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Small Airways Function Declines After Allogeneic Hematopoietic Stem Cell Transplantation

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ABSTRACT (Word count: 218 words)

Bronchiolitis obliterans (BO) following allogeneic hematopoietic stem cell transplantation (HSCT) affects peripheral airways. Detection of BO is presently delayed by the low sensitivity of spirometry.

We examined the relationship between peripheral airway function and time since HSCT, and compared it with spirometry and clinical indices in 33 clinically stable allogeneic HSCT

recipients. Measurements of lung function, exhaled NO, forced oscillatory respiratory system resistance and reactance, acinar (S_{acin}) and conductive airways (S_{cond}) ventilation heterogeneity and lung clearance index (LCI) measured by multiple breath nitrogen washout were performed. Twenty-two patients underwent repeat visits from which short term changes were examined.

Median time post HSCT was 12 months. Eight patients were clinically diagnosed as having BO. In multivariate analysis, time since HSCT was predicted by S_{acin} and FEV_1 %predicted. Twenty patients had abnormal S_{acin} with normal spirometry, whereas none had airflow obstruction with normal S_{acin}. S_{acin} and LCI were the only measures to change significantly between 2 visits, with both worsening. Change in S_{acin} was the only parameter to correlate with change in chronic graft-versus-host disease grade.

In conclusion, peripheral airways ventilation heterogeneity worsens with time after HSCT. S_{acin} may be more sensitive than spirometry in detecting BO at an early stage, which needs confirmation in a prospective study.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a procedure widely used for the treatment of a variety of haematological disorders. Pulmonary complications are a major cause of morbidity and mortality in HSCT recipients, with bronchiolitis obliterans (BO) being the most frequent non-infectious cause (2% to 40% of all recipients) [1-3]. Bronchiolitis obliterans is a progressive fibrinous obliteration of small airways that is strongly associated with chronic graft-versus-host disease (cGVHD) [1 3-6], and clinically characterised by the development of airflow obstruction which arises 100 days or more after transplantation, as evidenced by spirometry.

Gold standard diagnosis requires transbronchial, thoracoscopic or open lung biopsy, which are rarely performed because of sampling errors, the high degree of immunosuppression often present and risks associated with these diagnostic procedures [7]. Lung volume measurements and expiratory high resolution computed tomography are also useful to detect low-attenuation areas which suggest gas trapping as a manifestation of airways disease [8]. Symptoms and signs of airflow obstruction on spirometry do not occur until disease of the small airways is widespread and lesions are more likely to be irreversible [9]. Sensitive and reproducible measures that allow early detection and monitoring of peripheral airway function may allow a better understanding of the pathogenesis and natural history of BO and may contribute to the development of novel and more effective treatment. The lack of such measures is currently impeding progress in this disease. Increased exhaled nitric oxide (eNO), presumably representing increased airway inflammation, has recently been reported in early bronchiolitis obliterans syndrome (BOS) in lung transplant recipients [10]. Similarly, changes in single breath washouts were reported to precede changes in spirometry in a similar cohort [11]. There are no published studies on peripheral airway function in HSCT recipients.

The multiple breath nitrogen washout test (MBNW) is a measure of peripheral airway function that is highly reproducible [12] and sensitive to peripheral airway dysfunction in smokers who have normal spirometry [12-14]. It provides measurements of ventilation heterogeneity in the lung as a whole (Lung Clearance Index: LCI), in peripheral airways where gas transport is predominantly convective (S_{cond}) and in more peripheral airways still where gas transport is predominantly diffusive (S_{acin}) [15 16]. The rationale for the use of MBNW in this setting is based on the notion that ventilation becomes more uneven and heterogeneous early in peripheral airways disease [17]. Respiratory system resistance (Rrs) and reactance (Xrs) measured using the forced oscillation technique (FOT) have also been used as measures of peripheral airway function when using an oscillation frequency of 8Hz or less [18 19]. Airway narrowing may increase resistance [20] while airway closure and a more heterogeneous distribution of airway calibres may increase the respiratory system stiffness, and thus increase Xrs in absolute value [21 22]. In a lung transplant recipient with suspected acute rejection, Hamakawa et al. reported a good correlation between FOT measurements and clinical symptoms before and after steroid therapy [23].

