

Heterogeneity of respiratory disease in children and young adults with sickle cell disease

ABSTRACT

To detect and characterise different phenotypes of respiratory disease in children and young adults with sickle cell disease (SCD), 11 lung function and haematological biomarkers were analysed using k-means cluster analysis in a cohort of 114 subjects with SCD aged between 5 and 27 years. Three clusters were detected: cluster 1 had elevated pulmonary capillary blood volume, mixed obstructive/restrictive lung disease, hypoxia and moderately severe anaemia; cluster 2 were older patients with restrictive lung disease; and cluster 3 were younger patients with obstructive lung disease, elevated serum lactate dehydrogenase and bronchodilator reversibility. These results may inform more personalised management strategies to improve outcomes.

INTRODUCTION

Sickle cell disease (SCD) is one of the most common inherited disorders worldwide, affecting an estimated 300 000 newborns every year. The majority of children with SCD in developed countries will survive to adulthood, but may then suffer

multiorgan damage, including pulmonary complications. While lung function abnormalities are common in both children and adults, there is not a consistent picture. Furthermore, the aetiology and clinical consequences of the lung function abnormalities are unclear. It seems likely that the respiratory disease is not homogeneous.¹ Identification of different phenotypes of respiratory disease may provide a basis to stratify patients with regard to management. Cluster analysis is a method of detecting subgroups within multidimensional (ie, comprising many and/or varied measurements) data sets.² We hypothesised that cluster analysis of respiratory function and haematological biomarkers would reveal different phenotypes in patients with SCD.

METHODS

Study design

A secondary analysis of data from children and young adults with sickle cell anaemia (homozygous for sickle cell haemoglobin) and ages ranging from 5 to 27 years was conducted.^{3–5} The participants or their parents (in the case of children) gave informed written consent to take part.

Lung function measurements

Respiratory system resistance at a frequency of 5 Hz (R_{rs}) was measured, then spirometry (FEV_1 :vital capacity (VC)), static lung volumes using whole-body plethysmography (total lung capacity (TLC)) and gas transfer for carbon monoxide (DLCO) were assessed according to the American Thoracic Society/European Thoracic Society criteria.^{6–8} The transfer factor for nitric oxide was obtained by the addition of 40 ppm nitric oxide to the inspired gas mixture prior to commencing the single-breath DLCO measurement and used to estimate the pulmonary capillary blood volume (PCBV).³ Arterial oxygen saturation (SpO_2) was measured using pulse oximetry (Masimo Radical 7, Masimo, California, USA) (see online supplementary file).

Haematological data and SCD complications

The haemoglobin (Hb) concentrations, lactate dehydrogenase (LDH) levels, reticulocyte percentage (reticulocyte%) and white cell counts (WCC) were obtained from routine blood tests undertaken within 3 months of lung function testing. The medical records were reviewed and an acute chest syndrome (ACS) episode was diagnosed if the child had suffered chest pain, dyspnoea and fever, together

Table 1 Subject characteristics by cluster

	Cluster 1 (n=14)	Cluster 2 (n=55)	Cluster 3 (n=45)	P
FEV_1 :VC*	92.4 (88.05–100.9)	97.3 (91.1–102.6)	90.7 (85.9–96.2)	0.002
R_{rs} *	165.8 (140.8–186.5)	131.6 (112.9–160.3)	133.4 (110.2–153.6)	0.020
TLC*	84.8 (82.2–86.8)	85.6 (77.4–91.6)	94.1 (85.7–99.6)	0.003
DLCO*	98.1 (92.5–106.2)	77.8 (72.2–83.8)	96.6 (88.9–108.3)	<0.0001
PCBV (mL/L)	44.9 (41.4–47.0)	21.1 (17.9–23.5)	26.4 (23.4–29.7)	<0.0001
(Hb) (g/dL)	8.2 (7.4–8.9)	9.9 (8.7–10.8)	8.4 (5.7–9.2)	<0.0001
SpO_2 (%)	95.8 (95.0–97.8)	97.0 (95.8–99.0)	95.5 (93.0–98.0)	0.0327
LDH (IU/L)	495 (441–622)	399.5 (303.5–474.8)	639 (542–732.0)	<0.0001
WCC ($\times 10^9$ /L)	10.7 (10.3–12.4)	9.2 (7.3–10.8)	11.0 (8.8–12.3)	0.015
Reticulocyte%	12.7 (10.6–14.2)	8.5 (6.5–10.5)	10.4 (7.7–12.8)	0.018
Age (years)	14.2 (10.3–17.0)	17.5 (13.3–21.6)	11.3 (9.0–14.5)	<0.0001
% Obstructive†	0	9.1	26.7	0.027
% Restrictive†	14	29.1	4.4	0.023
% Mixed†	21	5.5	4.4	0.028
ACS ever†	35.7	33.3	33.3	0.9231
BDR %†	7.1	2.0	15.6	0.0338
% Hydroxyurea	14.3	10.9	15.6	0.7358
% Transfusion	7.1	20.0	31.1	0.1633

*Lung function tests are expressed as the percentage predicted for age and/or height.

