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Regional differences in bronchial reactivity assessed by respiratory impedance

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ABSTRACT

We used the Impulse Oscillometric System (IOS) to gain information concerning the distribution of hyper-reactivity along the bronchial tree during methacholine challenge test (MCT).

37 subjects underwent MCT until reaching the provocative dose (PD20). At each dose, we estimated respiratory resistance at 5 and 20 Hz (R_5, R_{20}), and reactance at 5 Hz (X_5). In non-responsive subjects ($N = 14$) no changes in R_5, R_{20}, and X_5 were observed during MCT. In responsive subjects, a wide spectrum of responses was found concerning frequency dependence and PD20. We describe two phenotypes representing the extremes of response. For PD20 > 400 μg ($N = 13$), MCT caused equal changes of resistance/reactance on varying oscillation frequencies, suggesting a homogeneous bronchoconstriction along the bronchial tree. For PD20 < 200 μg ($N = 10$), a remarkable frequency dependence was observed, with increase in R_5, no change in R_{20}, and decrease in X_5, suggesting hyper-responsiveness of the distal airways paralleled by a change in visco-elastic properties of lung parenchyma.

1. Introduction

Bronchial hyper-responsiveness (BHR) is a pre-clinical condition characterized by an excessive bronchial narrowing in response to various inhaled stimuli. BHR is frequently associated with airway inflammation preceding clinical manifestations, such as asthma (Koskela et al., 2003).

An appropriate broncho-provocation testing is of high clinical relevance particularly to detect the early phase of the developing disease. Indications for broncho-provocation also include assessment of the response to asthma therapy, and, less commonly, the identification of triggers or cases involving environmental or occupational asthma (Laprise et al., 1999; Weiss et al., 2000).

The clinical diagnosis of BHR is currently performed by measuring the decrease in FEV_1 as percent of baseline value, caused by a direct activation of airway smooth cells (Cockcroft and Davis, 2006) following inhalation of aerosolized bronco-constrictor agents, such as methacholine (methacholine challenge test, MCT) (Crapo et al., 2000). However, the fact that for many patients it is relatively difficult to perform reliable spirometric forced maneuvers, has led to combine the measurement of FEV_1, with the measurement of airways resistance (Raw) assessed by body plethysmography (Nensa et al., 2009) that is less dependent on patient's cooperation. The real problem underlying the response to MCT remains the lack of information concerning the distribution of the broncho-reactivity along the bronchial tree down to the terminal lung units. Indeed, neither FEV1 nor plethysmography does really allow the identification of sites of bronchoconstriction that is known to vary in the clinical context. Thus, the general diagnosis of hyper-reactivity remains nonspecific as far as the location of the reaction is concerned. This point is of great interest given the fact that BHR is a complex inflammatory process affecting to a various extent both the large airways as well as the distal lung that includes the terminal airways as well as the surrounding interstitial microenvironment.

The aim of this study was to assess inter-individual differences concerning the longitudinal distribution of airways flow resistive properties as well as the viscoelasticity of the lung tissue in response to methacholine stimulation. We relied on the use of impedenzometric indexes as a powerful tool to describe the lung mechanical response to methacholine. In particular, we adopted the Impulse Oscillometric System (IOS), a non-invasive method that has been widely used to study the lung function in children (Tomalak et al., 2006; Schulze et al., 2012; Shi et al., 2012), as well as in adults (Aronsson et al., 2011; Skloot et al., 2004).

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2. Materials and methods

2.1. Study subjects

Subjects ($N = 37$) were recruited among people undergoing functional evaluation for clinical purposes at the pneumological service of the San Gerardo Hospital. At time of examinations, none of the subjects had any cardiac or pulmonary disease other than suspected asthma and none was receiving any treatment. Methacholine challenge test was requested to assess bronchial hyper-reactivity.

Subjects were informed in detail about the operation of the equipment and it was proved that they were able to perform the required maneuvers correctly. We obtained informed consent from subjects for the procedure/treatment and for their medical data to be used in this study. The project was approved by the Ethics Committee of the San Gerardo Hospital.

Methacholine challenge test was performed according to standardized protocols through deep inhalations using a dosimeter method (Crapo et al., 2000). Subjects abstained from using β2-agonists or other drugs 24 h prior to the challenge. Subjects underwent 0.9% saline aerosol bolus (assumed as baseline) and after increasing doses of methacholine (corresponding to cumulative doses of 50, 100, 200, 400, 800, 1600 and 2400 μg, respectively) administered through aerosol inhalation (Markos Mefar Dosimeter MB3). Respiratory mechanics was evaluated by Impulse Oscillometric System (IOS, Jeager, MasterScreen System). The sequence of maneuvers was as follows: (a) three consecutive runs of IOS (Oostveen et al., 2003), (b) determination by plethysmography of total gas volume (ITGV) and airways resistance (Raw), (c) FEV_1, which currently represents the gold standard. This sequence was chosen to avoid influences by spirometric maximal maneuvers on the bronchial tone (Skloot et al., 2004). Tests were performed and reported by at least 2 physicians experienced in the field. At each methacholine dose, all measurements were performed within 5 min.

The methacholine dose was increased until the provocative dose was reached, corresponding to a 20% decrease in FEV_1 from baseline (PD20) or to a maximal dose of 2400 μg. PD20 was estimated from the linear interpolation of the measured FEV_1 values with the corresponding methacholine dose for a 20% decrease in FEV_1 relative to saline solution.

2.2. Plethysmography

The plethysmographic method (Bodyscope Ganshorn Medizin Electronic) was used to measure intra thoracic gas volume (ITGV) and airways resistance (Raw): while closing a shutter at functional residual capacity (FRC), the subject was invited to make a series of weak inspiratory and expiratory efforts against the shutter. The patients were instructed to support the cheek with the palms of the hands to minimize the artifacts due to cheek motion. We derived ITGV as the slope of the resulting correlation between variation of box pressure and the corresponding variation in pressure measured at mouth. FEV_1 and FVC were measured from a flow-volume forced maximum expiratory maneuver. Raw was obtained as a ratio between specific airways resistance and lung volume at FRC during panting.

