LCV} was assessed from stepwise quasi-static deflation P_L-V curves, whilst the dynamic condition of the SBN test can alter the shape of the P_L-V curve (Table 4). So far, only two studies have reported on simultaneous recordings of SBN washout and P_L-V curves in humans. Using a visual procedure conceptually close to our mono-exponential approach, Ingram et al. (1974) concluded that in healthy subjects CV_{SBN} and the volume at the inflection point coincide. They also showed that CV_{SBN} and P_L at the inflection point change coherently after methacholine inhalation. In contrast. Demedts et al. (1975) didn't find this coincidence both in healthy subjects and COPD patients. Nevertheless, their values of P_L at the onset of phase IV (P_{LSBN}) and at the inflection point (P_{LIP}) appear to be significantly correlated ($r=0.599$; $p=0.001$), provided three outliers (beyond 99% CI) are eliminated. Furthermore, P_{LIP}, assessed with a procedure closer to the sigmoid approach and hence analogous to P_{LCVsig}, was significantly larger than P_{LSBN} (0.24±0.23 *vs* 0.14±0.18 kPa; $p=0.004$). This difference is consistent with that between P_{LCVsig} and P_{LCVexp} (table 3), whereas P_{LSBN} and P_{LCVexp} coincide (0.14±0.18 *vs* 0.17±0.10 kPa; $p=0.354$). These similarities indicate that a coincidence between CV_{SBN} and volume at the inflection point should have occurred also in Demedts' study, provided the exponential approach had been used. The agreement between the present and previous studies, together with the observations that a) in youngsters the absence of inflection of the slow deflation P_L-V is paralleled by the absence of phase IV (Fig.1), and b) in COPD patients with or without phase IV, CV and P_{LCV} estimated with the exponential approach are similar (Table 2 and 3), support the conclusion that P_{LCVexp} is an adequate signal of airway closure.

Figure 5 illustrates the importance of a substitutive, reliable method for the assessment of CV, not limited to COPD patients, because absence of phase IV has been observed in elderly subjects (Knudson et al., 1977) and could also occur in patients with asthma, chronic heart failure, and obesity. While in young subjects airway closure takes place at lung volumes that are never reached during normal day-life, a consistent number of elderly subjects (~25%) should instead experience airway closure even during tidal breathing at rest. Moreover, the difference between ERV and CV is substantially reduced (Table 1), and tidal airway closure can easily occur during mild exercise, because of the expected fall of ERV. Compared with age-matched controls, COPD patients exhibit a much greater incidence of airway closure during resting tidal breathing (52%), which would be largely underestimated if assessment of airway closure had been based on SBN test alone. Furthermore, in these patients airway closure

involves a substantially greater fraction of the resting tidal volume; consequently, cyclic opening and closing should cause greater damage as it affects already altered airways.

REFERENCES

Baydur, A., Behrakis, P.K., Zin, W.A., Jaeger, M., Milic-Emili, J., 1982. A simple method for assessing the validity of the esophageal balloon technique. Am. Rev. Respir. Dis. 126, 788-791.

Berend, N., Skoog, C., Thurlbeck, W.M., 1961. Single breath nitrogen test in excised humans lungs. J. Appl. Physiol. 51, 1568-1573.

Buist, A.S., Ross, B.B., 1973a. Predicted values for closing volumes using a modified single breath oxygen test. Am. Rev. Respir. Dis. 107, 744-752.

Buist, A.S., Ross, B.B., 1973b. Quantitative analysis of the alveolar plateau in the diagnosis of early airway obstruction. Am. Rev. Respir. Dis. 108: 1078-1087.

Butler, J., White, H.C. Melville Arnott, W., 1957. The pulmonary compliance in normal subjects. Clin. Sci. 16, 709-729.

Cavagna, G.A., Stemmler, E.J., Dubois, A.B., 1967. Alveolar resistance to atelectasis. J. Appl. Physiol. 22, 441-452.

D'Angelo, E., Pecchiari, M., Baraggia, P., Saetta, M., Balestro, E., Milc-Emili, J., 2002. Low-volume ventilation causes peripheral airway injury and increased airway resistance in normal rabbits. J. Appl. Physiol. 92, 949-956.

D'Angelo, E., Pecchiari, M., Saetta, M., Balestro, E., Milc-Emili, J., 2004. Dependence of lung imjury on inflation rate during low-volume ventilation in normal open-chest rabbits. J. Appl. Physiol. 97: 260-268.

D'Angelo, E., Pecchiari, M., Gentile, G., 2007. Dependence of lung injury on surface tension during low-volume ventilation in normal open-chest rabbits. J. Appl. Physiol. 102, 174-182.

Davis, C., Campbell, E.J.M., Openshaw, P., Pride, N.B., Woodroof, G., 1970. Importance of airway closure in limiting maximal expiration in normal man. J. Appl. Physiol. 48, 695-701.

Demedts, M., Clément, J., Stanescu, D.C., van de Woestijne, K.P., 1975. Inflection point on transpulmonary pressure-volume curves and closing volume. J. Appl. Physiol. 38, 228-235.

Demedts, M., De Roo, M., Coseman, J., et al., 1976. Xenon and nitrogen single breath washout curves in patients with airway obstruction. J. Appl. Physiol. 41, 185-190.

Eltayara, L., Becklake, M.R., Volta, C.A., Milic-Emili, J., 1996. Relationship between chronic dyspnea and expiratory flow-limitation in patients with chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med. 154, 1726-1734.

Forkert, L., Dhingra, S., Anthonisen, N.R., 1979. Airway closure and closing volume. J. Appl. Physiol. 46, 24-30.

Frank, N.R., 1963. A comparison of static volume-pressure relations of excised pulmonary lobes of dogs. J. Appl. Physiol. 18, 274-278.

Fuller, S.D., Robinson, N.E., Scott, J.B., 1980. Effect of pulmonary venous pressure on steady-state collateral resistance. J. Appl. Physiol. 49, 643-648.