The likelihood of having BO increases not only with time post-transplantation but also with increased age at transplantation, with having had acute and/or chronic GVHD[3 24] and with myeloablative conditioning [7]. Moreover, once BO occurs, airflow obstruction progressively worsens in the majority of patients [9 25]. Thus, we hypothesised that measures of peripheral airway function would be more abnormal with increased time following HSCT. Our primary aims were to determine if ventilation heterogeneity, Rrs, Xrs and eNO, as measurements of peripheral airway function, related to time post HSCT in a cross section of HSCT recipients and to determine if they changed over a prospective observational period. The secondary aims

were to examine the clinical significance of these measurements by looking at the correlations with spirometry, cGVHD grade and respiratory quality of life.

METHODS

Study design

We conducted a prospective observational study of all patients from a single hospital who had received allogeneic HSCT three or more months previously. Anthropometric characteristics, smoking history and clinical data related to transplantation were collected. Patients had measurements of spirometry, lung volumes, carbon monoxide diffusing capacity (DLCO) and eNO at enrolment and again at least 3 months later, depending on their haematological follow-up. Peripheral airway function was measured by the MBNW and by FOT. A St George Respiratory Questionnaire (SGRQ) was completed as a measure of respiratory quality of life. We examined the relationship between time post transplantation and peripheral airway function, and related function to SGRQ and cGVHD grade as clinical correlates.

Patients

Patients were recruited from all HSCT recipients being managed at the Royal North Shore Hospital, Sydney, Australia. Subjects had to be clinically stable for at least 1 month with neither symptoms of chest infection, nor any acute change of respiratory symptoms. Written informed consent was obtained from all subjects and the study was approved by the Human Ethics Review Committee of the Northern Sydney, Central Coast Area Health Service (protocol number 0411-239M).

Anthropometric measurements and smoking history were recorded on the day of testing. Medical history, diagnoses, conditioning regimen, donor HLA match status, cGVHD grade, current or previous episodes of acute GVHD, and presence or absence of hypogammaglobulinemia were collected from patients' medical records. The staging of acute and chronic GVHD was performed by a haematologist, according to IBMTR staging system [26] and NIH consensus guidelines [27], respectively. These guidelines score cGVHD grade as absent, mild, moderate or severe, depending on the number of affected organs and the severity of symptoms. The criteria from the NIH consensus guidelines [27] were also used for the clinical diagnosis of BO: (1) $FEV_1/FVC < 0.7$ and $FEV_1 < 75\%$ of predicted, (2) radiologic, histologic or lung volume evidence of air trapping, and (3) absence of respiratory tract infection.

Pulmonary function testing

Spirometry, lung volumes by plethysmography and DLCO by single breath technique were measured according to the American Thoracic Society guidelines, using a Sensormedics V-Max Autobox 6200 (Sensormedics Co., Yorba Linda, California, USA). DLCO measurements were corrected for haemoglobin concentration. Predicted values were determined from the equations developed by the European Coal and Steel Community [28] for spirometry, and Crapo and co-workers [29] for lung volumes and DLCO. Airflow obstruction was defined by FEV_1/FVC lower limit of normal according to ATS/ERS consensus [30].