2.3. Impulse Oscillometric System (IOS)

This technique is based on the superimposition of small pressure oscillations with multiple frequency contents on the spontaneous breathing of the patient. The resulting changes in pressure and the corresponding flow, measured at the mouth of the subject under examination, depend on mechanical properties of airways, lung tissue and chest wall. To avoid interference from spontaneous breathing, the measurements are performed at frequencies at least one order of magnitude above normal breathing frequency. The technique allows estimating the absolute value of the impedance (Z_{rs}) of the respiratory system at different frequencies (in the range 5–20 Hz) that is given by the vector sum of resistance (R_{rs}) and reactance (X_{rs}). A full run of oscillation lasts 40 s. Subjects were instructed to support their cheeks with the palms of their hands to minimize shunt effects.

We considered the following IOS parameters: (a) resistance at 5 Hz (R_5), an index of total respiratory resistance, (b) resistance at 20 Hz (R_{20}), reported by most researchers as an index of resistance of the large airways; (c) the difference $R_5–R_{20}$ ($R_{5–20}$), index of frequency dependence of respiratory resistance, (d) reactance at 5 Hz (X_5), index of mechanical properties of the distal lung (Goldman et al., 2005). We considered the average inspiratory–expiratory values of resistance and reactance. In the case of reactance, on occasion we considered the expiratory value, X_{5exp}, that was proposed as a useful index to permit the early detection of flow limitation (Dellacà et al., 2004; Paredi et al., 2010).

2.4. Statistics

We performed linear regression and assessed the normality test for distribution as well as coefficient of skewness using Origin 8 software. To define the sensitivity and specificity of some IOS indexes, a receiver-operating characteristic (ROC) curve was plotted (Sigma plot 8). Cut-off levels at 95% confidence interval were calculated with the optimal combination of sensitivity and specificity using the Youden (Youden, 1950) index (sensitivity + specificity − 1).

3. Results

Patient recruitment and data collection and analysis extended from March 2012 to April 2013.

Table 1 reports the baseline subject's values of FEV_1, IOS indexes, as well as Raw and ITGV, and PD20. The first 3 lines report data concerning 3 subjects: a non-responsive control subject (A), and two patients whose response differed in terms of degree of frequency dependence of the impedenzometric indexes on increasing the methacholine dose: frequency dependence was not detectable in subject B-like while it was present to a various degree in subjects C-like. All other subjects were ranked in Table 1 based on increasing dose of PD20; considering this ranking, 62% of the subjects were considered positive (23 out of 37) having a PD20 lower than 2400 μg.

Fig. 1A shows R_{rs} and X_{rs} values at different oscillation frequencies for the three representative subjects. In a non-responsive subject (A) the R_{rs} and X_{rs} values (top and bottom panels, respectively) remained substantially unchanged at all frequencies of oscillation on increasing the methacholine dose up to 2400 μg. In subject B (Fig. 1B), the response to methacholine caused a similar increase in R_{rs} and corresponding decrease in X_{rs} at all frequencies. This caused a parallel shift upward and downward respectively for R_{rs} and X_{rs}, up to a maximal dose of 400 μg. In subject C (Fig. 1C), we observed a remarkable frequency dependent change in both R_{rs} and X_{rs} on decreasing frequency of oscillation upon reaching the maximal dose of 50 μg. The phenotypes of the two responsive subjects shown, essentially represent the extremes of a wide spectrum of response, characterized by a variable degree of frequency dependence and PD20 values.

Fig. 2A shows that a significant correlation was found by plotting $R_{5–20}$ at PD20 *vs.* the basal value of R_5. Furthermore, Fig. 2B shows that remarkable inter-individual differences were found when plotting the increase in $R_{5–20}$ at PD20, relative to baseline

Table 1
Anthropometric, spirometric, IOS and plethysmographic data in all subjects in basal condition.

