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

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ABSTRACT

To detect and characterise different phenotypes of respiratory disease in children and young adults with sickle cell disease (SCD), eleven lung function and haematological biomarkers were analysed using k-means cluster analysis in a cohort of 114 SCD subjects aged between 5 and 27years. Three clusters were detected: cluster one had elevated pulmonary capillary blood volume, mixed obstructive/restrictive lung disease, hypoxia and moderately severe anaemia; cluster two were older patients with restrictive lung disease; cluster three 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 commonest inherited disorders worldwide, affecting an estimated 300,000 new-borns every year. The majority of SCD children in developed countries will survive to adulthood, but may then suffer multiorgan damage, including pulmonary complications. Whilst 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 homogenous [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 (i.e. comprising many and/or varied measurements) datasets.[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 (SCA) (homozygous for sickle cell haemoglobin (HbSS)) and ages ranging from 5 to 27 years was conducted.[3-5] The studies were approved by King's College Hospital Research Ethics Committee and participants or their parents (in the case of children) gave informed written consent to take part.

Lung function measurements (see online supplement)

Respiratory system resistance at a frequency of 5Hz (R_{rs5}) was measured, then

spirometry (FEV_1:VC), static lung volumes using whole-body plethysmography (TLC) and gas transfer for carbon monoxide (DLCO) were assessed according to American Thoracic Society/European Thoracic Society criteria.[6-8] The transfer factor for nitric oxide (DLNO) was obtained by the addition of 40ppm NO 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 (S_pO_2) was measured using pulse oximetry (Masimo Radical 7, Masimo, CA).

Haematological data and SCD complications

The haemoglobin concentrations (Hb), lactate dehydrogenase (LDH) levels, reticulocyte percentage (reticulocyte%) and white cell counts (WCC) were obtained from routine blood tests undertaken within three months of lung function testing. The medical records were reviewed and an ACS episode was diagnosed if the child had suffered chest pain, dyspnoea and pyrexia together 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 FEV_1:VC, TLC, R_{rs5}, DLCO, PCBV, [Hb], S_pO_2, LDH, WCC, reticulocyte% and age at time of testing. The number of clusters was selected using the 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 14.5 (5.0-27.1) years were analysed (see online supplement Table A). A three-cluster solution was found to be optimal (see online supplement Figure S1). The clustering solution was robust under multiple imputations with the same three-cluster partition occurring in 96.4% of imputed datasets (see online supplement 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 supplement Figure S2). Physiological profiles for the three clusters are shown graphically (see online supplement Figure S3).

Cluster one had the largest proportion with mixed obstructive/restrictive lung disease and both the highest respiratory system resistance and pulmonary capillary blood volume. Cluster two comprised older patients with the highest FEV_1:VC ratio and a low TLC (i.e. restrictive pattern), the lowest gas transfer gas transfer, but highest haemoglobin. Cluster three comprised younger patients with the highest incidence of bronchodilator reversibility and the highest LDH levels (Table 1). Cluster three has a greater frequency of hospital admissions for vaso-occlusive crises, but not ACS episodes, than clusters one and two (both $p<0.05$) see on line supplement.

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 one, two and three 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 one had the highest respiratory system resistance and pulmonary capillary blood volume 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. Haemoglobin levels were significantly higher in cluster two 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 three 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 haemoglobin concentration was discriminative for cluster three. 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 three compared to 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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Authors contributions: AL and AG designed the study, AL, LM, DR and SH collected the data. AL analysed the data. All authors were involved in the development of the manuscript and approved the final version.

REFERENCES

1 Koumbourlis, A.C., Lung Function in Sickle Cell Disease. Paediatric Respiratory Reviews, 2014. **15**(1): p. 33-37.

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. *Pat Recog* 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.

Table 1 Subject characteristics by cluster.

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

†Supplementary data not used in the clustering.

	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_{rs5}*	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
D_LCO*	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
S_pO_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 (x10^9/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 (yrs)	14.2 (10.3 – 17.0)	17.5 (13.3 – 21.6)	11.3 (9.0 – 14.5)	<0.0001
%	0%	9.1%	26.7%	0.027
Obstructive†				
%	14%	29.1%	4.4%	0.023
Restrictive†				
% 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

%	14.3%	10.9%	15.6%	0.7358
Hydroxyurea				
%	7.1%	20.0%	31.1%	0.1633
Transfusion				

† BDR: bronchodilator reversibility.

FIGURE LEGENDS

Figure 1: Stratification algorithm to predict cluster membership