Multiple Breath nitrogen Washout

A closed circuit, bag-in-box breathing system to deliver 100% O_2 during inspiration with separate capture of exhaled breath was used to perform MBNW, as described previously [12]. The derivation of LCI and indices of ventilation distribution S_{cond} and S_{acin} were performed as previously reported [12]. Briefly, subjects inhaled 1L breaths of pure oxygen at 8-12 breaths/minute until mean nitrogen concentration reached $1/40^{th}$ of baseline. The washout was performed at least three times. The phase III slope of each breath is normalised to the mean nitrogen concentration (SnIII). Functional residual capacity was calculated by the total nitrogen washed out. The cumulative expired volume was normalised for lung size by dividing by FRC, i.e. for a 3L FRC, 1 turnover has occurred after 3 breaths of size 1L. S_{cond} was the least squares slope of points between turnovers 1.5 and 6, from the plot of SnIII versus turnover (see Figure 1). For quality control, any washouts in which an asymptote occurred before turnover 6 and had R^2 <0.85 were rejected. An SnIII asymptote can occur when conductive ventilation heterogeneity is such that the nitrogen from one part of the lung is completely washed out before turnover 6 and does not contribute further to gas mixing. This may then confound the Scond parameter.

Forced Oscillation Technique

Rrs was measured continuously with FOT at 6Hz during one minute of tidal breathing, with cheeks supported by the hands. The forced oscillation device provided measurements of flow and pressure at the mouth and was calibrated using calibration tubes of known resistance. The patients breathed room air through an exhaust port while a 6Hz oscillation was applied. Flow, differential pressure and mouth pressure were measured as described previously [20]. Mean Xrs and Rrs were calculated as previously reported [31] and expressed as predicted values using equations of Pasker et al. [32].

Exhaled nitric oxide

Exhaled NO was measured according to American Thoracic Society guidelines, using an offline technique. Patients exhaled over 5-15 s at a flow rate of 200L/min monitored by rotameter (Dwyer Flowmeter Model VFASS-25, AMBIT Instruments Pty Ltd, Parramatta, Australia), into a nitric oxide impermeable polyethylene bag (Scholle Industries Pty Ltd, Elisabeth West, Australia). A chemiluminescence analyser (Thermo Environmental Instruments Model 42C, Franklin, Massachusetts, USA) was used to analyse the exhaled gas. Our laboratory upper limit of normal is 13ppb.

Data analysis

Results are expressed as mean ± SD or median and range depending on the distribution of data. Where possible, the distributions of data were normalised via logarithmic or square root transformation. Depending on final data distribution, univariate correlations were determined using either Pearson's (r) or Spearman's (r_s) correlation analysis. Multivariate linear regression analyses were used to determine the independent relationships between peripheral airway function, SGRQ scores and duration post HSCT. Measured parameters were corrected for confounding factors, such as age, gender and smoking, by firstly determining their effects as independent variables in a multivariate linear regression. The resultant residuals from this

regression were then used in subsequent correlations and regression models. The proportion of patients that had abnormal Sacin was determined on uncorrected data since this is consistent with clinical practice. Paired T tests or Wilcoxon signed rank test were used to compare measured parameters between 2 visits, depending on the data distribution of these differences. The method of Holm [33] was used to correct the level of significance for multiple univariate correlations.

RESULTS

A total of 40 HSCT survivors were identified from Royal North Shore Hospital's transplanted cohort, from which 34 participated in the study. One patient was excluded because of a severe extra-pulmonary restrictive syndrome due to severe sclerodermatous GVHD. Thirty three patients were included in the analyses; their anthropometric characteristics, smoking history and clinical data related to HSCT are presented in Table 1. All but 4 patients were receiving immunosuppression in various combinations of prednisone, cyclosporine, mycophenolate mofetil, tacrolimus and azathioprine. Based on NIH consensus criteria [27], 8 patients (24%) were clinically diagnosed as having BO. The pre-transplant FEV1 was 96 ± 10 % predicted compared with 87 ± 20% predicted at the time of first MBNW testing. The median time post transplantation was 12 months (range: 3-73). Two patients had several allogeneic HSCTs, and time post transplantation was calculated from the date of the first transplant.

