ON-LINE SUPPLEMENT

MULTIPLE-BREATH INERT GAS WASHOUT AND SPIROMETRY VS. STRUCTURAL LUNG DISEASE IN CYSTIC FIBROSIS

Per M Gustafsson, Pim A de Jong , Harm AWM Tiddens , Anders Lindblad

METHODS

Subjects

This is a retrospective study of spirometry, MBW and HRCT recordings obtained over a 30-month period in 44 consecutive CF patients aged less than 20 years. All patients underwent these investigations as part of their routine annual review at the Göteborg CF center, where HRCT is done routinely every third year in patients over 5 years of age. Chronic colonisation with Pseudomonas aeruginosa was defined as three or more positive cultures over a 6-month period. This retrospective analysis was approved by the Ethics Committee for Human Research at the University of Göteborg.

Measurement of lung structure

Lung structure was evaluated using HRCT scans. For children a multi detector CT scanner (General Electric light speed ultra, 8 rows of detectors, GE Medical Systems, Milwaukee, WI) was used. Scans were obtained using a beam current of 120 mA, an exposure time of 0.5 s (60 mAs) and a beam potential of 120 kV from lung apex to base at 15 mm intervals using 1.25 mm thick slices in inspiration. In addition, three expiratory HRCT images were obtained through the upper, middle and lower lung zones. For adults a PQ 6000 scanner (Picker International Inc., Highland Heights, OH) was used. Scans were obtained using a beam current of 160 mA, a 1.0 s exposure time (160 mAs) and a beam potential of 120 kV from lung apex to lung base at 10 mm intervals using 1.5 mm thick slices in inspiration. In addition three expiratory HRCT images were obtained through the upper, middle and lower lung zones.

All scans were reconstructed with a high-spatial frequency algorithm (bone), blinded to date and patient identification, and scored in random order by an experienced observer (E1-4) using an adapted scoring system developed by Brody et al (E5). The scoring system evaluates the five lung lobes and the lingula as a sixth lobe on the inspiratory HRCT scans for: severity and extent of central and peripheral bronchiectasis; extent of central and peripheral mucus plugging; severity and extent of central and peripheral airway wall thickening; and parenchymal abnormalities (extent of opacities, ground glass pattern and cysts and bullae). On the expiratory HRCT scans hyperinflation (gas trapping) was scored in right and left middle, upper and lower lung zones.

We set an abnormal composite CT score as a score greater than 5 per cent. In 15 normal individuals that were scored intermixed with CF patients, all normal individuals had a CT score below 5% (unpublished data). We defined an abnormal amount of air trapping when more than 30% of the lung was involved. It is known that air trapping can be seen in normal individuals (E6), and in other studies in the bronchiolitis obliterans syndrome a 32% cut-off has been used (E7).

The maximum possible composite HRCT score is 207 (E5). Component HRCT scores were calculated by adding the component scores from the six lobes. For statistical analysis the composite HRCT score and component HRCT scores were expressed on a 0-100 scale (per cent) (E5).

Measurement of lung function

Spirometry: Forced expiratory maneuvers were measured using the Jaeger Masterslab equipment (Erich Jaeger AG, Wurzburg, Germany). A minimum of three technically acceptable maximal forced expiratory maneuvers were performed. Patients were encouraged to exhale for at least three seconds and were required to produce two reproducible FEV_1 results (lower being within 5% of the higher). The best forced vital capacity (FVC) and FEV_1 results were noted. The recording with the highest sum of FEV_1 and FVC was used to obtain the maximal expiratory flow when 75% of FVC was expired (FEF_{75}). Spirometry findings were related to Swedish normative data for children (6-18 years) (E8) and adults ($\geq$20.0 yrs of age) (E9, E10), respectively. The lower limits of normality (LLN) were defined as the predicted mean minus 1.96 residual standard deviations (RSD).