Subject no	Age (yrs)	Gender (M/F)	Height (cm)	Weight (kg)	FEV_1	% pred.	R_5	R_{5-20}	R_{20}	X_5	Raw	ITGV	PD20
Subject A	56	M	169	60	4.21	120	0.22	0.03	0.19	−0.05	0.65	2.29	Neg.
Subject B	42	F	160	54	3.25	122	0.31	0.01	0.30	−0.08	0.36	2.90	241
Subject C	64	F	153	77	1.64	90	0.44	0.09	0.36	−0.16	0.31	3.14	34
1	44	M	177	115	2.93	91	0.57	0.18	0.40	−0.23	0.34	2.57	5
2	9	M	144	35	2.16	100	0.59	0.15	0.45	−0.17	0.62	2.40	15
3	48	F	162	61	1.90	81	0.64	0.14	0.51	−0.19	0.47	4.21	30
4	29	M	167	78	3.76	100	0.40	0.09	0.31	−0.12	0.43	3.48	35
5	56	F	148	67	1.57	85	0.84	0.28	0.55	−0.24	0.22	2.79	65
6	39	F	165	61	2.09	75	0.57	0.13	0.44	−0.14	0.55	3.49	77
7	57	F	157	59	2.29	105	0.39	0.08	0.31	−0.14	0.26	3.80	77
8	30	F	160	50	2.65	101	0.41	0.05	0.36	−0.10	0.42	3.22	137
9	29	F	150	55	2.78	107	0.38	0.07	0.30	−0.13	0.65	2.22	186
10	51	F	158	55	2.65	111	0.47	0.03	0.45	−0.11	0.28	4.66	282
11	47	M	186	114	4.10	99	0.41	0.06	0.35	−0.04	0.27	2.46	246
12	35	M	169	82	3.43	91	0.40	0.02	0.38	−0.06	0.38	3.44	366
13	21	F	164	49	3.35	100	0.35	0.01	0.34	−0.10	0.23	3.07	400
14	41	M	175	84	3.64	101	0.37	0.05	0.33	−0.06	0.31	2.25	400
15	39	M	169	77	3.99	109	0.35	0.04	0.31	−0.09	0.32	3.65	400
16	23	F	150	64	2.33	85	0.51	0.07	0.44	−0.11	0.25	4.22	464
17	36	F	178	74	3.45	98	0.39	0.05	0.34	−0.08	0.35	3.11	597
18	25	M	173	73	4.32	102	0.50	0.08	0.41	−0.08	0.21	3.57	672
19	25	M	170	67	4.35	106	0.54	0.08	0.46	−0.12	0.32	2.95	1037
20	30	F	165	80	2.97	94	0.49	0.06	0.43	−0.07	0.48	1.71	1806
21	44	F	155	49	2.58	95	0.39	0.04	0.34	−0.14	0.18	4.29	2017
22	46	M	180	92	4.52	112	0.32	0.04	0.28	−0.11	0.31	4.18	Neg.
23	64	M	175	74	3.45	109	0.28	0.02	0.27	−0.04	0.36	4.01	Neg.
24	68	M	171	79	3.02	104	0.31	0.02	0.29	−0.06	0.3	3.82	Neg.
25	21	F	168	59	3.73	106	0.30	0.05	0.25	−0.09	0.17	4.18	Neg.
26	45	M	187	88	5.07	120	0.24	0.03	0.21	−0.03	0.28	4.52	Neg.
27	48	M	187	100	4.29	103	0.27	0.03	0.24	−0.02	0.31	4.53	Neg.
28	15	M	159	54	3.09	107	0.46	0.08	0.39	−0.07	0.31	4.31	Neg.
29	13	F	162	50	2.81	99	0.40	0.06	0.33	−0.11	0.33	4.24	Neg.
30	16	M	173	63	4.25	120	0.45	0.04	0.41	−0.05	0.25	4.42	Neg.
31	28	F	153	46	2.65	97	0.35	0.04	0.31	−0.14	0.21	3.88	Neg.
32	43	F	162	56	3.37	123	0.27	0.02	0.25	−0.09	0.31	4.4	Neg.
33	34	M	170	49	4.45	114	0.26	0.03	0.23	−0.07	0.27	3.94	Neg.
34	26	M	173	65	4.08	97	0.36	0.03	0.39	−0.05	0.24	4.22	Neg.

R_5, R_{5-20}, R_{20}, X_5 and *Raw* are expressed as kPa/(L s^{-1}); FEV_1 and ITGV are expressed in L; PD20 in μg.

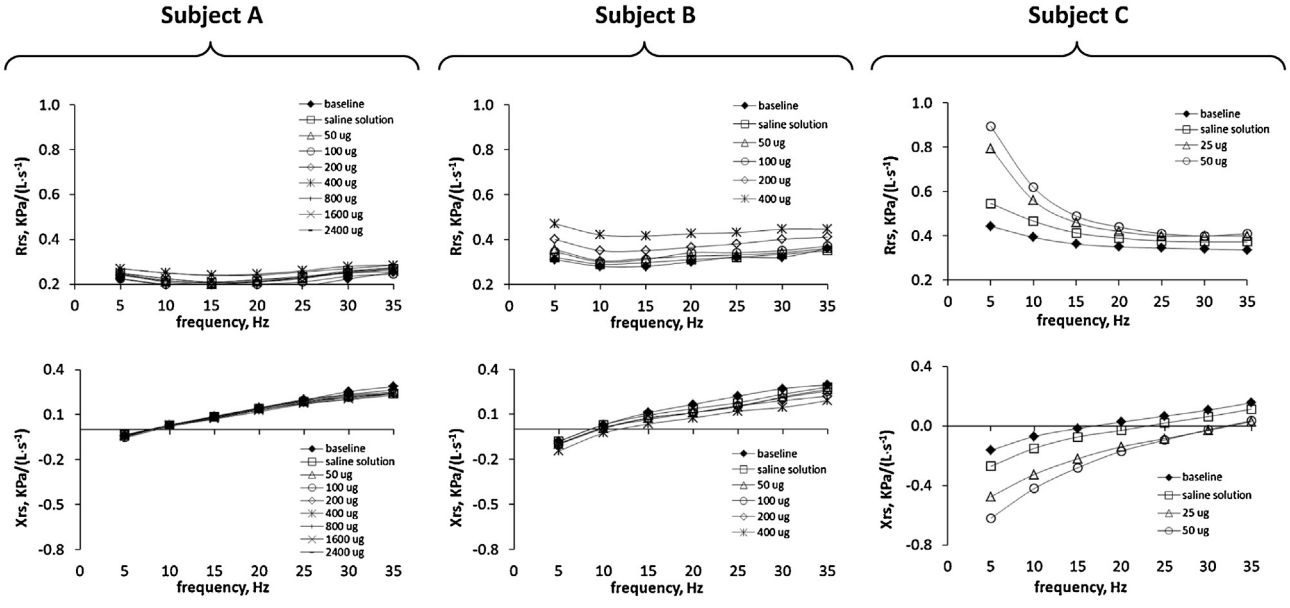

Fig. 1. Different effects of methacholine stimulation on impedenzometric indexes. Average values between inspiration and expiration are reported for respiratory resistance (R_{rs}) and reactance (X_{rs}). Different pattern of response on increasing oscillation frequency during methacholine stimulation (MCT) are presented for three representative subjects. (A) An unresponsive subject to MCT showing no changes in R_{rs} and X_{rs}; (B) a positive subject showing essentially equal changes of impedenzometric indexes at all frequencies, as a result R_{rs} and X_{rs} relationships are shifted in a parallel fashion upward and downward, respectively. (C) A positive subject showing a frequency dependence of impedenzometric indexes: R_{rs} and X_{rs} are remarkably affected on decreasing oscillation frequency below 20 Hz.

