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Expiratory flow-limitation detected by forced oscillation and negative expiratory pressure

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Running head: Detection of flow-limitation by FOT and NEP

Abstract

The within-breath change in reactance (DXrs) measured by forced oscillation technique (FOT) at 5Hz reliably detects expiratory flow-limitation (EFL) in COPD. In this study we compared this approach to the standard negative expiratory pressure (NEP) method.

We studied 21 COPD patients applying both techniques to the same breath and in 15 repeated the measurements after bronchodilator. For each patient and condition 5 NEP tests were performed and scored independently by three operators unaware of the FOT results.

On 180 tests, FOT classified 53.3% as flow-limited (FL). On average, the operators scored 27.6% of tests FL, 47.6% non-FL but could not score 24.8%. The methods disagreed in 7.9% and in 78% of these the NEP scores differed between operators. Bronchodilation reduced NEP and DXrs scores, only the latter achieving significance (p=0.02). Averaging the operators' NEP scores, a threshold between 24.6%-30.8% of tidal volume being FL by NEP produced 94% agreement between methods.

In conclusion, when NEP and FOT were both available they showed good agreement. As FOT is automatic and can measure multiple breaths over long periods, it is suitable for monitoring EFL continuously and identifying patients breathing close to the onset of EFL, where intermittent sampling may by unrepresentative.

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Keywords: chronic obstructive pulmonary disease, within-breath impedance, respiratory system reactance, forced oscillation technique.

Introduction

The presence of airflow obstruction is a defining feature of several lung diseases and its persistence over time and despite bronchodilator treatment is typical in COPD(1). When airflow obstruction develops lung emptying is delayed during forced maneuvers and there is a reduction in the ratio of FEV_1 to FVC, a change which also occurs with aging but to a lesser degree(2). As airflow obstruction worsens, expiratory flow limitation (EFL) appears at much lower flows for a given lung volume, and it becomes present at rest or at least develops early during exercise(3). This is important as changes in dynamically regulated lung volume that are characteristic of exercise in COPD are likely to be directly related to the presence of EFL(4). However, initial methods of detecting EFL proved either invasive involving balloon catheterisation, difficult to standardise because of variations in previous volume history or involved relatively complex and problematic plethysmographic techniques.

The development of the negative expiratory pressure (NEP) technique provided a relatively simple way of identifying flow limitation by comparing the expiratory flow volume profile of a control breath to that of a breath when additional negative pressure of about 3.5-5 cmH_2O was applied(5). Any increase in flow beyond the control data demonstrates that some expiratory flow reserve is present. This method is independent of volume and time history, non-invasive and relatively simple to apply in a variety of clinical settings e.g. ICU, exercise, and has been used in COPD(6-9) and other respiratory and systemic disorders(10-14). There is a possibility of upper airway artifacts in some patients and standardized methods of interpreting NEP data have not yet been published although individual investigators are familiar with these practical issues(8;11).

An alternative approach has recently been described in which within-breath changes in respiratory system reactance measured by forced oscillation technique (FOT), which increases abruptly during expiration in flow limited individuals, were used to define threshold values of reactance change that occurs in flow limited breaths(15;16). There was clear separation between flow limited and non-flow limited breaths when this method was compared with data from balloon catheters.

These approaches are based on different physical principles. NEP detects the condition in which all possible pathways between airway opening and the alveoli are choked. When this occurs, the total expiratory flow is independent of the expiratory pressure, a condition of ‘global’ expiratory flow-limitation. By contrast FOT assesses the amount of the lung that is choked during expiration only. This measures ‘regional’ flow-limitation, and a threshold value indicates when the regional flow-limitation reaches the condition of global flow-limitation. Therefore, when global expiratory flow-limitation is reached, the two techniques should produce the same response.

In this study we have used these methods to determine whether a given breath was flow-limited. We hypothesized that NEP and FOT methods would produce similar results when directly compared despite the different approaches adopted to identify EFL. To test this idea, we have compared NEP and FOT methods within the same breath and the impact each has on classifying an individual patient as being flow limited. In addition, we have examined whether bronchodilators drugs, which conventionally improve lung emptying, modify EFL detected by either methods.

Methods

Patients

We recruited 21 patients who met the standard diagnostic criteria for COPD(17) and were current or ex-smokers. They omitted their short- or long-acting bronchodilators for at least 3 and 12/24 hours as appropriate before the study. No patient had a history of a recent exacerbation or evidence of significant cardio/respiratory disease other than COPD. Spirometry and subdivisions of lung volume were measured in a constant-volume body plethysmograph (Medgraphic Autolink 1085D, Medical Graphics, St Paul, MN). Predicted values for flows and volumes were those recomended by the European Respiratory Society (18). The study was approved by the institutional research ethics committee, and written informed consent was given by each subject.

Measurements

Pressure and flow at the airway opening (Pao and $\dot{V}ao$) were measured by a transducer (PXLA0025DN, Sensym, Milpitas, CA) connected to the mouthpiece and by a screen-type pneumotachograph (3700A; Hans Rudolph, Kansas City, MO connected to a pressure transducer PXLA02X5DN, 0–2.5 cm H_2O; Sensym, Milpitas, CA). All the signals were sampled at 200 Hz by an analog-to-digital and digital-to-analog board (DAQCARD 6036-E, National Instruments, Austin, TX) and recorded by a personal computer. The flow signal was integrated to give lung volume (V_L). The volume drift resulted from the integration of the flow signal was removed by selecting 2-3 mins of stable quiet breathing and by estimating the linear trend on the integrated signal. This trend was then removed from the traces.

The frequency response of the measuring systems(19) was flat up to 30Hz.