Gennimata, S.A., Palamidas, A., Karakontaki, F., Kosmas, E., Koutsoukou, A., Loukides, S., Koulouris, N.G., 2010. Pathophysiology of evolution of small airways disease to overt COPD. COPD 7: 269-275.

George, R., Saumon, G., Lafosse, J.E., 1976. Volume de fermeture ete hétérogénéité du système méchanique ventilatoire. Bull. Eur. Physiopath. Resp. 12, 371-385.

Glaister, D.H., Schroter, R.C., Sudlow, M.F., Milic-Emili, J., 1973a. Bulk elastic properties of excised lungs and the effect of a transpulmonary pressure gradient. Respir. Physiol. 17, 347-364.

Glaister, D.H., Schroter, R.C., Sudlow, M.F., Milic-Emili, J., 1973b. Transpulmonary pressure gradient and ventilation distribution in excised lungs. Respir. Physiol. 17, 365-385.

Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD). Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease updated 2013. http://www.goldcopd.org/uploads/users/files/GOLD_Report_2013_Feb20.pdf

Greaves, I.A., Colebatch, J.H., 1980. Elastic behavior and structure of normal and emphysematous lungs post mortem. Am. Rev. Respir. Dis. 121, 127-136.

Hyatt, R.E., Rodarte, J.R., 1975. "Closing volume", one man's noise –other men's experiment. Mayo Clin. Proc. 50, 17-27.

Ingram, R.H. Jr, O'Cain, C.F., Fridy, W.W. Jr, 1974. Simultaneous quasi-static lung pressure-volume curves and "closing volume" measurements. J. Appl. Physiol. 36, 135-141.

Kitaoka, H., Kawase, I., 2007. A novel interpretation of closing volume based on single-breath nitrogen washout curve simulation. J. Physiol. Sci. 57, 367-376.

Knudson, R.J., Lebowitz, M.D., Burton, A.P., Knudson, D.E., 1977. The closing volume test: evaluation of nitrogen and bolus methods in a random population. Am. Rev. Respir. Dis. 115, 423-434.

Liebowitz, D., Massaro, G.D., Massaro, D., 1984. Adrenalectomy and surfactant in adult rats. J. Appl. Physiol. 56, 564-567.

Milic-Emili, J., Mead, J., Turner, J.M., Glauser, E.M., 1964. Improved technique for estimating pleural pressure from esophageal ballons. J. Appl. Physiol. 19, 207-211.

Milic-Emili, J., Torchio, G., D'Angelo, E., 2007. Closing volume: a reappraisal. Eur. J. Appl. Physiol. 99, 567-583.

Murphy, B.G., Engel, L.A., 1978. Models of the pressure-volume relationship of the human lung. Respir. Physiol. 32, 183-194.

Murphy, B.G., Plante, F., Engel, L.A., 1983. Effect of a hydrostatic pleural pressure gradient on mechanical behavior of lung lobes. J. Appl. Physiol. 55, 453-461.

Niewhoener, D.E., Kleinerman, J., 1973. Effects of experimental emphysema and bronchiolitis on lung mechanics and morphometry. J. Appl. Physiol. 35, 25-31.

Niewhoener, D.E., Kleinerman, J., Liotta, L., 1975. Elastic behavior of postmortem human lungs: affects of aging and mild emphysema. J. Appl. Physiol. 39, 943-949.

Pare, P.D., Boucher, R., Michoud, M.C., Hogg, J.C., 1978. Static lung mechanics of intact and excised rhesus monkey lungs and lobes. J. Appl. Physiol. 44, 547-552.

Quanjer, P.H. (ed), 1993. Standardized lung function testing. Report Working Party "Standardization of Lung Function Tests", European Community for Coal and Steel. Eur. Respir. J. 6 (suppl. 16), 1–100.

Robertson, B., 1984. Lung surfactant. In: Pulmonary surfactant, Robertson, B., Van Goulde, L., Batenburg, J., Amsterdam: Elsevier.

Robinson, P.D. (ed), 2013. Consensus statement for inert gas washout measurement using multiple- and single-breath tests. Eur. Respir. J. 41, 507-522.

Rodarte, J.R., Hyatt, R.E., Cortese, D.A., 1975. Influence of expiratory flow on closing capacity at low expiratory flow rates. J. Appl. Physiol. 39, 60-65.

Salazar, E., Knowles, J.H., 1964. An analysys of the pressure volume characteristics of lungs. J. Appl. Physiol. 19, 97-104.

Salmon, R.B., Primiano, F.P., Saidel, G.M., Niewhoener, D.E., 1981. Human lung pressure-volume relationships: alveolar collapse and airway closure. J. Appl. Physiol. 51, 353-362.

Silvers, G.W., Fine, R., Paul, G.W., Stanford, R.E., Petty, T.L., Filley, G.F., 1977. Effect of increased static lung recoil on ventilation distribution in excised human lungs. Am. Rev. Respir. Dis. 115, 435-442.

Silvers, G.W., Petty, T.L., Stanford, R.E., Filley, G.F., 1979. The elastic properties of lobes of excised human lungs. Am. Rev. Respir. Dis. 120, 207-209.

Susskind, H., Atkins, H.L., Klopper, J.F., Ansari, A.N., Richards, P., 1981. Comparison of airway closure measured in vivo and from single-breath washout curve. J. Appl. Physiol. 50, 587-596.

Warner, D.O., Hyatt, R.E., Rehder, K., 1988. Regional ventilation in excised lobes exposed to a transpulmonary pressure gradient. J. Appl. Physiol. 64, 771-780.

Wohl, M.E., Turner, J., Mead, J., 1968. Static volume-pressure of dog lungs–in vivo and in vitro. J. Appl. Physiol. 24, 348-354.