Multiple-breath sulphur hexafluoride washout (MBW): The patients were investigated in the sitting position. During the MBW tests the younger patients (<10 yrs) watched a video while the older patients watched a tidal volume trace on a computer screen and were instructed to breathe regularly with a tidal volume between 10 and 15 $mL*kg^{-1}$ body weight. All participants used a nose clip and breathed through a Fleisch no.1 pneumotachometer (PNT) (Metabo SA, Lausanne, Switzerland) via a mouthpiece. A sampling tube from a mass spectrometer was introduced into the middle of the air stream between the mouthpiece and the PNT through a short connecting piece. The external dead space was 15 mL. The PNT was connected to a differential pressure transducer (MP 45-14-87; Validyne Corp., CA, USA;$\pm$2 cmH_2O) and the flow signal was demodulated and amplified (CD12 C-2A; Validyne Corp.). Gas concentrations were measured using a respiratory mass spectrometer (AMIS 2000, Innovision A/S, Odense, Denmark). The PNT was calibrated with separate calibration constants for inspiratory and expiratory flows using a precision syringe. Recorded inspiratory and expiratory flows and volumes were converted to body temperature, ambient pressure, and saturated water vapor conditions. Gas samples and flow signals were aligned in time. The sample flow of the mass spectrometer was approximately 20 $mL*min^{-1}$ and the gas concentration signals were updated at a rate of 33.3 Hz. All signals were recorded at 100 Hz by a computer through a 16-channel AD-conversion board (DAS-1602; Keithley Metrabyte, Taunton, MA, USA). The software corrected the flow signal sample-by-sample for changes in dynamic viscosity caused by the variations in gas composition. One of the two inert tracer gases (SF_6) was used for the evaluations presented in this paper. Helium was included for other assessments of ventilation distribution not presented here.

Each test consisted of two phases: a washin phase during which a dry gas mixture containing 4% SF_6 (sulphur hexafluoride), 4% He (helium) 21% O_2 (oxygen), and balance N_2 (nitrogen) was administered using a bias flow applied via a T-piece on the external opening of the PNT. Washin was continued until the inspiratory and expiratory SF_6 concentrations were stable and equal, plus another 30 s. At this moment the bias flow was stopped during expiration by disconnecting the T-piece and washout was started. The

patients breathed room air during the washout phase. The washout phase continued until the end-tidal SF_6 concentration was <0.1% (i.e. 1/40th of the starting concentration) for at least three breaths. The functional residual capacity (FRC) was determined from the cumulative exhaled volume of marker gas (SF_6) divided by the difference in end-tidal SF_6 concentration at the start of the washout and end-tidal SF_6 concentration at completion of the washout. The number of lung volume turnovers, i.e. the cumulative expired volume of gas (sum of tidal volumes) divided by the FRC, was calculated for each subsequent breath. The cumulative expired volume was corrected for the external dead space in each breath. The lung clearance index (LCI) was calculated as the number of turnovers needed to lower the end-tidal tracer gas concentration to $1/40^{th}$ of the starting concentration. In a previous study including healthy subjects the mean, RSD and upper limit of normality (ULN; mean plus 1.96 RSD) for LCI were 6.33, 0.43 and 7.17, respectively (E11).

HRCT, MBW and spirometry were performed on the same day in 26 patients, and in all remaining patients but one, there was only one day between any of the tests.

Statistical analysis

Abnormal structure was defined as a composite HRCT-score >5%, presence of bronchiectasis, or air trapping >30%. Lung function was expressed as z-scores, which were calculated as (measured value - predicted value)/ RSD from the reference population. Abnormal lung function was defined as LCI above +1.96 z-scores, or FEV_1 or FEF_{75} below -1.96 z-scores. Proportions of patients with normal or abnormal FEV_1 or LCI results in relation to HRCT classifications (cross tabulations) were compared using the Yates corrected χ^2-test. The sensitivity and specificity were determined for LCI, FEV_1, and FEF_{75} with respect to abnormal HRCT composite score, the presence of bronchiectasis, and the presence of abnormal air trapping, as diagnosed by HRCT. Sensitivity was calculated as the proportion of the study population with abnormal HRCT findings that showed abnormal lung function results. Specificity was calculated as the proportion of the study population with normal HRCT finding that had normal lung function findings. The 95% confidence intervals around the sensitivity and specificity findings were calculated as follows: 95CI for $p = p +/- 1.96*SE$; SE for $p = (p* (1-p)/n)^{1/2}$; where p denotes the sensitivity or specificity expressed as a ratio.

Spearman rank correlation coefficients (R_s) were calculated for FEV_1, LCI and FEF_{75} with respect to HRCT composite score, bronchiectasis, mucus plugging, airway wall thickness, parenchyma and air trapping. A p-value <0.05 was accepted as statistically significant. Statistica 6.0 (StatSoft, Tulsa, OK, USA) was used for the statistical analyses.

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