Forced oscillations and NEP

In this study we measured the within-breath input impedance of a patient by applying a sinusoidal pressure oscillation at 5 Hz to the mouth. To allow the simultaneous assessment of a given breath by both FOT and NEP we used an experimental set-up for FOT modified from that described previously (15). The equipment comprised a 25 cm diameter loudspeaker (model HS250, Ciare, Ancona, Italy) mounted on a rigid box of ~2 l of internal volume used to generate the oscillatory pressure (Figure 1). The forcing pressure was transferred from the box through a connecting tube (22 cm long, 19 mm ID) and the pneumotachograph to the subject's mouthpiece. A low-resistance, high-inertance tube (25 mm of internal diameter and 1.5 m of length) in parallel with the loudspeaker allowed the subjects to breathe room air without significant loss of forcing pressure. The amplitude of the forcing signal was adjusted to provide an oscillatory pressure of ~1-2 cmH_2O peak to peak at the patient's mouth. A bias flow of ~15 l/min reduced the equipment deadspace to the volume of the pneumotachograph and the mouthpiece(20). Immediately before the pneumotachograph and the bias flow plug a three-ways pneumatic sliding valve (8500 series, Hans Rudolph, Kansas City, MO) allowed the connection of the measurement devices and the patient either to the loudspeaker and the high inertance tube or to a Venturi NEP valve (model 206A, Raytech Instruments, North Vancouver BC, Canada). The same computer and board used to sample flow and pressures signals was used to control both the three way and NEP valves and to generate the forcing signal which, amplified by a power amplifier (model RA 80, REVAC, Milano, Italy), drove the loudspeaker.

The system operated as follows: during quiet breathing, and thus during the NEP control breath, the three way valve connected the loudspeaker to the patient, which allowed the FOT measurement to be made. This configuration was maintained during the inspiratory phase of the breath after NEP control breath. As soon as expiration began, the three way valve switched into the NEP circuit and NEP was applied for the duration of the expiration. At the end of the expiration, the three way valve switched the circuit back to its original configuration for FOT measurement.

By using this set-up it has been possible to assess EFL on the NEP control breath by FOT and then to apply the negative pressure in the following expiration, allowing the simultaneous assessment of EFL on the NEP control breath by both the techniques.

Protocol

Firstly spirometry and subdivision of lung volumes were assessed. After that, patients were connected to the modified FOT-NEP device while seated, wearing a nose clip and with an operator firmly supporting the cheeks to reduce upper airways shunt. Patients were asked to breath spontaneously while submitted to FOT. After at least 60-90 s of quiet breathing the first NEP manoeuvre was performed. A total of 5-6 NEP tests were recorded, each separated by at least 30-60 s of quiet breathing from the previous one. FOT measurements were recorded continuously throughout the study period which lasted approximately 7 mins.

In 15 patients 5mg of nebulized Salbutamol was administered, after which patients were asked to rest for approximately 30 mins. Finally, all measurements (spirometry, lung volumes, FOT and NEP tests) were repeated as described above.

Data analysis

For each measurement the first five NEP maneuvers that did not present evidence of leaks or other major abnormalities were selected. Each one was analyzed by both FOT and flow-volume loops as follows:

FOT: Within-breath Xrs was computed for each breath from Pao and $\dot{V}ao$ as previously described (15). The mean values of Xrs during inspiration ($\overline{Xinsp}$) and expiration ($\overline{Xexp}$) were computed. Their difference ($\overline{\Delta Xrs} = \overline{Xinsp} - \overline{Xexp}$) was used to detect EFL. A breath was considered flow-limited (FL) if $\overline{\Delta Xrs}$ was greater than a threshold of 2.8 cmH_2O*s/L, a value that in our previous study(15) was able to identify FL breaths with 100% sensitivity and specificity when compared to Mead Whittenberger method(21).

Manoeuvres in which the Xrs tracing showed spikes or oscillations due to swallowing or glottis closure were discarded.

NEP: To compare the quantitative measurement provided by FOT with NEP, the five flow-volume loops for a given patient where plotted on a single page. The 36 pages obtained (21 for baseline conditions and 15 for post bronchodilators) where organized in random order and sent to three operators who independently scored each loop blind to the FOT results. They followed the following criteria:

1) If there were no overlapping regions between the control expiratory trace and the NEP expiratory trace throughout expiration the breath was scored with a 0 (no EFL, Figure 2A);

2) If the two lines overlapped throughout expiration, excluding the short and sharp spike of extra-flow due to upper airway shunt, we scored the breath with a 100% (complete EFL, Figure 2C).

3) In those cases in which the two traces overlapped for only part of the expiration, the breath was scored according to the percentage of the tidal volume in which overlapping occurred (partial EFL, Figure 2B).

A NEP manoeuvre was discarded if one or more of the following four conditions were present:

1) The volume time course showed air leaks during the NEP application. Leaks during NEP introduce a clear stepwise drop in the volume trace that does not recover after the application of the negative pressure.

2) The duration of the NEP breath is not as long as that of the control breath (Figure 2D).

3) The control and the NEP loops are clearly different, in particular the volume range of the two loops is markedly different and the loops are only partially overlapped, probably due to a volume drift that cannot be corrected (Figure 2E).

4) The flow trace shows wide oscillations during the application of NEP, probably due to upper airways instability induced by the application of negative pressure which prevent the clear identification of the onset of EFL (Figure 2F).

As the presence of EFL with NEP is detected by the presence of overlapping between the control and the NEP expiratory flow-volume loops, it is essential to remove the oscillatory signal from flow and volume signals. We used a moving average filter with a window of 40 samples, providing a narrow stop-band filter to the frequency components at 5Hz and all the relative harmonics. To avoid alteration in the shape of the flow-volume loops due to the high frequency components present in the spike at the beginning of the application of NEP, we filtered the whole flow signal and we used the filtered data to plot the control breath and the inspiration of the NEP breath, while we used the unfiltered data to plot the expiratory flow and volume during the application of the negative pressure.

Significance of differences between physical characteristics, spirometric data, DXrs and NEP scores between before and after bronchodilators were tested by paired T-test. Values of $p>0.05$ were considered nonsignificant (NS). The agreement of NEP and FOT in classifying a given patient as flow-limited or not flow-limited was evaluated by the kappa statistic. Data are expressed as mean (SD) unless otherwise stated.

Results

The characteristics and lung function data of the COPD patients are shown in Table 1. Figure 3 shows a representative time course of volume, airway opening pressure, Rrs, Xrs and DXrs for a few breaths before and after a NEP manoeuvre obtained with our experimental set-up. This patient was classified as flow limited by DXrs. By using the raw data to plot a flow-volume loop we obtain the loop shown in Figure 4, left panel. After the filtering procedure, the same manoeuvre produced the plot in Figure 4, right panel. These graphs were used for the NEP scoring. Altogether, 180 breaths were studied in this way. Of these, 105 were recorded in the 21 patients under baseline conditions and 75 from the 15 patients who repeated the test after bronchodilator. On average, each observer discarded 24.8% of the flow-volume loops because they did not meet the acceptance criteria. Altogether 11.2% of discarded breaths met criterion 1, 18.6% criterion 2, 14.2% criterion 3 and 55.2% criterion 4.

