

# **Pulmonary function, computed tomography and echocardiographic abnormalities in sickle cell disease**

On line data supplement

## **Lung function assessments**

Patients were assessed in the Amanda Smith Pulmonary Function Laboratory at King's College Hospital NHS Foundation Trust. No subject underwent lung function testing within two weeks of an upper respiratory tract infection or within a month of suffering a vaso-occlusive crisis. A history was taken of past and current respiratory symptoms and medication for respiratory problems. Standing height was measured using a wall-mounted stadiometer (Holtain Ltd, Crymych, Dyfed, UK) and weight using electronic weighing scales (Seca Ltd, Birmingham, UK).

Measurements were performed using a pneumotachograph based system (Jaeger Masterscreen PFT, Carefusion Ltd, Basingstoke UK). Results were expressed as percent predicted for height, age, and sex using the ethnic-specific reference equations for spirometry-[E1] and the European Community for Steel and Coal Statement of the European Respiratory Society reference equations for lung volumes and gas transfer.[E2] Spirometry, static lung volumes using whole-body plethysmography and transfer factor for carbon monoxide were assessed according to American Thoracic Society/European Thoracic Society guidelines.[E3-E5] The mean respiratory system resistance ( $R_{rs}(0)$ ) and frequency dependence of resistance ( $R_{rs}(1)$ ) from 5-25Hz were measured using impulse oscillometry and expressed as the percent predicted for height, weight, and age.[E6] The frequency dependence of resistance was assessed using the mean slope of the resistance-frequency

curve over the range 5 to 25Hz. Respiratory resistance was considered to be elevated if  $R_{rs}(0)$  was greater than the upper limit of normal. Spirometry was repeated following administration of a bronchodilator (400 $\mu$ g salbutamol via a MDI and spacer) and a positive response was defined as an increase in FEV<sub>1</sub> of greater than or equal to 12% from baseline and an increase of at least 200ml. Oxygen saturation was measured using a pulse oximetry (Masimo Radical 7 and a rainbow probe, Masimo, California, USA).

The predicted values for total lung capacity were reduced by 12%, and residual volume by 7% to correct for ethnicity.[E7] The lower and upper limits of normal were defined as the fifth and ninety-fifth percentiles respectively of the appropriate reference range. Patients were diagnosed as having a restrictive abnormality if their TLC was less than the lower limit of normal (LLN), with a normal FEV1:VC. An obstructive abnormality was diagnosed if the FEV1:VC was less than the LLN with a normal VC, or if VC was less than the LLN with a normal TLC and an RV:TLC greater than the upper limit of normal (ULN). A mixed abnormality was diagnosed if the TLC and FEV1:VC were less than the LLNs .

### **Computed tomography**

Patients were scanned on a 64-channel multidetector CT machine (GE 64 VCT Lightspeed machine; GE Healthcare, Waukesha, Wisconsin, USA; 100 kV, Auto mA/Smart mA; pitch

1.375, 0.5 sec tube rotation, beam collimation 40 mm, detector size 0.65 mm). Contrast was injected at a rate of 4-5 ml/s and scanning was performed cranio-caudally with the subject in a supine position and breathing suspended at maximal inspiration. The period of CT scanning was timed to coincide with optimal contrast opacification of the pulmonary arterial tree using bolus-tracking. In the earlier study-[E8] patients had been imaged using a dual-detector CT machine (HiSpeed NX/I; GE Medical Systems, Waukesha, Milwaukee, Wisconsin, USA). Interspaced HRCT images (1.5mm collimation at 10 mm intervals) had been acquired at total lung capacity, in the supine and prone positions. Images were reconstructed using a high-spatial-frequency (bone) algorithm. All CT studies were securely stored on CD for subsequent review.

CT patterns-[E9] were quantified i) to the nearest 5%; ii) on a semiquantitative scale or iii) for their presence or absence, as appropriate. The following CT patterns were quantified to the nearest 5%: i) reticular pattern defined as innumerable interlacing line shadows which, by summation, produce an appearance resembling a mesh; ii) ground-glass opacification (defined as a hazy increased lung opacity in which the visibility of bronchial and vascular margins were preserved); iii) areas of decreased attenuation as part of a mosaic attenuation pattern defined as a patchwork of areas of differing lung densities and iv) consolidation defined as a homogeneous increase in lung parenchymal attenuation which obscures the margins of airway and vessel walls. In patients with consolidation, the extent to the nearest 5% of sub-pleural consolidation was also recorded. For ground glass opacification, a score was assigned based on the observed extent (0=none; 1= <10% extent; 2= 10-50% extent; 3 >50% extent). The following CT signs were scored semi-quantitatively: i) thickening of interlobular septa (0=none; 1= <5 thickened interlobular septa [ILS]; 2= >5 thickened ILS or

<50% pleural surface involved; 3= >50% pleural surface involved or 4 = diffusely thickened ILS), ii) lobar volume loss (0=none; 1=mild or 2=severe). The presence or absence of the following signs were recorded: i) traction bronchiectasis defined as irregular bronchial/bronchiolar dilatation caused by surrounding retractile pulmonary fibrosis in a reticular pattern and/or ground-glass opacification; ii) linear bands of attenuation; iii) sub-pleural curvilinear lines defined as a thin curvilinear opacity of 1-3 mm thickness and lying within one centimetre of and parallel to the pleural surface and iv) pulmonary infarcts defined as a peripheral irregular opacity associated with a linear opacity no more than 2 cm in length. Whole-lung scores were produced by taking the mean of all lobes for signs which had been measured to the nearest 5% and by summing the lobar scores for signs recorded as present/absent. Vascular dimensions were assessed in two ways. First, proprietary electronic callipers were used to measure the widest short axis diameters of the upper lobe apical or apico-posterior segmental arteries and the lower lobe posterobasal segmental arteries together with the widest external short axis diameter of the corresponding segmental bronchi on the same axial image in at least three out of four lobes.[E10] Based on those measurements, the mean segmental artery/ bronchus (A/B) ratio in at least three out of four lobes was calculated.