Table 1
Anthropometric and lung function data

	YOUNG (#1)	ELDERLY (#2)	COPD phase IV (#3)	p-value #3 *vs* #2	COPD no phase IV (#4)	p-value #4 *vs* #2	p-value #4 *vs* #3
M/F	14/6	17/3	19/3		16/5		
age, yrs	23±3	71±4	73±6		74±8		
height, m	1.76±0.10	1.75±0.08	1.70±0.08		1.66±0.09		
weight, kg	73±16	79±12	78±14		67±13		
mMRC			1.5±0.9		2.1±1.0		0.027
GOLD II/III/IV			11/9/2		12/9		<0.001
spirography							
IC, L	3.41±0.84	3.00±0.56	2.37±0.60		1.97±0.51		
% predicted	107±23	101±13	90±15	n.s	81±17	0.001	n.s.
ERV, L	1.90±0.46	1.10±0.50	0.76±0.38		0.60±0.30		
% predicted	102±19	93±38	66±31	0.022	53±23	0.005	n.s.
VC, L	5.31±1.08	4.10±0.78	3.13±0.85		2.57±0.65		
% predicted	103±11	105±11	87±16	0.020	76±12	<0.001	<0.001
FEV_1, L	4.44±0.86	2.89±0.58	1.32±0.35		0.81±0.26		
% predicted	105±11	100±16	50±12	<0.001	33±9	<0.001	<0.001
FVC, L	5.28±1.09	3.85±0.69	2.83±0.68		2.02±0.71		
% predicted	106±11	103±12	83±13	<0.001	62±15	<0.001	<0.001
FEV_1/FVC, %	85±7	76±11	47±11		41±7		
% predicted	101±8	102±15	64±14	<0.001	55±10	<0.001	0.009
plethysmography							
TLC, L	6.83±1.19	6.81±1.01	6.77±1.03		6.98±1.30		
% predicted	103±10	102±12	108±16	n.s.	110±16	n.s.	n.s.
ITGV, L	3.49±0.85	3.70±0.90	4.41±0.86		5.14±1.24		
% predicted	102±20	99±23	120±23	0.013	141±30	<0.001	n.s.
ITGV/TLC, %	51±9	54±7	65±7		73±7		
% predicted	105±19	93±13	111±11	0.001	124±13	<0.001	0.018
RV, L	1.45±0.30	2.67±0.55	3.53±0.84		4.52±1.09		
% predicted	94±18	104±20	139±36	0.005	181±47	<0.001	<0.001
sR_{AW}, kPa·s	0.93±0.64	1.04±0.20	2.88±1.41		5.80±2.08		
% predicted	84±55	91±20	250±117	<0.001	503±181	<0.001	<0.001

Values are mean±SD; M, male; F, female; mMRC, modified Medical Research Council dyspnoea scale; GOLD, Global Initiative for Obstructive Lung Disease; FEV_1, forced expiratory vital capacity in one second; FVC, forced vital capacity; IC, inspiratory capacity; ERV, expiratory reserve volume; VC, slow expiratory vital capacity; FEV_1, forced expiratory vital capacity in one second; FVC, forced vital capacity; TLC, total lung capacity; ITGV, intrathoracic gas volume; RV, residual volume; sR_{AW}, total specific airway resistance.

Table 2
Parameters of the SBN test

	YOUNG (#1)	ELDERLY (#2)	p-value #2 *vs* #1	COPD phase IV (#3)	p-value #3 *vs* #2	COPD no phase IV (#4)	p-value #4 *vs* #2	p-value #4 *vs* #3
phase III								
slope, $N_2\%\cdot L^{-1}$	0.8±0.5	1.9±0.8	n.s.	6.9±3.0	<0.001	14.1±4.6	<0.001	<0.001
% predicted	79±42	121±50	n.s.	445±166	<0.001	872±275	<0.001	<0.001
elevation, $N_2\%$	17±3	20±3	n.s.	19±5	n.s.	18±6	n.s.	n.s.
phase IV								
slope, $N_2\%\cdot L^{-1}$	4.0±2.4	9.4±2.7	<0.001	12.9±5.2	0.012			
N_2 at RV, %	22±4	36±3	<0.001	44±5	<0.001	52±5	<0.001	<0.001
CV, L	0.48±0.32	0.72±0.24	0.039	0.79±0.30	n.s.			
CV, %VC	9±6	19±6	<0.001	24±8	0.013			
% predicted	109±65	79±23	n.s.	93±28	n.s.			
CC, L	1.95±0.42	3.37±0.63	<0.001	4.43±1.03	<0.001			
CC, %TLC	28±5	50±6	<0.001	64±9	<0.001			
% predicted	107±19	99±10	n.s.	125±18	<0.001			
aDS, L	0.23±0.05	0.24±0.05	n.s.	0.22±0.05	n.s.	0.20±0.05	n.s.	n.s.
expiratory flow								
average, $L\cdot s^{-1}$	0.26±0.12	0.26±0.09	n.s.	0.26±0.11	n.s.	0.24±0.15	n.s.	n.s.
at CV, $L\cdot s^{-1}$	0.17±0.10	0.18±0.20	n.s.	0.20±0.21	n.s.			

Values are mean±SD. CV, closing volume; RV, residual volume; VC, slow expiratory vital capacity; CC, closing capacity; TLC, total lung capacity; aDS, anatomical dead space.