Comparison Between FOT and NEP methods

Breath-by-breath comparison: In Table 2 the results of DXrs data and the individual flow-volume loop scores produced by the three observers are reported for all 180 breaths. Using DXrs it was possible to classify all the breaths studied. DXrs classified 46.7% of the analysed breaths as not flow-limited and 53.3% as flow-limited. For comparison with these data, a breath was considered flow-limited by NEP if the score produced by the observer was above 50%. Although this threshold is arbitrary, most of the scores (91.4%) were either 100% or 0%. On average, the three observers classified 85.7/180 (47.6%) of breaths as being not flow-limited and only 49.7/180 (27.6%) of breaths as flow-limited, the remaining 44.6/180 (24.8%) being unsuitable for NEP analysis by our criteria. This implies that most of the flow-volume loops rejected by the observers were classified as being flow-limited by DXrs method (61.1%, 62.2%, 81.1% for operators one, two and three, respectively).

The three operators scored 29 flow-volume loops differently. These breaths constituted the majority (78%) of the 37 breaths where FOT and NEP disagreed, suggesting that intra-observer variability was the most important source of disagreement between the techniques. Only 95/180 NEP manoeuvres were accepted and classified similarly by all three observers. If we limit the analysis to these breaths and considering NEP as reference method, DXrs showed a sensitivity of 93% and a specificity of 91%. Of the 8 misclassified breaths, 6 were false positive. Of these, 4 breaths showed a DXrs value that only exceeded the threshold for EFL (2.8 cmH_2O*s/L,(15)) by less than 0.22 cmH_2O*s/L and, therefore, were borderline.

Patients classification analysis: To reduce the impact of intra-operator variability and to test the ability of the two methods to identify expiratory flow limitation in a given patient, we performed a 'patients level' analysis by averaging all the scores obtained from all the observers and all the accepted NEP tests from a given patient. This implies that the number of scores averaged for a given patient was variable depending on the number of tests discarded by the observers. In this way we obtained an estimate of the average degree of flow-limitation for that patient. Of the 36 averaged data sets (21 from patients at baseline and 15 after bronchodilation) two could not be used because all observers discarded all of the 5 NEP graphs. We compared these averaged NEP values to the mean DXrs values obtained in all 5 manoeuvres from the same patient (Table 3). We applied this procedure to all patients both before and after bronchodilator and the results are reported in Figure 5. This approach produced a good degree of agreement between the methods. From the data in Figure 5 we identified a threshold for the NEP scores, which lay between 24.6% and 30.8% of the breath showing flow-limitation. Using this value there was 94% agreement with DXrs criteria in the identifying flow-limited patients, with 32 of the 34 available assessments being classified in the same way. A kappa statistic has been performed to assess the statistical power of the agreement. We found that k=0.87, confirming that there was excellent agreement between the methods.

Effect of a Bronchodilator

On average bronchodilation reduced the degree of flow-limitation of the patients assessed by both the techniques, as shown in Table 3. However, the difference measured by NEP did not reach statistical significance. Conversely, DXrs values fell significantly after bronchodilator. Although the changes were significant, there was a large variability in the response to bronchodilator: 13/15 reduced DXrs while 2 increased. However, only 3 of the 13 patients where DXrs fell passed from values above to values below the threshold of EFL. Of these, two showed a similar change in the NEP data, the other patient being considered as not flow–limited by this method. In the two patients who showed an increase of DXrs, in one case the value passed from below to above the threshold for EFL, and this was also identified by NEP scoring.

Discussion

The detection of expiratory flow limitation during tidal breathing is a potentially important measurement which has been substantially simplified by the development of the NEP and, more recently, the FOT measurements described here. Both methods define EFL independently of the previous volume history of the test, are non-invasive and easily repeated. Despite these similarities, each method exploits different physical principles to identify EFL, which might affect their ability to classify individual breaths or individual patients as being flow-limited. Our data, in which the methods are compared using the same breath, are reassuring but highlight several factors which can influence the categorisation of individual breaths and patients as being flow-limited or not.

Our protocol compared the two methods using the same breath to measure DXrs and as a reference breath for the subsequent NEP application. Thus any possible effect of FOT on breathing pattern, which has not previously established, would affect both methods to a similar degree. The FOT method uses empirically derived criteria for identifying flow-limitation, which are applied automatically. As the decision to classify a given breath as being flow-limited using the NEP method could be influenced by the observer, we used three independent observers who were unaware of the DXrs data to score the NEP traces. We developed a set of rules to determine EFL by the NEP method, something which has not been explicitly stated in previous reports. This is the first occasion when a comparison of blinded inter-observer agreement has been reported for the NEP method.

To compare quantitative results between FOT and NEP we scored the degree to which the expiratory flow volume loops of the control and NEP breaths overlapped. However, applying NEP produces an additional expiratory flow from the upper airways (as shown in Figure 2) making it impossible to determine whether the initial period of the breath is flow-limited or not. The use of a lower negative expiratory pressure might reduce this artifact but this phenomenon cannot be avoided entirely. As the duration of the artifact depends on the amplitude of the applied pressure and the extra-thoracic airways compliance, this introduces a variability in the scores that is unrelated to the degree of flow-limitation. For this reason we arbitrarily decided to assign a score of 100% (breath flow-limited) to the breaths in which flow-limitation is clearly present as soon as the artifact disappears.

We applied a NEP pressure of approximately 7 cmH_2O, which is somewhat greater than that usually used. This might have increased the number of breaths discarded because of upper airway collapse artifacts, as suggested by other workers (11;22). Although this increased the number of loops discarded by the observers it did not affect the reliability of NEP or change the relationship between NEP and FOT in the remaining tests, and in fact only two evaluations on 36 were not possible because all the breaths were discarded by all the observers. Finally, to permit appropriate comparison with the control breath we developed a special filtering procedure to electronically subtract the superimposed FOT fluctuations on the flow-volume loops.