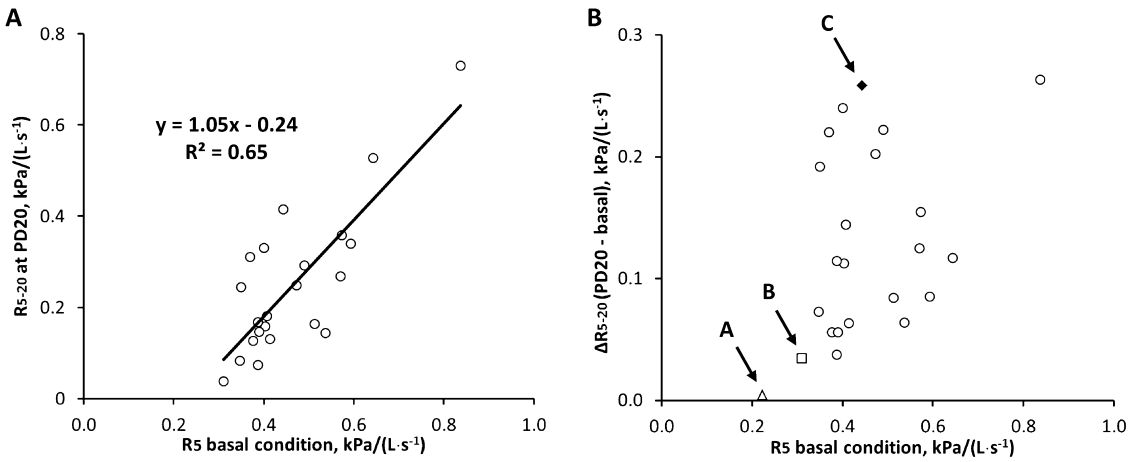

Fig. 2. Relationship between frequency dependence of respiratory resistance at PD20 and baseline respiratory resistance. (A) Regression between R_{5-20}, an index of frequency dependence, and baseline values of R_5 that reflect the baseline airways caliber. B. This panel shows the remarkable inter-individual variability encountered when plotting the increase in R_{5-20} at PD20, *vs*. the baseline values of R_5; letters A, B and C refer to the three subjects shown in Fig. 1.

values, *vs*. the basal values of R_5 (letters A, B and C refer to the three subjects shown in Fig. 1).

Fig. 3A shows that the relationships R_{20} *vs*. R_5 for subjects A, B and C could be fitted with linear regressions whose slopes decreased remarkably going from subject A to C during MCT. In fact, in negative subject (A) the increase in R_5 and R_{20} were exactly equal during MCT, in case of subject B the increase in R_{20} was 0.75 of that of R_5, while in subject C the increase in R_{20} was only 0.14 compared to R_{5-20}. Fig. 3B shows that also the slope of the relationships R_{5-20} *vs*. R_{20} increased remarkably going from subject A to C; the distribution of the slopes of these relationships for all positive subjects showed a wide spectrum of values and was remarkably skewed toward higher values with a coefficient of skewness of 1.24.

Fig. 4A highlights that for the same 20% decrease in FEV_1, corresponding to PD20, a large variability in frequency dependence of respiratory resistance, as expressed by R_{5-20}, was found in positive subjects. Fig. 4B shows that a great variability was also found when considering the individual relationships obtained by plotting R_{5-20} *vs*. *Raw* during MCT.

Fig. 5A shows that by plotting the values of R_{5-20} *vs*. $|X_5|$ for the three reference subjects, data essentially scattered along the same relationship; indeed, data from subject C simply extended the relationship to higher values of R_{5-20} and $|X_5|$. Fig. 5B shows that in all positive subjects the individual R_{5-20} *vs*. $|X_5|$ relationships essentially followed a similar trend so that the overall average linear regression was calculated.

Fig. 6A and B shows the semilog plot of R_{5-20} and $|X_{5exp}|$ at PD20 *vs*. the corresponding PD20 values for all positive subjects. The average value for negative subjects was indicated with an open square and referred to a maximal dose of 2400 μg. The vertical dashed lines refer to 200 and 400 μg of methacholine and it can be appreciated that a sharp increase in the values of R_{5-20} and $|X_{5exp}|$ were observed in subjects with PD20 < 200 μg.

In patients with PD20 > 400 μg, ITGV averaged 3.17 ± 1.1 L and 3.3 ± 0.9 L in baseline and at PD20, respectively (not significant increase). In patients with PD20 < 400 μg, baseline ITGV averaged 3.05 ± 0.7 L and increased to 3.57 ± 0.7 L at PD20 (significant 18% increase).

Fig. 7 presents the ROC analysis for R_{5-20}, and $|X_{5exp}|$, the two indexes that showed the greatest changes at PD20, aiming to discriminate the subjects with PD20 < 200 μg to those with 200 μg < PD20 < 400 μg. Concerning R_{5-20}, for the optimal Youden index of 0.6, corresponding to a cutoff of 0.32 kPa/(L s^{-1}), one reaches a sensitivity of 1 and a specificity of 0.6. Considering now $|X_{5exp}|$, for the optimal Youden index of 0.55 corresponding to a cutoff of 0.31 kPa/(L s^{-1}), one has a sensitivity of 0.7 and a specificity of 0.85.

4. Discussion

In physiological conditions, airways resistance mostly incorporates central airways, while distal airways, due to their large total cross-sectional area and low flow velocity, only contribute to a small fraction of total airway resistance (Mead, 1970). However, the contribution of small airways to total airways resistance and to its frequency dependence may substantially vary when their bronchomotor tone is increased. In this study we have extended the use of impedenzometric indexes to characterize individual differences in lung mechanics during MCT to provide indications as to the site of action of methacholine. As suggested by Fig. 2A, the frequency dependence of the response of the airways to the agonist drug related with baseline value of total respiratory resistance, as indexed by R_5, that obviously reflects baseline airways caliber (Ding et al., 1987). However, as shown by Fig. 2B, the increase in frequency dependence was highly variable among responsive subjects on reaching PD20, and therefore does not appear to simply be a function of baseline airways caliber. Furthermore, Fig. 4A highlights that for a 20% decrease in FEV_1, corresponding to PD20, a large variability of R_{5-20} was indeed found in positive subjects and, moreover, data from Fig. 4B also show that a similar variability for R_{5-20} could be found for a given *Raw* value. Thus, a wide spectrum of variability concerning the lung mechanical changes caused by the agonist drug can be detected by impedenzometric indexes already on the occasion of the first MCT.