Distal vessel dimensions, including both arteries and veins at the subsegmental level-[E11] were measured using the method of Matsuoka *et al.*[E12, E13] Three CT slices were selected from each examination. The upper cranial slice was located approximately one centimetre above the upper margin of the aortic arch, the middle slice approximately one centimetre below the carina, and the lower caudal slice approximately one centimetre below the right inferior pulmonary vein. The measurements were performed using the Java-based semi-automated image analysis software 'ImageJ' (Rasband, W.S., ImageJ, U. S. National

Institutes of Health, Bethesda, Maryland, USA, <http://imagej.nih.gov/ij/>, 1997-2012). Before processing, each selected image was smoothed using Gaussian blurring to filter image noise. The lung field was then segmented using a thresholding technique to include all pixels between -500 and -1024 Hounsfield Units (HU) (Figure 1) and the segmented image was converted to a binary image with a window level of -760 HU (Figure 1). The 'Analyze Particles' function was then used to count and measure the cross-sectional area of vessels within a range of 0-5mm<sup>2</sup>. The 'Circularity' function was then used to select only those vessels running approximately perpendicular to the scan plane based on their apparent shape in the slice image, setting the circularity range to 0.9-1.0. The total summated cross-sectional area for those vessels was then expressed as a percentage of the total lung area of the three selected slices using threshold values between -500 HU and -1024 HU (CSA<5mm%).

The segmental A/B ratio has been shown to correlate with pulmonary artery pressure in a mixed cohort of patients with lung disease [E14]. CSA<5mm% has been measured in patients with HRCT-defined emphysema due to COPD and has been shown to correlate with pulmonary artery pressure in this group.[E12, E13]

CT total lung volume (TLV<sub>CT</sub>), comprising air plus tissue components, was derived using a proprietary lung segmentation algorithm (Apollo, Vida Core Lab Services, Vida Diagnostics Inc, Iowa, USA) which uses a localized and adaptive threshold method to delineate lung tissue on a lobar basis from surrounding tissues (Figure 2). The TLV<sub>CT</sub> was not available for the scans obtained in 2003-2005.

For the longitudinal analyses, initial and follow-up CT scans were compared. Two observers identified anatomically comparable sections as judged by vascular and bronchial landmarks on the two CT scans from each individual and recorded their impression of whether the overall appearance of the interstitium on the follow-up scan compared to the initial scan had

deteriorated. Patients who had deteriorated were assigned a 'gestalt' change score of 'one' and those who did not were scored as zero.

### **Statistical analysis**

Data were tested for normality using the D'Agostino and Pearson omnibus normality test. Comparisons, as appropriate, were made with t-tests or Mann-Whitney U tests and paired analyses were performed using paired-sample t-tests or Wilcoxon matched-pairs signed rank tests. In the longitudinal cohort, we used non-parametric tests for baseline-follow up comparisons as we felt that the number of patients was too small to meaningfully perform normality testing. For the descriptive data in the larger cohort (n=35), who were tested once, data were displayed as median (range) to demonstrate the wide range in lung function. On HRCT, whilst GGO score, bands, curvilinear bands, and A/B ratio were not normally distributed, the residuals in all of the final regression models did not differ significantly from a normal distribution (Pearson omnibus normality test  $p>0.05$ ). The strength of relationships were assessed using the Pearson or Spearman rank correlation. Stepwise linear regression with backward elimination was used to identify HRCT parenchymal and vascular results which correlated with the results of the lung function tests. HRCT variables examined in the preceding bivariate analyses were entered as initial predictors, unless predictors were multicollinear. All regression models were built in the sample size n=35. All final models satisfied the assumptions of multiple linear regression as determined by assessment of homoscedasticity, no multicollinearity between predictors and normal distribution of errors. In the longitudinal cohort, exploratory models were generated to test whether baseline vascular markers predicted subsequent deterioration of parenchymal disease and progression of lung function abnormalities. Logistic regression was used to assess the effect of baseline

segmental A/B ratio and CSA<5mm on the deterioration in the overall CT appearance ('Gestalt' change score) and linear mixed model analysis (LMM) to predict individual decline in lung function results.

### **Haematological data**

Haemoglobin, lactate dehydrogenase (LDH), bilirubin levels and reticulocyte counts were obtained from routine blood tests taken within one month of testing when patients were clinically stable.

Thirty-five patients with a median age of 43 (range 17-73) years were assessed. Twenty of the 35 patients (median age at initial assessment 38, range 17 – 66 years) had been assessed at a median of 6.6 (range 5.5-6.7) years previously. Of the thirty-three patients who participated in the original study 20 were tested at follow-up: three had died, six were lost to follow-up three declined to participate in the follow-up study and one declined to undergo HRCT scanning and was excluded. The twenty patients who were tested at follow-up were included in the 2009-2013 cohort, together with fifteen new patients making a total of thirty-five patients.

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