†supplementary data not used in the clustering.

ACS, acute chest syndrome; BDR, bronchodilator reversibility; DLCO, gas transfer for carbon monoxide; Hb, haemoglobin; LDH, lactate dehydrogenase; PCBV, pulmonary capillary blood volume; R_{rs} , respiratory system resistance at a frequency of 5 Hz; SpO_2 , arterial oxygen saturation; TLC, total lung capacity; VC, vital capacity; WCC, white cell counts.

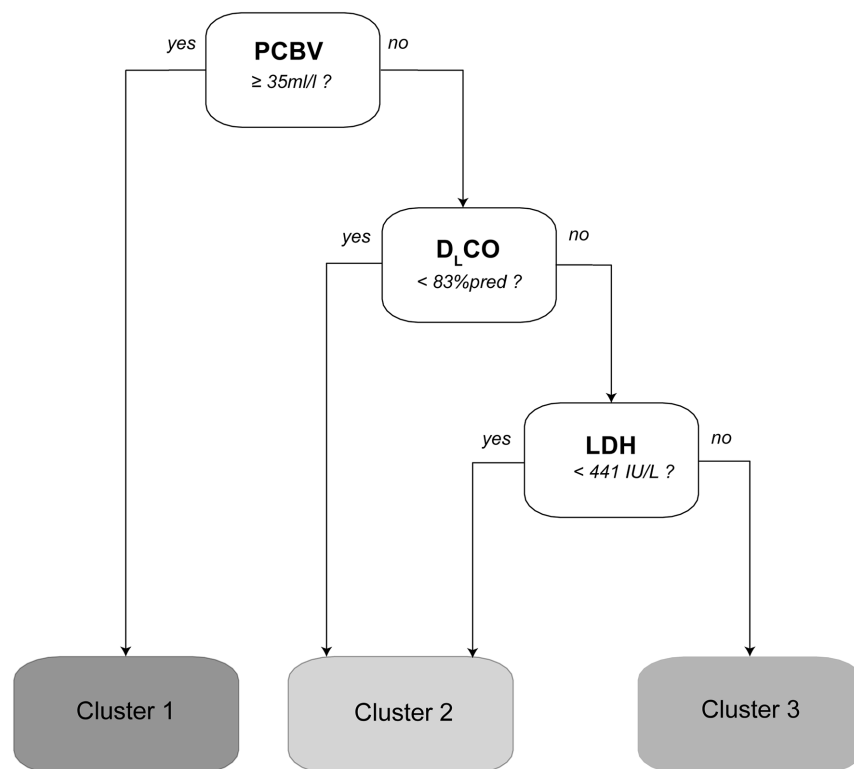


Figure 1 Stratification algorithm to predict cluster membership. DLCO, gas transfer for carbon monoxide; LDH, lactate dehydrogenase; PCBV, pulmonary capillary blood volume.

with a new pulmonary infiltrate on chest radiograph. To determine the impact of cluster membership on future morbidity, the medical records were reviewed for the period subsequent to the lung function testing, and the occurrence of individual ACS episodes and non-elective hospital admissions were recorded and expressed as events/year.

Statistical analysis

Variables included in the cluster analysis were FEV₁:VC, TLC, R_{rs}, DLCO, PCBV, (Hb), SpO₂, LDH, WCC, reticulocyte% and age at time of testing. The number of clusters was selected using the criterion-for-clustering function (CritCF) index,⁹ which ranks partitions comprising different numbers of clusters; a higher CritCF indicates a better clustering.

RESULTS

Data from 114 patients with complete lung function results and a median age of 14.5 (5.0–27.1) years were analysed (see online supplementary table A). A three-cluster solution was found to be optimal (see online supplementary figure S1). The clustering solution was robust under multiple imputations with the same three-cluster partition occurring in 96.4% of imputed data sets (see online supplementary figure S1). For all lung function and haematological variables,

the proportion of patients with obstructive, restrictive or mixed lung function defects and the prevalence of bronchodilator reversibility, but not the proportion taking hydroxyurea, receiving regular transfusions or with a history of ACS episodes, differed significantly between clusters (table 1). Clusters were well separated, with little overlap on a discriminant coordinates plot (see online supplementary figure S2). Physiological profiles for the three clusters are shown graphically (see online supplementary figure S3).