Table 3
Parameters of the slow deflation P_L-V curve

	YOUNG (#1)	ELDERLY (#2)	p-value #2 *vs* #1	COPD phase IV (#3)	p-value #3 *vs* #2	COPD no phase IV (#4)	p-value #4*vs* #2	p-value #4 *vs* #3
P_{LTLC}, kPa	3.6±0.6	2.6±0.6	<0.001	2.2±0.3	n.s.	1.9±0.4	<0.001	n.s.
P_{LTLC}, %pred	104±18	113±23	n.s.	98±12	n.s.	86±17	<0.001	n.s.
$P_{L0.5TLC}$, kPa	0.46±0.12	0.20±0.10	<0.001					
$P_{L0.5TLC}$,	97±24	106±52	n.s.					
P_{LRV}, kPa	-0.5±0.4	-2.0±1.0	<0.001	-2.9±1.4	0.027	-3.2±0.9	0.001	n.s.
exponential fit								
V_{exp}, %VC	87±6	79±6	0.001	74±6	0.049	74±7	0.035	n.s
P_o, kPa	0.04±0.12	0.05±0.08	n.s.	0.03±0.11	n.s.	0.03±0.08	n.s.	n.s.
K, kPa^{-1}	1.40±0.36	1.72±0.43	0.012	2.17±0.42	0.007	2.45±0.48	<0.001	n.s.
P_{LCV}, kPa	0.16±0.10	0.21±0.09	n.s.	0.17±0.12	n.s.	0.16±0.10	n.s.	n.s.
CV, L	0.50±0.32	0.68±0.24	n.s.	0.78±0.29	n.s.	0.62±0.24	n.s.	n.s.
CV, %VC	10±5	18±6	0.001	24±6	0.008	24±7	0.005	n.s.
V'_{CV}, $L \cdot s^{-1}$	0.20±0.11	0.21±0.14	n.s.	0.19±0.06	n.s.	0.16±0.06	n.s.	n.s.
sigmoid fit								
P_{LCV}, kPa	0.41±0.17	0.42±0.10	n.s.	0.33±0.11	n.s.	0.29±0.07	0.009	n.s.
CV, L	1.56±0.60	1.44±0.24	n.s.	1.31±0.30	n.s.	1.06±0.29	0.027	n.s.
CV, %VC	31±10	38±3	<0.001	41±2	n.s.	41±2	n.s.	n.s.
V'_{CV}, $L \cdot s^{-1}$	0.24±0.14	0.24±0.15	n.s.	0.22±0.06	n.s.	0.22±0.15	n.s.	n.s.

Values are mean±SD. P_L, transpulmonary pressure at total lung capacity (TLC), residual volume (RV), and closing volume (CV). Predicted values are from Quanjer (1993); V_{exp}, fraction of the vital capacity fitted with the exponential function $V/VC=1-e^{-K\cdot(P_L-P_o)}$; P_o and K. coefficients of the exponential function; VC, slow expiratory vital capacity; P_{LCV}, pressure at the inflection point according to the exponential or sigmoid function $V/VC=a+b\cdot EXP(-e^{-(P_L-P_{LCVsig})/c})$; V'_{CV}, expiratory flow at CV.

Table 4
Analysis of the deflation P_L-V curve of isolated human and animal lungs or lobes

	P_{Lsig} kPa	Vinfl %VC	P_{Lmx} kPa	V_0 %TLV	P_o kPa	K kPa^{-1}	Vinfl %VC	P_{Lexp} kPa	N	Ref.
Stepwise quasi-static P_L-V curve – Normal lungs										
	-0.63	-94	2.45		-0.02	1.634	0	0	37[a]	Niewhoener, 1975
	-1.54	-1739	1.97	28.4	0.01	1.586	0	0	4[a]	Silver et al., 1977
	-0.80	-280	1.96	16.8	0.02	1.641	0	0	5[a]	Silver et al., 1979
	-0.68	-362	2.43	36.0	0.00	2.001	0	0	14[a]	Greaves & C., 1980
	-0.72	-1160	2.52	37.5	0.01	2.149	0	0	13[a]	Berend et al., 1961
	-1.42	-1384	2.45		-0.01	2.104	0	0	5[a]	Salmon et al., 1981
	-0.02	-5	2.94	12.5	0.02	1.540	0	0	29[b]	Frank, 1963
	-0.81	-110	2.94	23.9	0.03	1.211	0	0	19[b]	Murphy & E., 1983
	-0.35	-52	1.96	13.6	0.02	1.570	0	0	12[b]	Fuller et al., 1980
	-1.00	-748	2.94	12.1	0.01	1.524	0	0	10[b]	Wohl et al., 1968
	-1.14	-1219	2.45	6.4	0.00	2.261	0	0	10[c]	Cavagna et al., 1967
	-1.23	-1237	2.96	8.3	-0.03	2.159	0	0	12[d]	Pare et al., 1978
	-1.16	-201	3.92	7.6	0.02	1.151	0	0	9[f]	Liebowitz et al., 1984
	-0.82	-169	1.96	15.1	0.01	1.515	0	0	16[g]	Niewhoener, 1973
mean	-0.88	-626	2.56	18.5	0.01	1.718	0	0		
SD	0.41	600	0.55	11.2	0.02	0.356				
Stepwise quasi-static P_L-V curve – Emphysematous lungs										
	-0.77	-1280	2.45		-0.05	3.251	0	0	12[a]	Niewhoener, 1975
	-0.81	-550	2.39	52.3	-0.01	2.646	0	0	19[a]	Greaves & C., 1980
	-0.98	-791	2.57	46.7	0.04	2.536	0	0	5[a]	Berend et al., 1961
	-1.05	-1391	1.96	33.2	0.00	2.838	0	0	16[g]	Niewhoener, 1973
mean	-0.91	-1003	2.33	44.0*	-0.01	2.818*	0	0		
SD	0.13	399	0.25	9.8	0.04	0.315				
Slow dynamic P_L-V curve – Normal lungs										
	0.19	34.0	2.45		-0.04	1.977	8.4	0.03	5[a]	Salmon et al., 1981
	0.58	30.9	2.96		0.08	1.131	16.0	0.14	4[b]	Glaister et al., 1973a
	0.37	31.9	2.88		0.10	1.498	13.7	0.13	4[e]	#
	0.32	30.3	3.07		0.07	1.760	12.2	0.10	6[f]	#
	0.29	29.1	2.45	7.1	0.05	1.942	9.1	0.11	4[h]	Cavagna et al., 1967
mean	0.35*	31.2*	2.76		0.05	1.662	11.9*	0.10*		
SD	0.15	1.8	0.29		0.05	0.352	3.2	0.04		

