Respiratory impedance in healthy subjects: baseline values and bronchodilator response

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METHODS

Impedance measurements

Respiratory impedance (Zrs) was measured at centres C1-C5 by using the setups described below, with strict adherence to the manufacturer’s instructions regarding calibration and maintenance, wherever available.

C1: In the custom-made setup, a loudspeaker was connected to the mouthpiece through a wide-bore tube (ID= 3.2 cm). The airway opening pressure and pressure drop across a Fleisch no. 2 pneumotachograph were measured with matched pressure transducers (Celesco LCVR ± 2 hPa, Honeywell, Plymouth, MN). The common mode rejection ratio (1) was >60 dB at 26 Hz. The flow signal was corrected for the time constant of the pneumotachograph. To prevent rebreathing, a bias flow was flushed continuously through the breathing tube. The characteristics of the setup were compatible with recent ERS guidelines (1). The setup generated pseudorandom pressure variations containing all harmonics of 2 Hz from 4 to 32 Hz. Pressure and flow signals were measured for 16 s, and both signals were time-averaged over 0.5-s blocks before fast Fourier transformation (FFT) was performed. The values of Zrs were corrected for the mouthpiece impedance. The reference impedance was measured at intervals in order to check the FOT system.

C2: A device formerly commercially available (ROS Oscilink, SensorMedics, The Netherlands) was used. A pseudorandom noise signal containing integer multiples of 1 Hz from 4 to 30 Hz was generated by a loudspeaker. Mouth flow was sensed by
a pneumotachograph (4700, Hans Rudolph, Kansas City, MO, USA) connected to a
differential transducer (DP45 ± 2 hPa, Validyne, Northridge, CA, USA). Both signals
were low-pass filtered and sampled at 128 Hz for measurement periods of 16 s. Auto-
and cross-spectra of flow and pressure were calculated for adjacent 4-s blocks and
averaged over each 16-s period to yield a mean estimate of Zrs for each frequency
component. Static calibration of the pneumotachograph and pressure sensor was
performed daily.

C3: A pseudorandom signal consisting of a 2-Hz fundamental and its multiples up to
26 Hz was employed in a custom-made setup. The signal was delivered by a
loudspeaker-in-box system via the flowmeter and a bacterial filter to the mouthpiece
of the subject during 20 s of quiet breathing. The flowmeter consisted of a cylindrical
(29-mm-ID) screen pneumotachograph and a differential transducer (MP-45 ± 2 hPa,
Validyne, Northridge, CA, USA). The pressure drop across the respiratory system
plus the pneumotachograph and the mouthpiece was sensed by an IC Sensors
transducer (model 33NA002D, Milltopa, CA, USA). This FOT setup was calibrated as
follows. The flow into and the pressure inside a 3-l rigid-walled container were
measured with the flowmeter and the pressure sensor, respectively, and the
theoretical input impedance of the container was calculated; the ratio of these two
spectra was used as the calibration function of the setup. The instrumental
impedance between the pressure port and the subject was determined and removed
from the measured Zrs spectra. Each Zrs spectrum was computed by FFT on 8-s time
windows and 75% overlapping.
**C4:** A commercially available device (I2M, Chess mT, Ghent, Belgium) was used. The measured Zrs spectra were corrected for the impedance of the bacterial filter. The loudspeaker of the setup generated pseudorandom noise pressure variations, containing a 2-Hz fundamental and its multiples up to 48 Hz. Mouth pressure and flow were recorded during 8 s with the use of identical differential pressure transducers (IC Sensors, Model 1220, Measurement Specialties, VA, USA). Data were sampled at 128 Hz, and FFT was performed with moving window averages of 1024 samples. The accuracy of the equipment was verified daily by using a known resistance of ~ 2 hPa.s.L⁻¹.