Cluster 1 had the largest proportion with mixed obstructive/restrictive lung disease and both the highest respiratory system resistance and PCBV. Cluster 2 comprised older patients with the highest FEV₁:VC ratio and a low TLC (ie, restrictive pattern), the lowest gas transfer, but the highest Hb. Cluster 3 comprised younger patients with the highest incidence of bronchodilator reversibility and the highest LDH levels (table 1). Cluster 3 has a greater frequency of hospital admissions for vaso-occlusive crises, but not ACS episodes, than clusters 1 and 2 (both P<0.05) (see online supplementary file).

A conditional inference tree analysis was performed on the subset of patients assigned to the training set (n=85). A model using three variables (PCBV, DLCO and LDH) (figure 1) was found to be optimal and

assigned 90% of patients from the unseen validation set to the correct cluster. Classification success rates for clusters 1, 2 and 3 were 80%, 93% and 90%, respectively.

DISCUSSION

Analysis of lung function and haematological data detected three distinct clusters in children and young adults with SCD. Cluster 1 had the highest respiratory system resistance and PCBV, which suggest they may have peripheral airways disease mediated by pulmonary vascular engorgement. We speculate that this interaction may arise from direct compression of distal airways by adjacent pulmonary vessels within peribronchial sheaths or from bronchovascular congestion due to elevated pulmonary venous pressure. Hb levels were significantly higher in cluster 2, which suggests that their restrictive lung disease might be related to increased blood viscosity, leading to impaired microvascular blood flow and an increased likelihood of occult vaso-occlusion in the pulmonary capillary plexus with consequent cumulative lung injury. Cluster 3 had the highest LDH levels and the highest incidence of bronchodilator reversibility. Those findings are consistent with the observation that airway hyper-reactivity, as assessed by methacholine challenge testing, was associated with elevated LDH levels.¹⁰ Serum LDH, but not reticulocyte count or Hb concentration, was discriminative for cluster 3. This may reflect LDH had a greater sensitivity as a marker of haemolysis. LDH, however, is thought to primarily reflect intravascular haemolysis; thus in cluster 3 compared with the other two clusters, perhaps a greater proportion of overall haemolytic activity was occurring in the intravascular compartment.

Conditional tree analysis demonstrated that a subset of the variables could reliably predict cluster membership. This suggests that a method for phenotyping of SCD respiratory subclasses could be based on those biomarkers.

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REFERENCES

- 1 Koumbourlis AC. Lung function in sickle cell disease. *Paediatr Respir Rev* 2014;15:33–7.

- 2 Wardlaw AJ, Silverman M, Siva R, *et al.* Multi-dimensional phenotyping: towards a new taxonomy for airway disease. *Clin Exp Allergy* 2005;35:1254–62.
- 3 Wedderburn CJ, Rees D, Height S, *et al.* Airways obstruction and pulmonary capillary blood volume in children with sickle cell disease. *Pediatr Pulmonol* 2014;49:716–22.
- 4 Lunt A, Ahmed N, Rafferty GF, *et al.* Airway and alveolar nitric oxide production, lung function, and pulmonary blood flow in sickle cell disease. *Pediatr Res* 2016;79:313–7.
- 5 Lunt A, McGhee E, Sylvester K, *et al.* Longitudinal assessment of lung function in children with sickle cell disease. *Pediatr Pulmonol* 2016;51:717–23.
- 6 Miller MR, Hankinson J, Brusasco V, *et al.* Standardisation of spirometry. *Eur Respir J* 2005;26:319–38.
- 7 Wanger J, Clausen JL, Coates A, *et al.* Standardisation of the measurement of lung volumes. *Eur Respir J* 2005;26:511–22.
- 8 Macintyre N, Crapo RO, Viegi G, *et al.* Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J* 2005;26:720–35.
- 9 Breaban M, Luchian H. A unifying criterion for unsupervised clustering and feature selection. *Pattern Recognit* 2011;44:854–65.
- 10 Field JJ, Stocks J, Kirkham FJ, *et al.* Airway hyperresponsiveness in children with sickle cell anemia. *Chest* 2011;139:563–8.