Although both the methods detect the presence of EFL, the physical principles they use to do so are different and this may contribute to some of the discrepancies in classification that we observed. During EFL, the impedance measured by FOT is a measure of the mechanical properties of airways downstream from the choke points. This is because a change in pressure cannot be transmitted upstream through the choke points and only the downstream airways are oscillated (26). As airway wall compliance is one order of magnitude greater than lung compliance, the reduction of Xrs during expiration reflects the number of choke points that occurred and their distribution within the bronchial tree. Therefore DXrs reflects the overall distribution of flow-limitation within the lung, and the threshold indicates the value above which all the pathways between airway opening and the alveoli are choked. Conversely, NEP technique detects only the latter condition, i.e. when all pathways are choked, in which further increase in alveolar pressure cannot lead to increase in expiratory flow.

This difference could have an important impact on the clinical meaning of the measurements provided by the two methods. For example, DXrs starts increasing with the progressive development of choke points, associated with a decrease in expiratory flow reserve. In these conditions some airways are not yet flow limited and would increase their flow by increasing alveolar pressure, thus making the phenomenon go undetected by NEP.

Moreover, as FOT provides a 'quantification' of number and location of choke points, we found very different values for DXrs within the fully flow-limited patients (from 2.89 to 15.20). Even if the clinical meaning of the value of DXrs has still to be investigated, bronchodilation affected significantly DXrs, suggesting that the degree of EFL has been modified although the changes were too small to modify the classification of the patient to not flow-limited for most of the patients flow-limited at baseline. This information cannot be provided by any method to detect EFL based on the comparison of expiratory flows.

All these differences might explain the higher sensitivity that DXrs showed in assessing the effects of bronchodilators, which could change the number of airways in which the choke points occur or cause the choke points to move distally within the airway without necessarily changing the NEP score.

Another important difference between FOT and NEP is that FOT does not depend on the evaluation of an observer but provides a number obtained by an automatic algorithm. This might be useful especially for the classification of borderline patients which, in our study, showed the largest disagreement between observers when using NEP.

Given all these differences between the methods, the comparison of the results can obviously be performed by considering their ability to detect full expiratory flow limitation, and this was the main goal of this study. In these conditions there was good agreement between the methods in identifying flow-limited and non-flow-limited breaths when the observers all agreed about the scoring. The small number of false positive breaths reported in this comparison were largely the result of using a single threshold value of DXrs to classify flow-limitation rather than the range of data noted in our original empirical study(15). In the majority of cases where there was disagreement between NEP and DXrs in the whole dataset there was also disagreement between the observers about the NEP scores, suggesting that these breaths were difficult to score using the NEP method.

We also used the methods to determine whether individual patients had EFL by averaging the NEP scores from all the observers for a given patient. Since the number of averaged NEP scores was variable and there were always five DXrs scores available, the comparison between the average of NEP scores and DXrs values might be statistically biased. However, it reflects the way in which the two techniques are performed in clinical practice and, therefore, we believe that it provides a sensible approach for the comparison of the two methods.

In this analysis we observed two sources of variability in our tests results: breath-to-breath variation in the degree of EFL within a patient and between-operator differences in scoring of NEP loops. Both methods identified between-breath changes in the degree of flow-limitation in the same patient, a process likely to reflect spontaneous variation in dynamically regulated end-expiratory lung volume. Data presented in Figure 6 support this view where the within breath reactance change over several minutes in three different patients illustrates both breath-to-breath variability and the way in which an individual close to the threshold value for flow-limitation (middle panel) can change between the two states. FOT appears best suited to detect these changes as NEP requires at least 45 seconds of stable breathing between measurements which makes following flow-limitation dynamically more difficult. Differences in NEP scoring between operators meant that we had to derive an empirical threshold for agreement between NEP scores and DXrs. When the average NEP score was greater than 30.8% there was agreement between NEP and DXrs in determine EFL and, similarly, patients were reliably classified as not flow-limited when the average score was below 24.6%. These empirically derived thresholds might differ if different observers were used. However, the improvement seen in the agreement between methods when the NEP data were averaged suggests that much of the variance lies in how the NEP are interpreted.

Bronchodilator drugs would be expected to modify the degree of expiratory flow-limitation and this has been proposed as an explanation of the reduced end-expiratory lung volume during exercise that usually(23;24), but not always(25), follows bronchodilator treatment in COPD. However, studies to date have been disappointing with no change in NEP score after high dose bronchodilators administered to severe COPD patients at rest(6). Our data confirmed these findings, three patients changing from FL to non-FL using the DXrs criteria, 2 of whom also showed a change in NEP scores. However one patient increased the degree of EFL by both the methods. Overall there was a significant reduction in within-breaths reactance after bronchodilator the clinical significance of which is still to be determined.

In conclusion, both methods have good measurement properties and, thanks to the recent technological advances in digital and power electronics, similar complexity and production costs.

Despite the subjective nature of the NEP response there is good inter-observer agreement, especially when the breath is clearly flow-limited or not. In contrast FOT is relatively 'objective' and the values distinguishing flow-limitation from non flow-limited breaths derived from balloon catheter data are in good agreement with the classification of flow-limitation using the NEP method. The ability to measure multiple breaths over longer periods and to do so automatically means that the DXrs method is well suited to continuously monitoring EFL, which may be desirable in the intensive care unit or when an intervention such as non-invasive ventilation is planned(16). Our data with both methods suggests that the degree of flow-limitation varies from breath to breath in patients with stable COPD. Thus individuals can move through a transition state where flow-limitation may or may not be detected in an individual breath. This emphasizes the need for testing several breaths in the evaluation of a patient's flow-limitation status. Further studies of the factors that determine this variability are now possible using the FOT technique.

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Figure legend

Figure 1: Experimental set-up for simultaneous EFL assessment by FOT and NEP. See text for details.