Analyses were performed on data obtained from digitized figures or tables. P_{Lsig} and P_{Lexp}, transpulmonary pressure at the inflection point according to the function $V/VC=a+b\cdot EXP(-e^{-(P_L-P_{Linfl})/c})$ and $V/VC=1-e^{-K\cdot(P_L-P_o)}$; V_{infl}, volume at the inflection point, expressed as percent vital capacity, *i.e.* the volume difference between maximal (P_{Lmx}) and zero P_L, values of P_{Lsig} and $V_{infl} \leq 0$ indicating absence of inflection in the P_L-V curve; V_0, volume at zero P_L, expressed as percent total lung volume (TLV); P_o and K, parameters of the mono-exponential function; N, number of lungs or lobes used in the analysis; [a]man; [b]dog; [c]cat; [d]monkey; [e]rabbit; [f]rat; [g]hamster; [h]3 rabbits and 1 dog; #E. d'Angelo unpublished data. *$p \leq 0.003$, compared with normal lungs under quasi-static condition; r-values were ≥ 0.991 for both the sigmoid and exponential function.

LEGENDS

Fig. 1 Single-breath N_2 test tracings (*left panels*) and simultaneously recorded transpulmonary pressure volume curves (*right panels*) in two youngsters (A and D), an elderly subject (B), and two COPD patients (C and E). In the left panels, vertical bars limit the part of the tracing used for the assessment of dead space (1), slope of phase III (2-3) and phase IV (4-5); squares indicate volume of the dead space (a), elevation of phase III (b), onset of phase IV (c), and end-expiratory N_2 concentration (d). In the right panels, thin continuous and dotted lines are the fitted sigmoid and mono-exponential functions (see text), while squares indicate the corresponding inflection points (sig and exp, respectively).

Fig. 2 Relationship between closing volume assessed with the exponential approach (CV_{exp}) and transpulmonary pressure at the end of maximal expiration (P_{LRV}) in healthy subjects and COPD patients. Numbers are slope ±SE.

Fig. 3 Relationship between closing volumes (CV; *upper panel*) or transpulmonary pressures at CV (P_{LCV}; *lower panel*) obtained according to the exponential approach for the assessment of the inflection point (exp), and the corresponding values assessed from the onset of phase IV (SBN) in the four groups of subjects. The dotted lines indicate 99% CI. Numbers are slope ±SE.

Fig. 4 Average slow deflation P_L-V curve of the four groups of subjects with the position of the residual volume (RV), intra-thoracic gas volume (ITGV), and inflection point according to the sigmoid (P_{LCVsig}) and the exponential approach (P_{LCVexp}).

Fig. 5 *Upper panel:* difference between expiratory reserve (ERV) and closing volume (CV) assessed either from the onset of phase IV (SBN) or the simultaneously recorded deflation P_L-V curve according to the exponential approach (exp) in the four groups of subjects. *Lower panel:* relative amount of the resting tidal volume affected by airway closure and presumably involved in cyclic opening and closing of small airways. Bars are SD. Numbers indicate subjects in whom CV exceeded ERV.

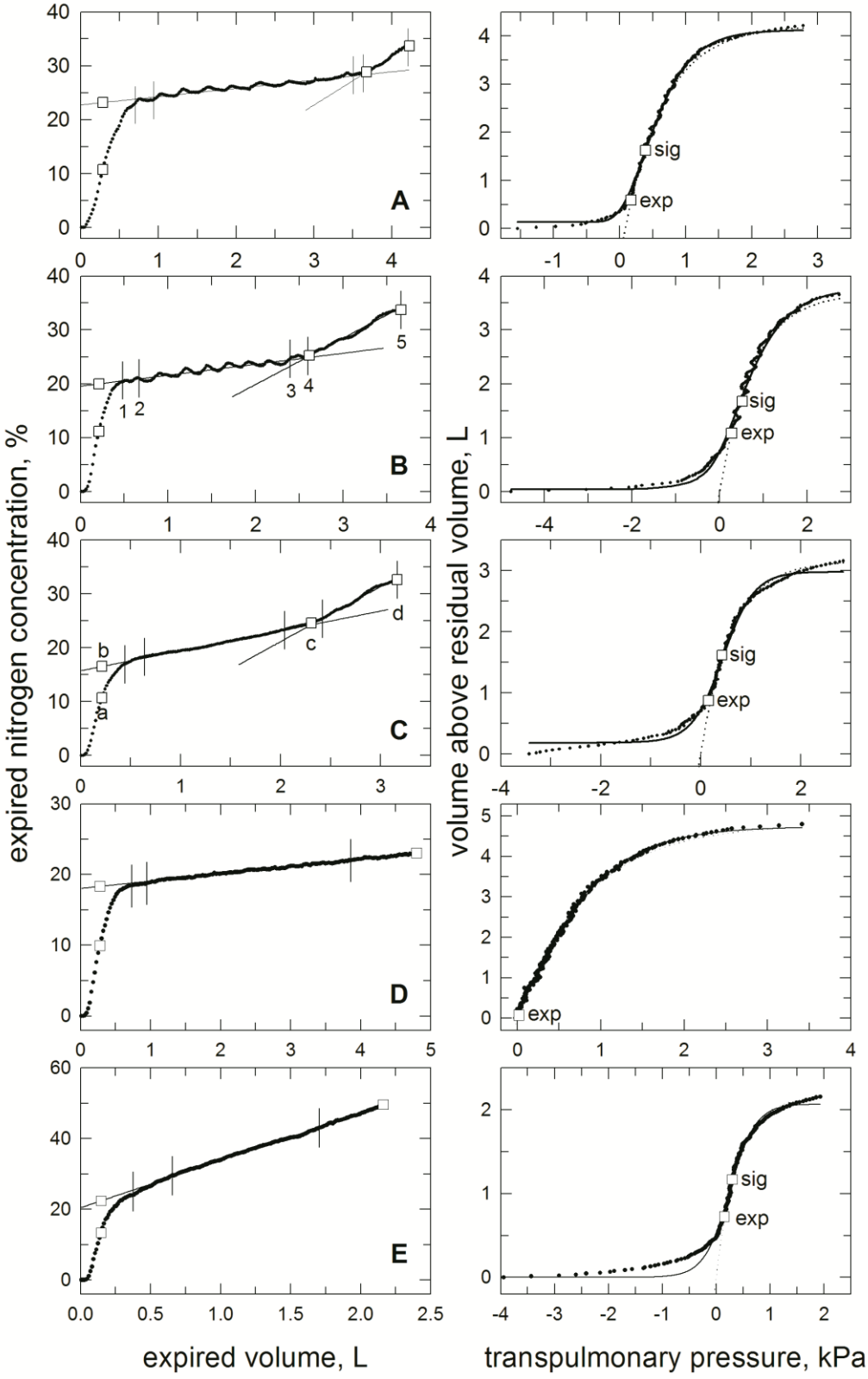
expired nitrogen concentration, %
volume above residual volume, L
expired volume, L
transpulmonary pressure, kPa
A
B
C
D
E
1 2
3 4
5
a
b
c
d
sig
exp