The phenotypic responses of the B-like and C-like patients, reveal differences in the reactivity down the bronchial tree going from large to distal airways as well as changes in tissue mechanics elicited by methacholine. The interpretation of the changes in impedenzometric indexes during MCT is based on the various contribution of three main factors: (a) the inhomogeneous constriction of the airways (Farré et al., 1998, 1999a; Tgavalekos et al., 2005), (b) airways shunt proximal to an elevated airway resistance, and (c) decreased compliance due to loss of ventilated units (Skloot et al., 2004) and/or greater stiffness of the lung tissue. Since hyperinflation may occur when bronchoconstriction develops, a comment is also due on how this may affect the impedenzometric indexes. Indeed, an increase in ITGV may blunt the increase in airways resistance (Ding et al., 1987) but would actually increase tissue resistance due to lung overdistension.

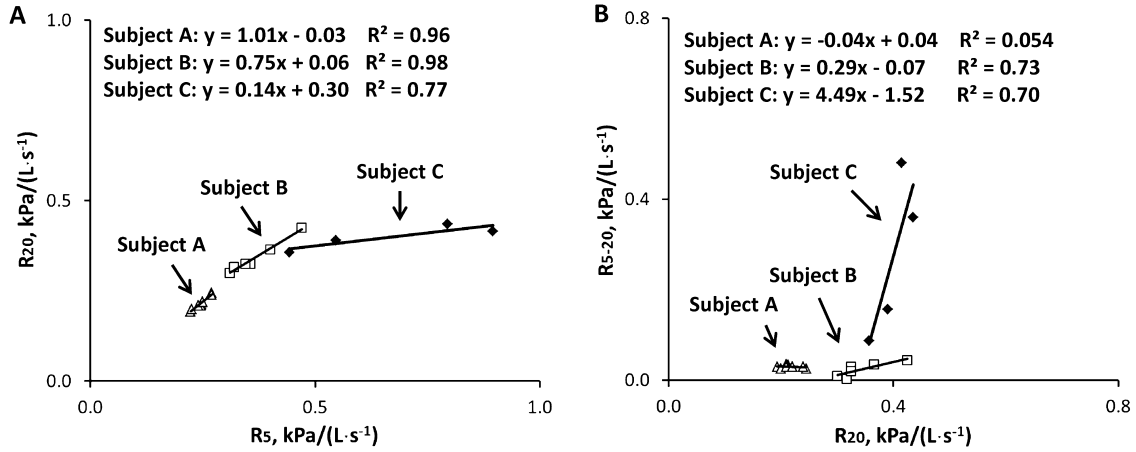

Fig. 3. Relative changes of R_{20} and R_5 during MCT for the three subjects shown in Fig. 1. (A) Minimal changes are observed for R_{20}, an index of central airways resistance and R_5 in the non-responsive subject A on MCT. In subject B, R_{20} increased almost as much as R_5, unlike subject C where the increase in R_5 was much greater than that of R_{20}. (B) R_{5-20}, an index of frequency dependence, increased remarkably more than R_{20} in MCT going from subject A to C.

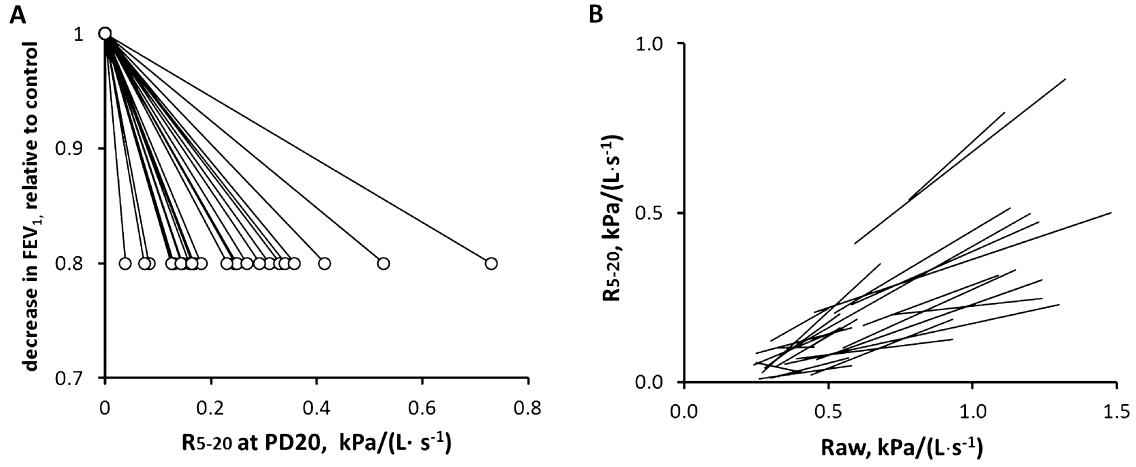

Fig. 4. Relationship between FEV1 and R_{5-20} at PD20 and between R_{5-20} and Raw. (A) This panel highlights the inter-individual variability of R_{5-20} at PD20 for the same 20% decrease in FEV_1. (B) Individual R_{5-20} *vs. Raw* relationships during MCT: note the large variability of R_{5-20} for the same value of Raw.