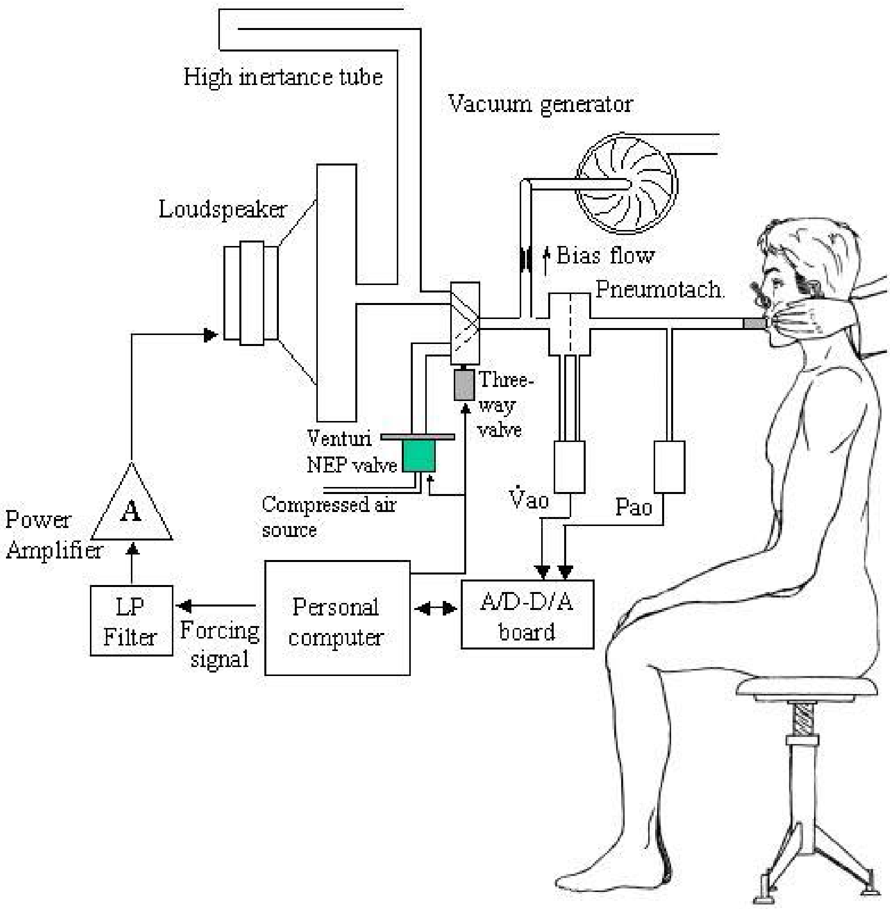


Figure 2: Upper panels: Representative examples of flow-volume loops during NEP maneuver for a non flow-limited (A), partially flow-limited (B) and fully flow-limited breath (C). Lower panels: Examples of NEP flow-volume loops that were discarded because of: D) the application of the negative expiratory pressure was too short compared to the breath; E) the control breath and the NEP breath lung volumes were too different; F) presence of wide oscillations of the expiratory flow during NEP, indicating possible upper airways instability.

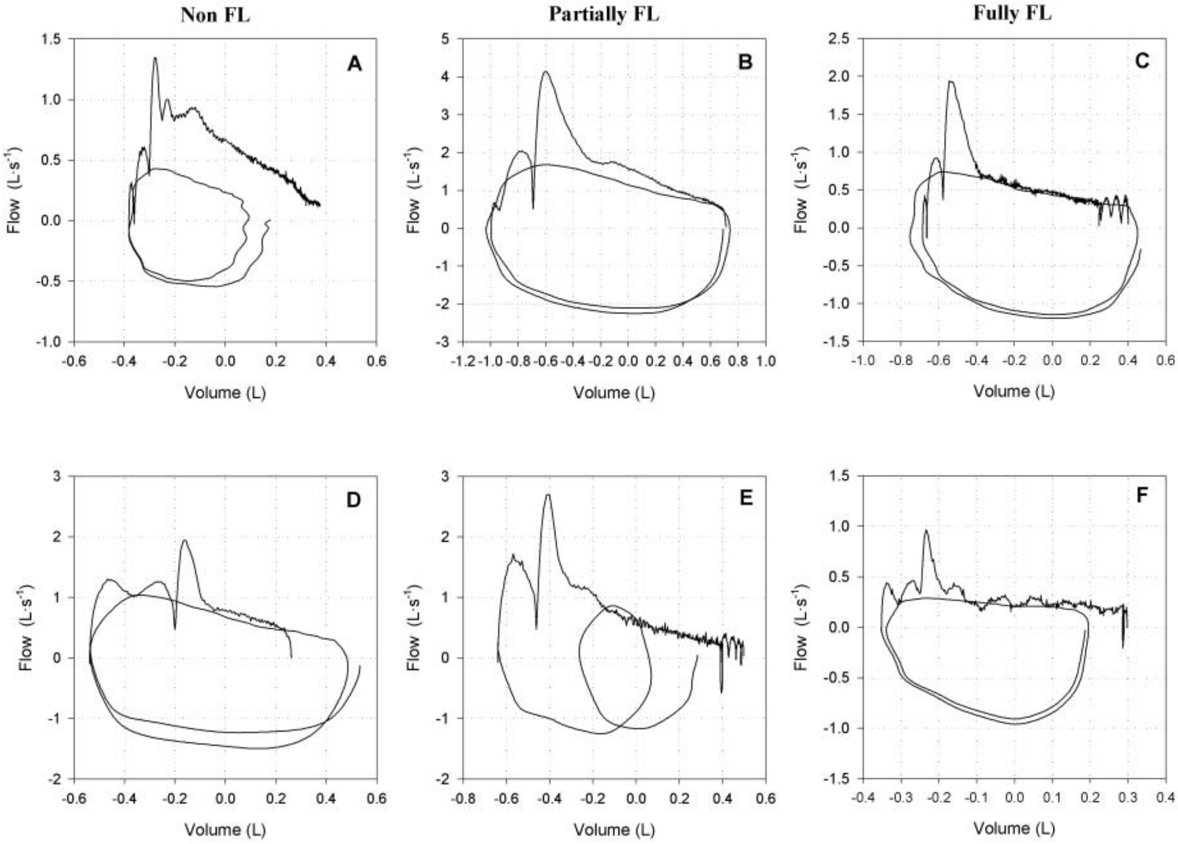


Figure 3: Experimental tracings from a representative flow-limited COPD patient during simultaneous FOT and NEP assessment of EFL. Pao: pressure at the airway opening. Zrs: total respiratory input impedance expressed as resistance (Rrs, continuous line) and reactance (Xrs, dashed line). DXrs indicates the presence of expiratory flow-limitation when its value is above the threshold of 2.8 cmH_2O*s/l, dotted line. See text for details.