Figure 1

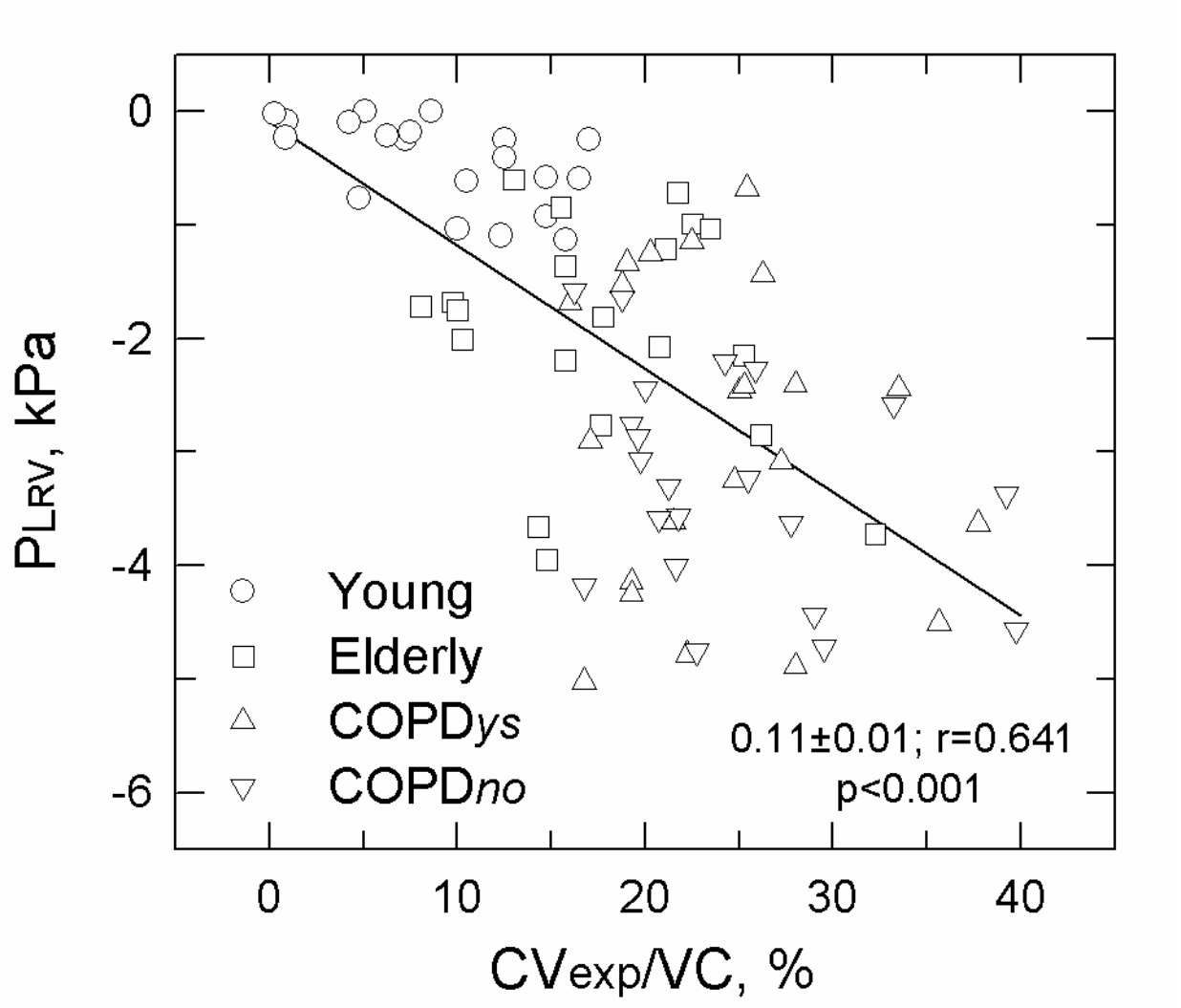
0
-2
-4
-6
P_{LRV}, kPa
0
10
20
30
40
CVexp/VC, %
Young
Elderly
COPDys
COPDno
0.11±0.01; r=0.641
p<0.001