**C5:** A commercially available device (IOS, Jaeger, GmbH, Hoechberg, Germany) was employed. The pneumotachograph of the device was calibrated before every measurement and the equipment accuracy was checked by using a known resistance of 2 hPa.s.L⁻¹. The loudspeaker of this setup generated an impulse-shaped pressure fluctuation at intervals of 200 ms. Pressure and flow signals were recorded for 30 s with the use of identical pressure transducers (SensorTechnics SLP004D, Germany). For measurements and data analysis the manufacturer’s default settings were used.

In all setups except that at C5, the oscillatory forcing signal was constructed in such a manner that the energy content of the lower frequencies was enhanced and the relative energy content decreased hyperbolically or exponentially with increasing frequency. In each setup, the forcing signal was kept within ± 1.5 hPa.
The Zrs measurements were performed according to recent ERS guidelines (2). In brief, subjects were measured while seated, with the head in the neutral position. The nose was clipped and the investigated subject firmly supported his/her cheeks and the floor of the mouth with both hands. The subject was instructed to breathe quietly at FRC. Between the measurements, the subject came off the mouthpiece. Individual measurements were excluded as technically unacceptable if any of the following was noticed: swallowing, glottic closure, an incomplete seal around the mouthpiece or irregular breathing. The average and standard deviation of 3 to 5 technically acceptable Zrs measurements were used for further analysis.

**Reference impedance**

A mechanical impedance device was constructed by fitting a high-resistance wire mesh screen into a 14-cm-long, 18-mm-ID tube. The resistance of this reference impedance was measured with dc flow (R= 8.9 hPa.s.L⁻¹). The measured impedance of the device was independent of oscillatory amplitude up to an inlet pressure of ± 2.2 hPa, and increased by <3% at ± 3.3 hPa. The impedance data collected on this reference device are illustrated in Fig. S1. Although the impedance of this resistive-inertive device did not mimic the typical spectra of the human respiratory system, its magnitude was large enough to reveal the possible magnitude and phase distortions introduced by the setups used in this study.

**Forced expiration**

Spirometry was performed with the following setups: C1: MasterScreen PFT, Viasys Healthcare GmbH, Hoechberg, Germany (software version JLAB 4.52); C2 and C5:
MasterScreen PFT (JLAB 4.53a), C3: Spirobank–Winspiro (Mir, Rome, Italy); C4: Welch Allyn Pneumocheck (Skaneateles Falls, NY, USA). The highest values of the forced expiratory volume in 1 s (FEV₁) and the (forced) vital capacity ((F)V)C from 3 technically acceptable and reproducible manoeuvres were retained for further analysis.

**Characteristics of the participating subjects**

A total of 368 subjects (51% females) were included in the study; the characteristics of the subjects enrolled at the different centres are presented in Figs S2-S5.

**Mixed-model analysis of Zrs data**

Mixed-model analysis (3) was applied to choose the autoregressive covariance structure with which to model the correlation between spectra. First the dependences of the Rrs and the Xrs data on sex, frequency (f) and centre were analysed on the transformed resistance and reactance data, ln(Rrs) and ln(4-Xrs), respectively. As the 3-way interaction and the 2-way interaction that involved sex and f were significant, separate analyses were performed for the males and the females. The dependences of the Rrs and Xrs data on f and centre were examined with adjustment for the covariates height (H), age (A) and weight (W). The mean values of Rrs and Xrs at the different centres after the adjustment exhibited some systematic differences in the frequency dependences of Rrs and Xrs between centres. Subsequently, a mixed model with the interaction centre × f and the covariate combinations H-A-W and H-A-body mass index (BMI) was addressed. Since the latter combination did not
improve the model performance, H, A and W were retained in the subsequent analysis. Both Rrs and Xrs were significantly influenced by the covariates f, H and W and the interaction centre × f for females and males. Since the centre × f interaction was significant, i.e. the inter-centre frequency dependence of Zrs differed significantly, the full set of Zrs spectra from all the centres could not be pooled; however, they could be pooled at some frequencies. The Zrs data from C1, C3 and C4, which were obtained only at even values of frequency, were supplemented with the linearly interpolated values at 5, 15 and 25 Hz. Therefore, data became available at 5 Hz and its multiples from all centres, whereas at even frequencies between 4 and 26 Hz, the data from 4 centres could potentially be combined. The mean Rrs (Rmean), the resonant frequency (fres) and the reactance curve area (AX, (4)) were also included in the analysis. There was generally no centre dependence in Rrs, except at 20 and 25 Hz in the females, and at 25 Hz in the males, whereas Xrs was centre-independent up to 14 Hz in both sexes. Exclusion of the data from C5 eliminated the significant differences in Rrs, but not in Xrs.