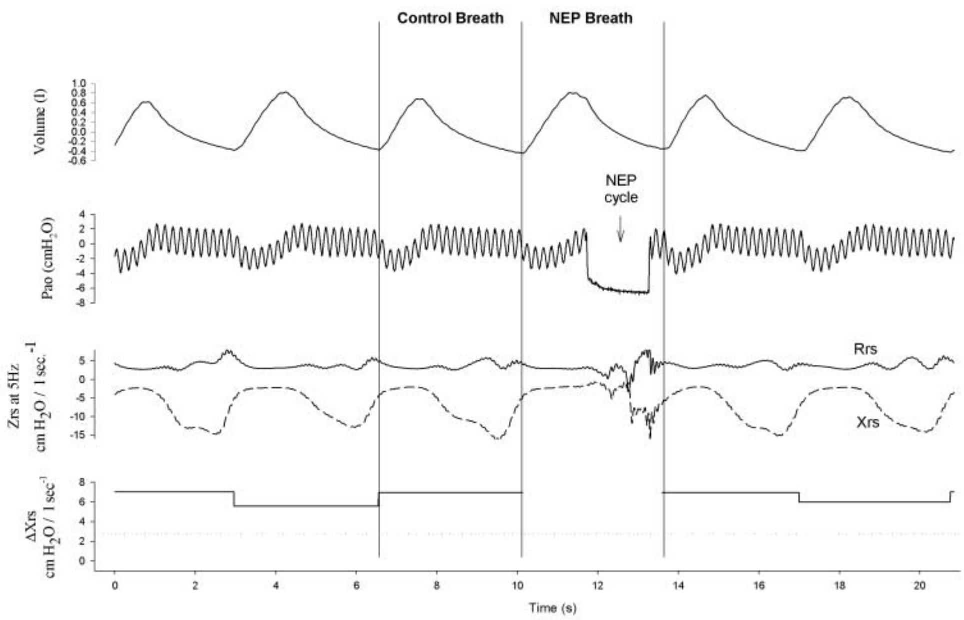


Figure 4: Effects of forced oscillations on flow-volume loops during NEP before (left panel) and after (right panel) filtering the data. The loops are from a representative maneuver from a flow-limited patient.

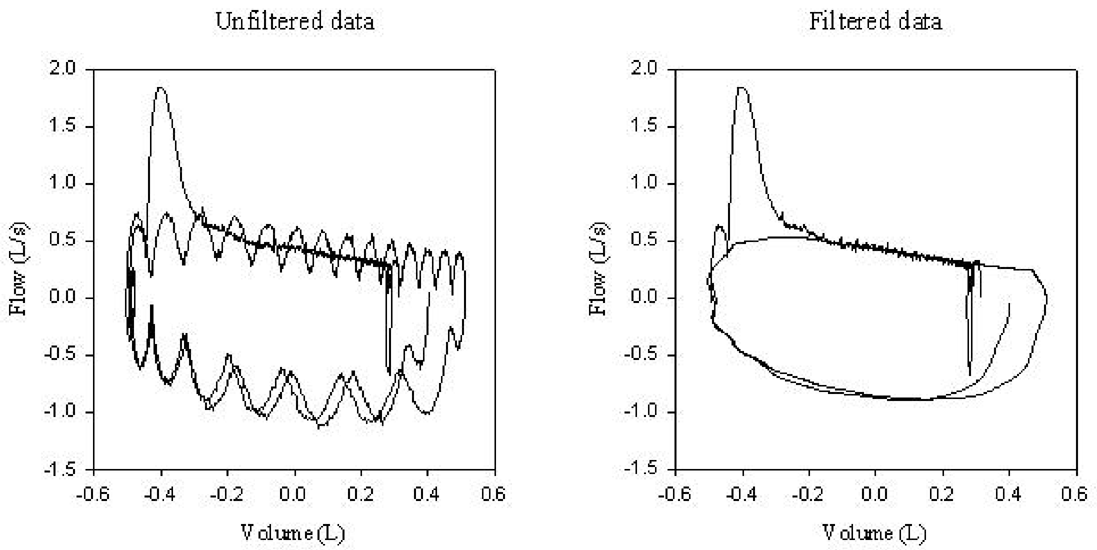


Figure 5: DXrs values vs. NEP scores plotted for all the considered breaths before (closed circles) and after (open circles) bronchodilators. The vertical dashed line indicates the threshold for flow-limitation on DXrs.