Figure 2

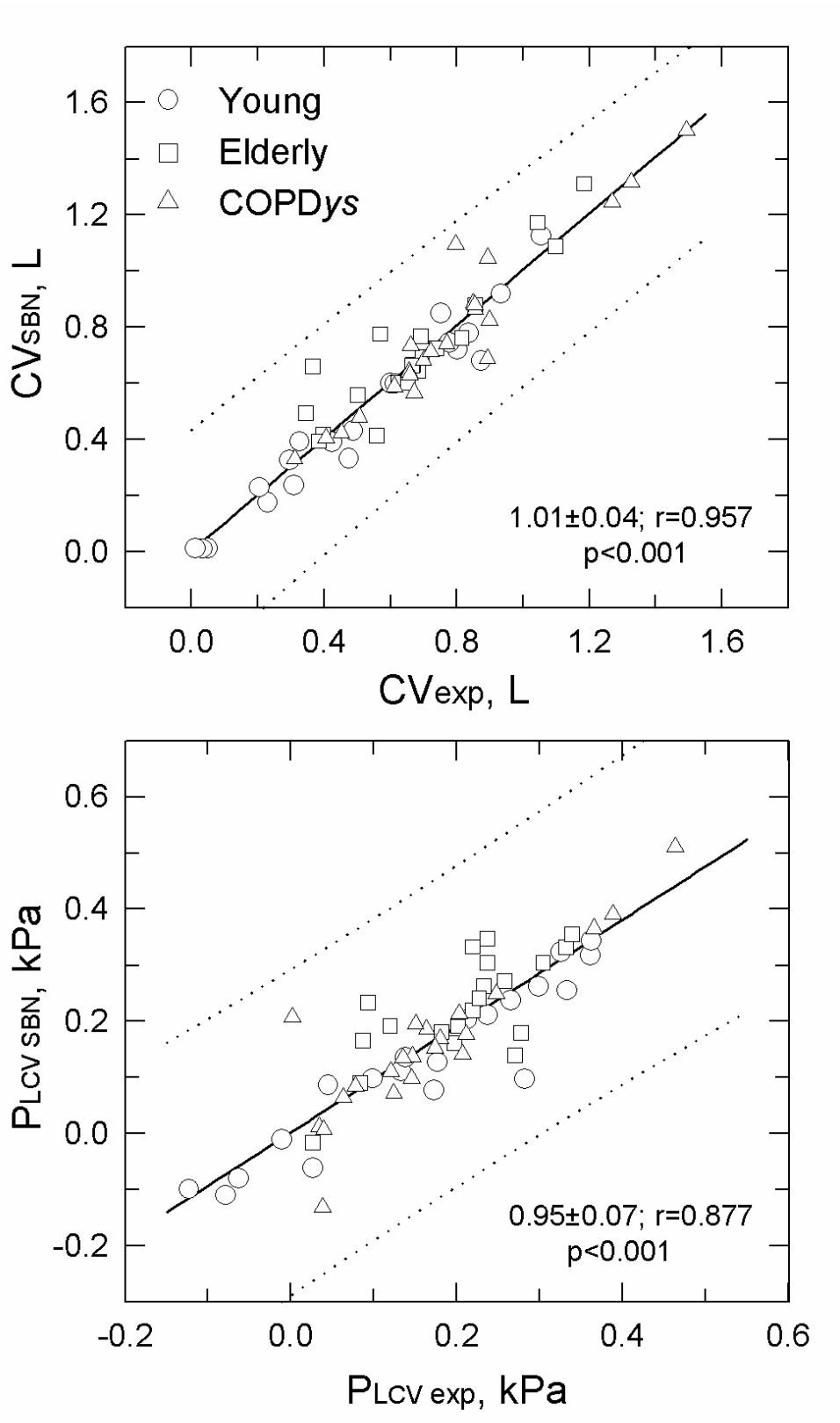
Young
Elderly
COPDys
1.01±0.04; r=0.957
p<0.001
CVSBN, L
CVexp, L
0.95±0.07; r=0.877
p<0.001
PLCV SBN, kPa
PLCV exp, kPa

Figure 3

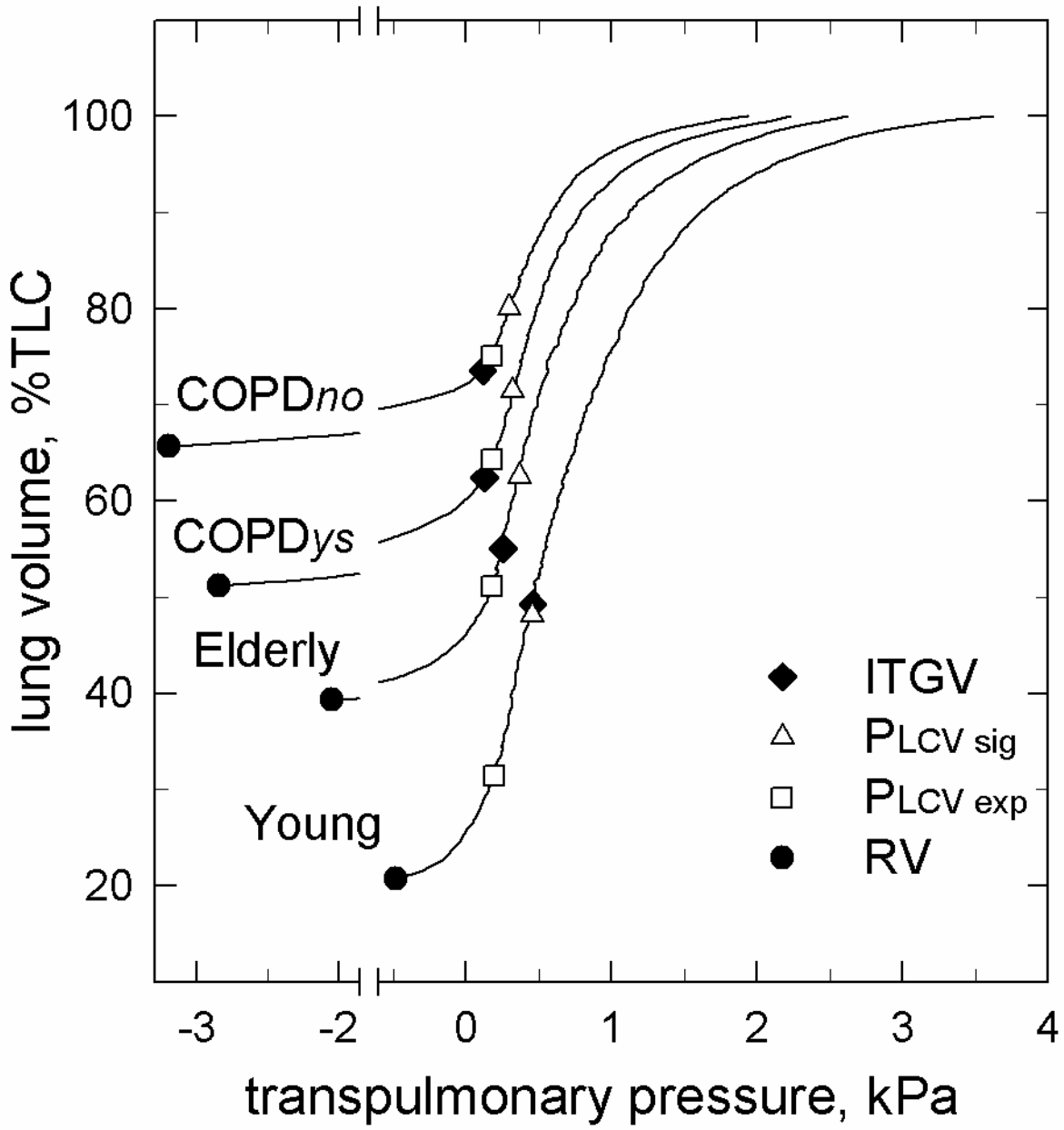
100
80
60
40
20
lung volume, %TLC
COPDno
COPDys
Elderly
Young
ITGV
PLCV sig
PLCV exp
RV
-3
-2
0
1
2
3
4
transpulmonary pressure, kPa