In subjects B-type, concerning point (a), we can deduce that, based on a fairly similar increase in R_5 and R_{20}, airway resistances increased homogeneously along the bronchial tree showing little or no frequency dependence. As a consequence of homogeneous increase in resistance along the bronchial tree, factor (b) should not appreciably contribute to a change in respiratory resistance as no specific point exists to be considered proximal to an elevated airway resistance. Concerning point (c), the changes in X_5 and

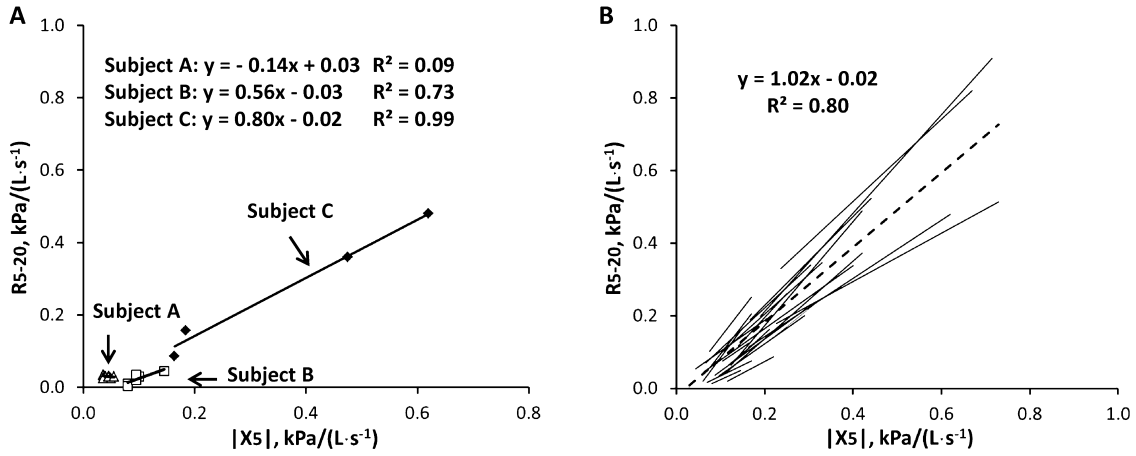

Fig. 5. Relationship between frequency dependence (R_{5-20}) and reactance (X_5). (A) Note that plotting the values of R_{5-20} *vs.* $|X_5|$ for the three reference subjects, data essentially scattered along the same relationship; indeed, data from subject C simply extended the relationship to higher values of R_{5-20} and $|X_5|$. (B) The individual R_{5-20} *vs.* $|X_5|$ relationships essentially followed a similar trend so that the overall average linear regression, having a slope close to unity, was calculated.

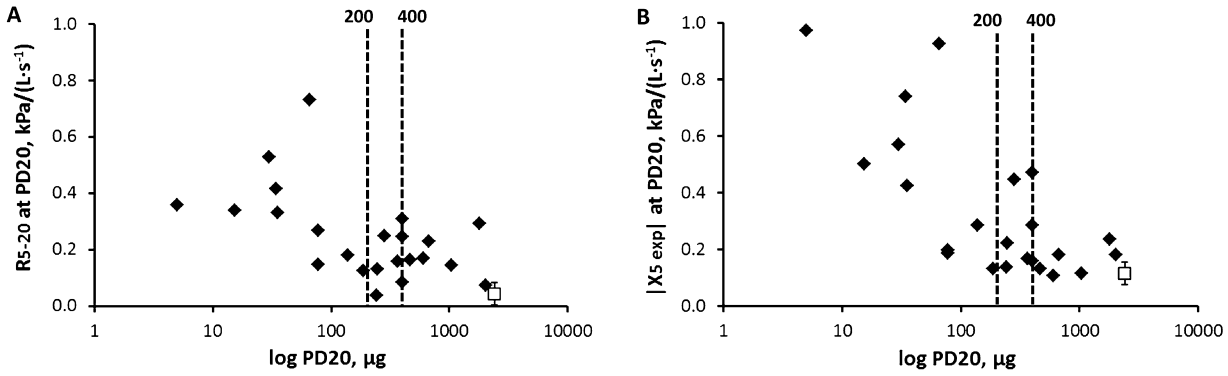

Fig. 6. Relationships between resistance and reactance and PD20. (A) Semilog plot of R_{5-20} at PD20 for all positive subjects. (B) Semilog plot of $|X_{5exp}|$ at PD20. The average values referring to negative subjects was indicated in both panels with an open square and referred to a maximal dose of 2400 μg. Vertical dashed lines refer to 200 and 400 μg of methacholine.

R_{5-20} were both of small amplitude, suggesting that no appreciable changes occurred in tissue viscoelasticity and gas compliance. Finally, for subjects having PD20 > 400 μg (essentially B-type) the increase in ITGV was not significant. Thus, in this phenotypic characteristic, the absence of an increase in frequency dependence of resistance and reactance stems for a homogeneous response of the broncho-constriction along the bronchial tree.

In C-like subjects (having a PD20 < 400 μg), most of the total respiratory system resistance (R_5) on MCT came from the increase in R_{5-20}. The remarkable increase in R_{5-20} with minor change in R_{20} suggests hyper-responsiveness at the lung periphery causing heterogeneity of regional distribution of ventilation and resistance, while the upper airways are largely not changing diameters enough to affect flow resistance. In these subjects ITGV increased significantly by 18% at PD20, therefore the actual increase in R_{5-20} would have been greater than that actually measured (Ding et al., 1987). A contribution of factor (b) cannot be excluded. However this effect was documented in case of severe bronchoconstriction (Farré et al., 1999b; Peslin et al., 1985), which was not the case in our subjects as we only considered a 20% decrease in FEV_1. Interestingly, since in C-like subjects the slope of the average relationships between R_{5-20} and $|X_5|$ is 1 (Fig. 5B), this supports the hypothesis that the increase in frequency dependence of resistance involving the peripheral airways is paralleled by changes in viscoelastic properties of the lung tissue. One may recall that in the experimental model, reactance measurements were significantly altered in interstitial edema causing an increase in extravascular lung water not exceeding 10% and a remarkable perturbation of the macromolecular organization of the extracellular matrix (Dellacà et al., 2008; Miserocchi, 2009). An increase in $|X_5|$ may also be interpreted as a consequence of a loss of ventilated alveolar units. In summary, in subject C-like, the characteristic phenotypic response to the agonist drug mostly involves the peripheral lung district.

Previous works have addressed the greater specificity and sensitivity of various impedenzometric indexes *vs.* the spirometric indexes (Schulze et al., 2012; Shi et al., 2012) to evaluate the response to methacholine challenge test.