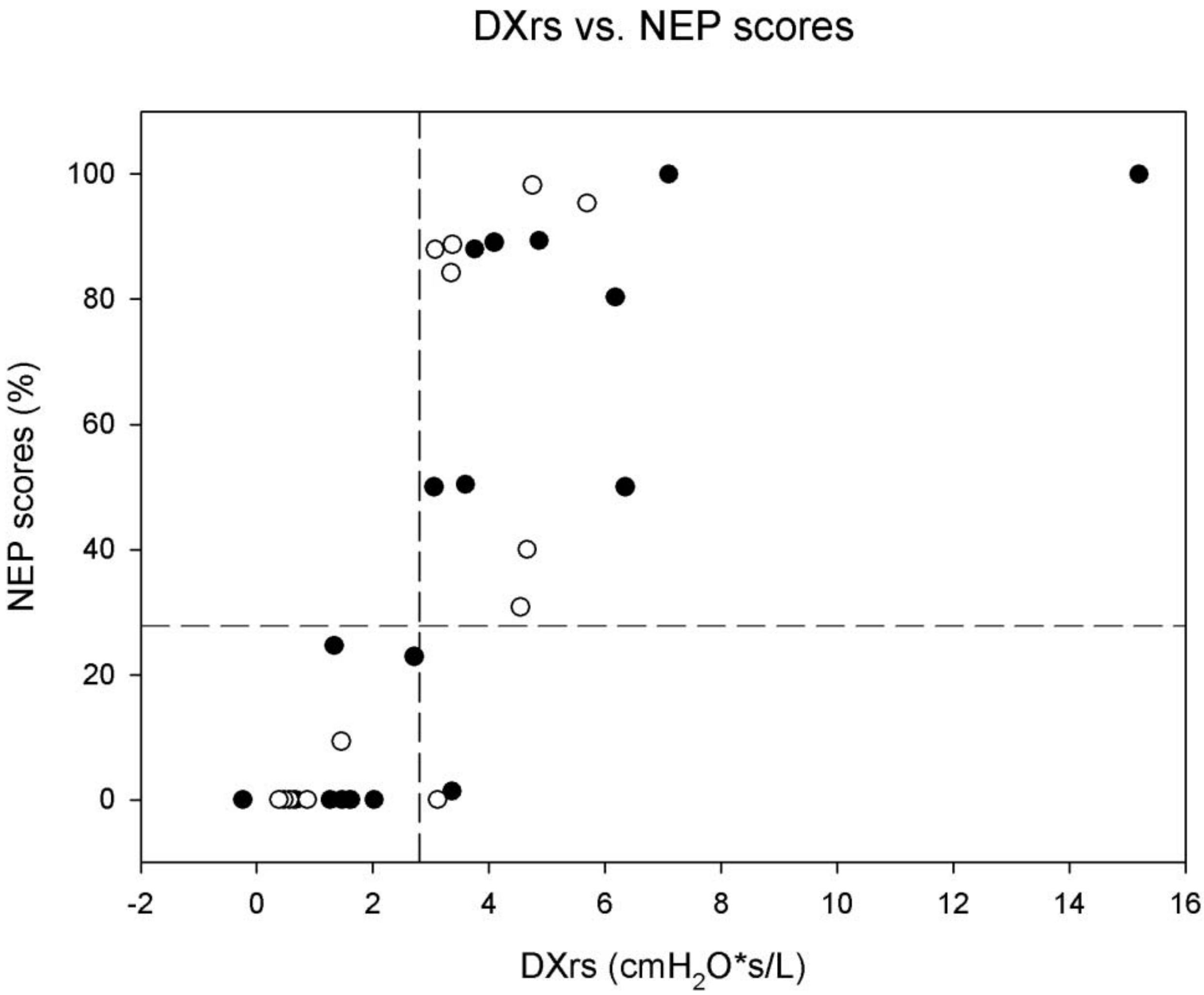
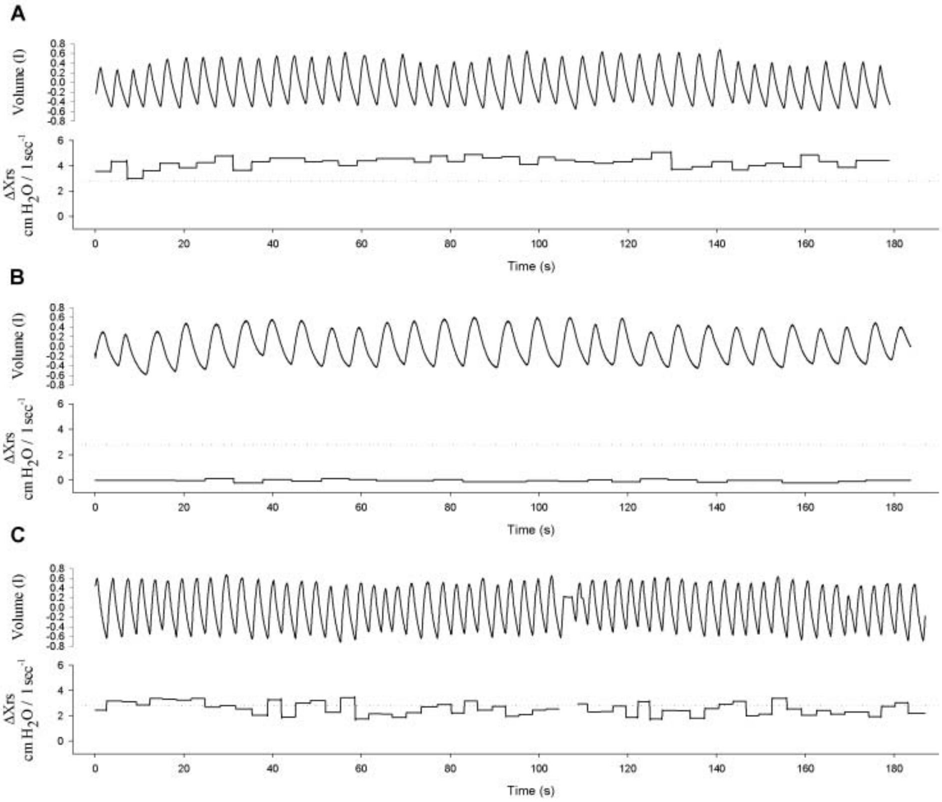


Figure 6: Variability of expiratory flow limitation assessed by DXrs during a period of three minutes of quiet breathing for representative flow-limited (A), not flow-limited (B) and intermediate (C) patients.



A
Volume (l)
ΔXrs cm H2O / l sec-1
Time (s)
B
Volume (l)
ΔXrs cm H2O / l sec-1
Time (s)
C
Volume (l)
ΔXrs cm H2O / l sec-1
Time (s)

Table 1: Patients characteristics and spirometric data of the patients.