Prediction example

The predicted median value of Rrs at 6 Hz (Rrs6) for a 50-yr-old male subject, measuring 1.80 m and 80 kg, is \( \exp(5.454 - 3.079 \times 1.80 - 0.00482 \times 50 + 0.01366 \times 80) \)
\( = \exp(0.7636) = 2.15 \) hPa.s.L\(^{-1}\). The upper limit of the normal (ULN) value \( \text{ULN}(Rrs6) \)
\( = \exp(0.7636 + 1.64 \times \text{RSD}) = \exp(0.7636 + 1.64 \times 0.2662) = 3.32 \) hPa.s.L\(^{-1}\).

The predicted median value of Xrs at 6 Hz (Xrs6) for the same subject is \( \{4 - \exp(2.407 -0.606 \times 1.80 + 0 \times 50 + 0.00234 \times 80)\} = 4 - \exp(1.503) = -0.50 \) hPa.s.L\(^{-1}\). The lower limit
of the normal (LLN) value \( \text{LLN}(\text{Xrs6}) = 4 - \exp(1.503 + 1.64 \times \text{RSD}) = 4 - \exp(1.503 + 1.64 \times 0.0701) = 4 - \exp(1.618) = 4 - 5.045 = -1.05 \text{ hPa.s.L}^{-1}. \)

**Short-term variability and bronchodilator response**

The short-term variability of \( Zrs \) was assessed in 302 subjects and that of \( \text{FEV}_1 \) in 179 subjects. The variability in \( Rrs \) was expressed as the coefficient of repeatability (CR), defined as twice the standard deviation of the difference of the two baseline measurements made 15 min apart (5). CR was expressed in absolute and relative (as a percentage of the average baseline value) terms. The relative values of CR were used as threshold values to identify responders to bronchodilation. Table S1 reports on the positive and negative responders as identified with the use of the CR of \( \text{Rrs} \) at low frequency and \( \text{FEV}_1 \).
Figure S1. The resistance \( (R) \) and reactance \( (X) \) of a mechanical analogue, as measured with the different FOT setups at the 5 centres (C1-C5) involved in the study.
Figure S2. Age distribution of the subjects participating at the different centres.
Figure S3. Height distribution of the subjects participating at the different centres.
Figure S4. Weight distribution of the subjects participating at the different centres.
Figure S5. Body mass index (BMI) distribution of the subjects participating at the different centres.
Figure S6. Comparison of the predicted mean values of Rrs (Rmean) for males (left panels) and females (right panels) from the present study and those from previously published studies (6-10), as functions of weight at different ages. Height was fixed at a value 1.75 and 1.65 m in males and females, respectively. Predictions for Rrs at 19 Hz were computed from Brown et al. (9) since Rrs at 20 Hz was closest to Rmean in our data set.
Table S1. Sensitivity of respiratory resistance and reactance at low frequency and FEV₁ to detect responders to salbutamol by the use of the relative coefficient of repeatability of the baseline measurement as the threshold value.

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R4, R5, R6 and R10: respiratory resistance at 4, 5, 6 and 10 Hz, respectively (hPa.s.l⁻¹). X4, X5 and X6: respiratory reactance at 4, 5 and 6 Hz, respectively (hPa.s.L⁻¹). AX4 and AX5: area under the reactance curve calculated from 4 and 5 Hz, respectively (hPa.L⁻¹). FEV₁: Forced expiratory volume in 1 s (L).
REFERENCES