As Fig. 6 shows, the remarkable changes in impedenzometric indexes, particularly R_{5-20} and $|X_{5exp}|$ were observed in subjects with PD20 < 200 μg that have a greater hyper-responsiveness with a major involvement of the distal lung. We therefore thought it useful to develop a ROC analysis in order to partition between subjects with PD20 < 200 μg, that should be considered as more severe patients, relative to subjects with PD20 < 400 μg but >200 μg. Based on the ROC analysis for R_{5-20}, assuring sensitivity of 1 and a specificity of 0.6, for a cutoff of 0.32 kPa/(L s^{-1}) we counted 6 C-like subjects out of 23 (26%). On considering $|X_{5exp}|$, for a cutoff of 0.31 kPa/(L s^{-1}), a sensitivity of 0.7 and a specificity of 0.85, the number of C-like subjects would increase up to 8 (about 35%) as two subjects with 200 μg < PD20 < 400 μg would be included. In Table 2 we report for these 8 subjects, to show that their average impedenzometric indexes in basal conditions are higher compared to positive subjects with PD20 > 200 μg as well as to negative subjects.

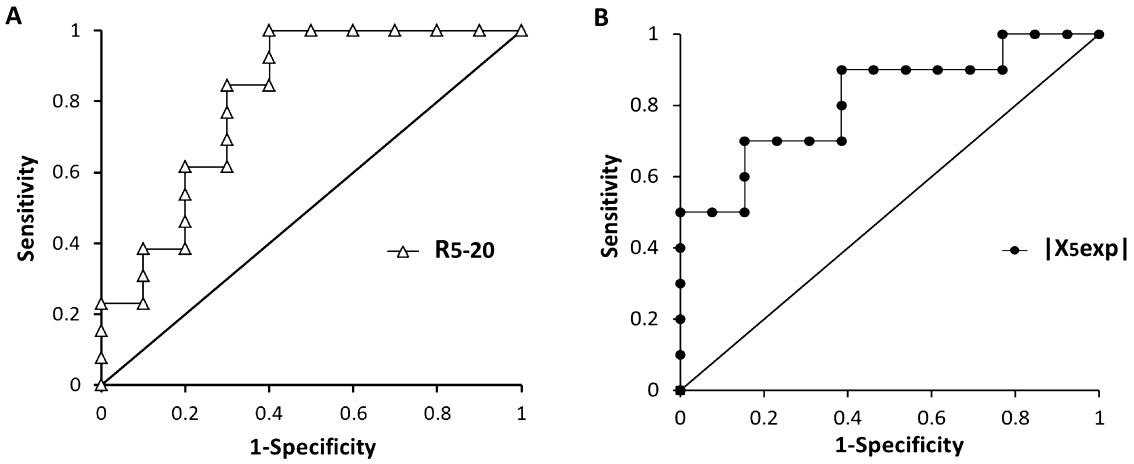

Fig. 7. Receiver-operating characteristic curves (ROC). Receiver-operating characteristic (ROC) curve for R_{5-20} (A) and $|X_{5exp}|$ (B) at PD20 for subjects with PD20 < 200 μg. Areas under the curves (AUC) were 0.8 and 0.82, respectively.

Table 2
Average basal values of IOS indexes for negative and positive subjects.

	R_5	R_{5-20}	R_{20}	X_{5exp}
Negative	0.32 ± 0.07	0.03 ± 0.02	0.29 ± 0.07	−0.04 ± 0.02
Positive PD20 > 200 μg	0.41 ± 0.07	0.05 ± 0.02	0.37 ± 0.06	−0.07 ± 0.03
Positive PD20 < 200 μg	0.56 ± 0.15	0.14 ± 0.07	0.42 ± 0.08	−0.22 ± 0.04

R_5, R_{5-20}, R_{20} and X_{5exp} are expressed as kPa/(L s^{-1}).
Data are expressed as mean ± standard deviation.

4.1. Limitation of the study

This study includes a relatively limited number of subjects, yet enough to identify a group of very sensitive patients, identified as C-like, having a major involvement of the distal lung in the response to broncho-constrictor agents.

The interest of adopting forced oscillations to gather longitudinal data to assess the state/progression of the morbidity has been highlighted to follow the degree of the remodeling and/or the entity of the inflammation process (Hackett, 2012).

A limited number of studies have been published on IOS accuracy compared to FOT, which generally suggest that the two methods yield similar but not identical measures of R_{rs} and X_{rs} (Hellinckx et al., 2001). IOS was shown to overestimate R_{rs} at low frequency with increasing resistance values, compared to mixed sinusoidal or broad-band multi-frequency wave forms; this should not invalidate our estimates as this factor would equally impact on the C-like and B-like behavior.

5. Conclusions

Assessment of respiratory resistance and reactance through forced oscillation allows to distinguish between main involvement of peripheral lung units as opposed to homogeneous response of the broncho-constriction along the bronchial tree in response to a broncho-constrictor agent.

Contributorship statement

Egidio Beretta, medical doctor, PhD, main role in study design, IOS data collection and analysis, paper writing, took responsibility for the integrity of the work as a whole.

Francesco Tana, pneumologist, involved in subject recruitment and in plethysmography evaluation.

Gabriele Simone Grasso, medical doctor, role in IOS data collection/analysis and pulmonary function data integration.

Manuela Bartesaghi, medical doctor, IOS data collection.

Luca Novelli, medical doctor, helped in the plethysmography evaluation.

Alberto Pesci, pneumolgist, supervisor of the pneumological evaluation.

Giuseppe Miserocchi, contributed to study design, integration of informations.

Competing interests

No competing interests for any author.