PATIENT #	SEX	AGE (yrs)	FEV_1			FVC			FEV_1/FVC			TLC			TGV			RV	
			PRE	POST	diff	PRE	POST	diff	PRE	POST	diff	PRE	POST	diff	PRE	POST	diff	PRE	POST
#1	M	72	30	34	4	77	94	17	39	36	-3	134	122	-12	192	170	-22	237	187
#2	M	67	30	33	3	63	70	7	49	48	-1	126	129	3	171	164	-7	224	219
#3	F	51	24	30	6	54	84	30	44	35	-9	151	148	-3	213	190	-23	294	243
#4	F	67	31	46	15	59	76	17	53	60	7	129	131	2	190	184	-6	227	207
#5	M	61	50	62	12	74	100	26	68	63	-5	130	116	-14	150	117	-33	213	159
#6	M	75	32	30	-2	57	60	3	56	51	-5	119	120	1	169	166	-3	221	214
#7	M	72	30	34	4	77	94	17	39	36	-3	134	122	-12	192	170	-22	237	187
#8	M	79	40	56	16	80	113	33	49	48	-1	117	108	-9	167	135	-32	176	126
#9	M	80	50	51	1	86	92	6	57	55	-2	120	117	-3	147	141	-6	172	163
#10	M	73	24	23	-1	48	53	5	50	44	-6	119	125	6	173	189	16	217	248
#11	F	62	29	39	10	67	83	16	42	47	5	148	143	-5	206	192	-14	257	236
#12	M	81	46	54	8	66	77	11	69	70	1	129	140	11	172	182	10	226	248
#13	M	63	27	32	5	61	81	20	44	40	-4	158	154	-4	256	233	-23	326	285
#14	M	57	32	33	1	75	82	7	43	41	-2	124	125	1	182	178	-4	223	203
#15	F	67	48	46	-2	73	75	2	64	61	-3	113	112	-1	140	132	-8	158	137
Mean		**68,5**	**34,9**	**40,2**	**5,3**	**67,8**	**82,3**	**14,5**	**51,1**	**49,0**	**-2,1**	**130,1**	**127,5**	**-2,6**	**181,3**	**169,5**	**-11,8**	**227,2**	**204,1**
SD		**8,7**	**9,3**	**11,5**	**5,8**	**10,7**	**15,3**	**9,8**	**10,0**	**10,9**	**4,1**	**13,2**	**13,4**	**7,0**	**29,3**	**29,3**	**14,2**	**43,2**	**44,9**
p-value				**0,003**			**<0.001**			**0,069**			**0,175**			**0,006**			**0,005**
#16	F	59	36			63			57			170			240			325	
#17	M	51	84			100			84			113			151			171	
#18	M	53	22			43			50			97			140			208	
#19	M	70	26			57			46			153			239			284	
#20	M	74	24			44			55			121			175			225	
#21	F	75	48			80			60			151			179			236	
Mean		**67,1**	**36,3**			**66,9**			**53,2**			**131,2**			**183,0**			**231,3**	
SD		**9,3**	**14,4**			**14,3**			**11,2**			**17,9**			**32,7**			**45,9**	

FEV1: forced expiratory volume in one second; FVC: forced vital capacity; TLC: total lung capacity; TGV: thoracic gas volume; RV: residual volume; PRE: befor bronchodilator; POST: after bronchodilator; diff: differences between PRE and POST: p-values are for COPD before *versus* after bronchodilator. All pulmonary function data are expressed as percentage of predicted.

Table 2: Comparison between EFL breaths classification by FOT (DXrs) and NEP before and after bronchodilator (BD). OP1-3: operator 1-3

	PRE BD					POST BD					ALL				
	DXrs	NEP				DXrs	NEP				DXrs	NEP			
		OP 1	OP 2	OP 3	mean		OP 1	OP 2	OP 3	mean		OP 1	OP 2	OP 3	mean
Accepted	105	84	79	72	78.3	75	60	56	55	57.0	180	144	135	127	135.3
FL	58	34	39	14	29.0	38	22	25	15	20.7	96	56	64	29	49.7
NFL	47	50	40	58	49.3	37	38	31	40	36.3	84	88	71	98	85.7
NFL FOT, FL NEP		5 (6%)	1 (1.3%)	0 (0%)			3 (5%)	2 (3.6%)	2 (3.6%)			8 (5.6%)	3 (2.2%)	2 (1.6%)	
FL FOT, NFL NEP		6 (7.1%)	7 (8.9%)	16 (22.2%)			1 (1.7%)	9 (16.1%)	5 (9.1%)			7 (4.9%)	16 (11.9%)	21 (16.5%)	
Misclassified (%)		11 (13.1%)	8 (10.1%)	16 (22.2%)	14.7	0.0	4 (6.7%)	11 (19.6%)	7 (12.3%)	13.4	0.0	15 (10.4%)	19 (14.1%)	22 (18.1%)	14.2

Table 3: Patients classifications by the average values of the five tests before (pre) and after (post) bronchodilator. Values in brackets indicate the number of classified (non rejected) NEP maneuver for operator one, two and three, respectively.

Patient #	DXrs (cmH_2O*s/L)			NEP(%)				
	PRE	POST	diff	PRE		POST		diff
#1	3,75	3,35	-0,40	88,0	(5,5,5)	84,2	(5,5,5)	-3,8
#2	15,20	4,66	-10,54	100,0	(5,5,0)	40,0	(3,2,0)	-60,0
#3	7,10	4,75	-2,34	100,0	(3,4,0)	98,2	(2,3,1)	-1,8
#4	3,60	0,57	-3,04	50,4	(5,5,3)	0,0	(5,5,5)	-50,4
#5	1,34	3,08	1,74	24,6	(4,4,5)	87,9	(5,5,4)	63,3
#6	1,62	0,47	-1,15	0,0	(5,5,5)	0,0	(5,5,5)	0,0
#7	4,10	1,46	-2,64	89,1	(4,3,3)	9,3	(5,5,5)	-79,7
#8	4,87	3,37	-1,49	89,4	(5,5,5)	88,7	(5,4,4)	-0,7
#9	3,06	4,54	1,49	50,0	(2,3,5)	30,8	(4,3,2)	-19,2
#10	3,37	0,87	-2,50	1,4	(4,2,5)	0,0	(5,5,5)	-1,4
#11	0,66	0,39	-0,27	0,0	(5,5,5)	0,0	(5,5,5)	0,0
#12	6,18	5,69	-0,49	80,3	(4,4,4)	95,3	(5,4,1)	15,0
#13	6,35	2,89	-3,46	50,0	(2,2,0)	-	(0,0,0)	-
#14	4,45	3,12	-1,33	-	(0,0,0)	0,0	(2,0,0)	-
#15	2,71	0,39	-2,32	22,9	(4,5,5)	0,0	(5,5,5)	-22,9
Mean	**4,56**	**2,64**	**-1,92**	**53,28**		**38,18**		**-12,42**
SD	**3,47**	**1,83**	**2,83**	**38,26**		**42,66**		**36,10**
p-value		**0,020**			**0,239**			
#16	1,27			0	(5,5,5)			
#17	-0,24			0	(5,5,5)			
#18	2,02			0	(2,0,5)			
#19	1,47			0	(5,4,5)			
#20	0,67			0	(5,5,5)			
#21	1,61			0	(4,3,5)			
Mean	**3,58**			**37,30**				
SD	**3,33**			**40,36**				

p-values are for before (PRE) *versus* after (POST) bronchodilator. diff: difference between PRE and POST