Figure 4

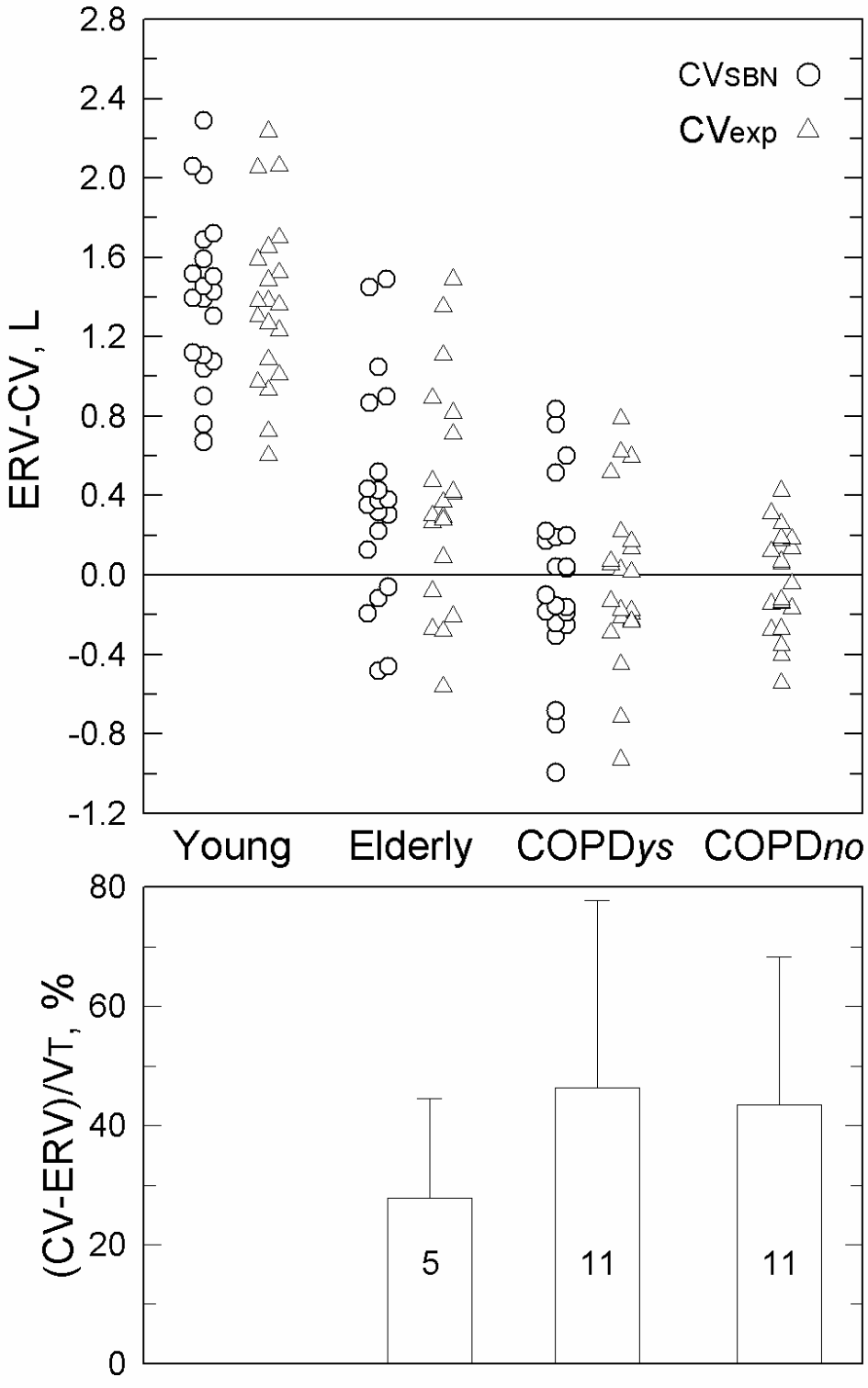
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0.0
-0.4
-0.8
-1.2
ERV-CV, L
CVSBN
CVexp
Young
Elderly
COPDys
COPDno
80
60
40
20
0
(CV-ERV)/VT, %
5
11
11

Figure 5