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References

Aronsson, D., Tufvesson, E., Bjermer, L., 2011. Comparison of central and peripheral airway involvement before and during methacholine, mannitol and eucapnic hyperventilation challenges in mild asthmatics. Clin. Respir. J. 5, 10–18.
Cockcroft, D.W., Davis, B.E., 2006. The bronchoprotective effect of inhaling methacholine by using total lung capacity inspirations has a marked influence on the interpretation of the test result. J. Allergy Clin. Immunol. 117, 1244–1248.
Crapo, R.O., Casaburi, R., Coates, A.L., Enright, P.L., Hankinson, J.L., Irvin, C.G., MacIntyre, N.R., McKay, R.T., Wanger, J.S., Anderson, S.D., Cockcroft, D.W., Fish, J.E., Sterk, P.J., 2000. American Thoracic Society Guidelines for methacholine and exercise challenge testing – 1999. Am. J. Respir. Crit. Care Med. 161, 309–329.
Dellacà, R.L., Santus, P., Aliverti, A., Stevenson, N., Centanni, S., Macklem, P.T., Pedotti, A., Calverley, P.M.A., 2004. Detection of expiratory flow limitation in COPD using the forced oscillation technique. Eur. Respir. J. 23, 232–240.
Dellacà, R.L., Zannin, E., Sancini, G., Rivolta, I., Leone, B.E., Pedotti, A., Miserocchi, G., 2008. Changes in the mechanical properties of the respiratory system during the development of interstitial lung edema. Respir. Res. 9, 51–60.
Ding, D.J., Martin, J.G., Macklem, P.T., 1987. Effects of lung volume on maximal methacholine-induced bronchoconstriction in normal humans. J. Appl. Physiol. 62, 1324–1330.
Farré, R., Ferrer, M., Rotger, M., Torres, A., Navajas, D., 1998. Respiratory mechanics in ventilated COPD patients: forced oscillation versus occlusion techniques. Eur. Respir. J. 12, 170–176.
Farré, R., Peslin, R., Rotger, M., Barberá, J.A., Navajas, D., 1999a. Forced oscillation total respiratory resistance and spontaneous breathing lung resistance in COPD patients. Eur. Respir. J. 14, 172–178.
Farré, R., Rotger, M., Marchal, F., Peslin, R., Navajas, D., 1999b. Assessment of bronchial reactivity by forced oscillation admittance avoids the upper airway artefact. Eur. Respir. J. 13, 761–766.
Goldman, M.D., Saadeh, C., Ross, D., 2005. Clinical applications of forced oscillation to assess peripheral airway function. Respir. Physiol. Neurobiol. 148, 179–194.
Hackett, T.L., 2012. Epithelial-mesenchymal transition in the pathophysiology of airway remodelling in asthma. Curr. Opin. Allergy Clin. Immunol. 12, 53–59.
Hellinckx, J., Cauberghs, M., De Boeck, K., Demedts, M., 2001. Evaluation of impulse oscillation system: comparison with forced oscillation technique and body plethysmography. Eur. Respir. J. 18, 564–570.
Koskela, H.O., Hyvarinen, L., Brannan, J.D., Chan, H.K., Anderson, S.D., 2003. Responsiveness to three bronchial provocation tests in patients with asthma. Chest 124, 2171–2177.
Laprise, C., Laviolette, M., Boutet, M., Boulet, L.P., 1999. Asymptomatic airway responsiveness: relationship with airway inflammation and remodelling. Eur. Respir. J. 14, 63–73.
Mead, J., 1970. The lung's quiet zone. N. Engl. J. Med. 282, 1318–1319.
Miserocchi, G., 2009. Mechanisms controlling the volume of pleural fluid and extravascular lung water. Eur. Respir. Rev. 18, 244–252.
Nensa, F., Marek, W., Marek, E., Smith, H.J., Kohlhäufl, M., 2009. Assessment of airway hyperreactivity: comparison of forced spirometry and body plethysmography for methacholine challenge tests. Eur. J. Med. Res. 14, 170–176.
Oostveen, E., MacLeod, D., Lorino, H., Farré, R., Hantos, Z., Desager, K., Marchal, F., 2003. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. Eur. Respir. J. 22, 1026–1041.
Paredi, P., Goldman, M., Alamen, A., Ausin, P., Usmani, O.S., 2010. Comparison of inspiratory and expiratory resistance and reactance in patients with asthma and chronic obstructive pulmonary disease. Thorax 65, 263–267.
Peslin, R., Duvivier, C., Gallina, C., Cervantes, P., 1985. Upper airway artifact in respiratory impedance measurements. Am. Rev. Respir. Dis. 132, 712–714.
Schulze, J., Smith, H.J., Fuchs, J., Herrmann, E., Dressler, M., Rose, M.A., Zielen, S., 2012. Methacholine challenge in young children as evaluated by spirometry and impulse oscillometry. Respir. Med. 106, 627–634.
Shi, Y., Aledia, A.S., Tatavoosian, A.V., Vijayalakshmi, S., Galant, S.P., George, S.C., 2012. Relating small airways to asthma control by using impulse oscillometry in children. J. Allergy Clin. Immunol. 129, 671–678.
Skloot, G., Goldman, M., Fischler, D., Goldman, C., Schechter, C., Levin, S., Teirstein, A., 2004. Respiratory symptoms and physiologic assessment of Ironworkers at the World Trade Center disaster site. Chest 125, 1248–1255.
Tgavalekos, N.T., Tawhai, M., Harris, R.S., Musch, G., Vidal-Melo, M., Venegas, J.G., Lutchen, K.R., 2005. Identifying airways responsible for heterogeneous ventilation and mechanical dysfunction in asthma: an image functional modeling approach. J. Appl. Physiol. 99, 2388–2397.
Tomalak, W., Radliński, J., Pawlik, J., Latawier, W., Pogorzelski, A., 2006. Impulse oscillometry vs. body plethysmography in assessing respiratory resistance in children. Pediatr. Pulmonol. 41, 50–54.
Weiss, S.T., van Natta, M.L., Zeiger, R.S., 2000. Relationship between increased airway responsiveness and asthma severity in the childhood asthma management program. Am. J. Respir. Crit. Care Med. 162, 50–56.
Youden, W.J., 1950. Index for rating diagnostic tests. Cancer 3, 32–35.