Results of pulmonary function, MBNW, FOT and eNO measurements are shown in Table 2. One patient could not perform MBNW because of an inability to use the mouthpiece due to severe mouth GVHD. To illustrate the wide spectrum of ventilation heterogeneity measured by MBNW in this population, normalized slope curves of 2 patients with normal and highly abnormal ventilation heterogeneity are shown on Figure 1. No individual washouts were rejected based on the presence of an asymptote.

Correlations between airway function and time post transplant

Correlations between measured parameters and time post transplant are shown in Table 3. FEV_1, FVC and RV/TLC in % predicted, as well as LCI and S_{acin} were significantly correlated with time post transplant in univariate analysis. FEF 25-75 % predicted, Xrs % predicted and S_{cond} had borderline correlations with time post transplant since they were no longer significant after removing the 2 patients with highest measured values or longest time post transplant. FEV_1/FVC as absolute values and as % predicted, TLC % predicted, DLCO % predicted, Rrs % predicted and eNO were not related to time post transplant.

Age, sex, BMI, conditioning regimen (myeloablative vs non myeloablative) and smoking history were assessed as potential confounding factors for all small airways measurements. S_{acin} was correlated with age and smoking history and after correction for these, the correlation between S_{acin} and time post transplant remained highly significant ($r=0.55$, $p=0.001$) (Figure 2). Corrected S_{acin} was used in subsequent analyses. None of the other measurements were related to any of these potential confounders.

To determine which measures of airway function were independently related to time post transplant, a backwards, stepwise multiple linear regression analysis was performed, including all significant parameters from the univariate analyses, corrected for confounding factors where applicable. The time post transplant was predicted independently by both S_{acin}

(partial r^2=0.18, p=0.04) and FEV_1 % predicted (partial r^2=0.24, p=0.02, p<0.001 for overall model).

As age at transplant has also been found to be associated with the development of BO [24], we looked for correlations with measured lung function parameters. S_{acin} was the only parameter to correlate with age at transplant (r_s=0.50, p=0.004). After correction for smoking history this correlation was still significant (rs=0.41, p=0.02). However, in a multivariate analysis with time post transplant and age at transplant, time post transplant was the sole independent predictor of S_{acin}.

Correlations between measured parameters

There were significant interrelationships between peripheral airway function and both spirometry and lung volumes measurements. FEV_1 % predicted correlated inversely with corrected S_{acin} (r=-0.50, p=0.004), S_{cond} (r=-0.68, p<0.0001), LCI (r=-0.69, p<0.0001) and Xrs % predicted (r=-0.54, p=0.001). RV/TLC % predicted correlated with S_{cond} (r=0.54, p=0.002) and LCI (r=0.43, p=0.01). Twenty patients (61%) without airflow obstruction on spirometry (FEV1/FVC > lower limit of normal) had abnormal S_{acin} (using an upper limit of normal of 0.13 L^{-1})[12], whereas none had airflow obstruction with a normal S_{acin} (Figure 3). There were five patients who had gas trapping on static lung volumes (RV/TLC >120% predicted) all of whom had abnormal S_{acin}, while 20 patients (61%) who had no gas trapping (RV/TLC <120% predicted) had abnormal S_{acin}. The remaining 8 patients had normal RV/TLC and S_{acin}.

Clinical correlates

<u>*St George Respiratory Questionnaire*</u>:
SGRQ total score was significantly correlated with time post transplantation (r=0.48, p=0.006), but was unrelated to age, sex, BMI, or smoking history. SGRQ total score was significantly correlated with FEV_1 % predicted (r=-0.50, p=0.004), RV/TLC % predicted (r=0.37, p=0.04), S_{cond} (r=0.55, p=0.001) and LCI (r=0.59, p<0.001), but not to corrected S_{acin}. In multivariate analysis, LCI was the only independent predictor of SGRQ total score.

<u>*Chronic GVHD grade*</u>:
Chronic GVHD grade was related to time post transplantation (r_s=0.55, p=0.002) and SGRQ total score (r_s=0.40, p=0.03). Corrected S_{acin} was the sole airway function measurement to correlate with cGVHD grade (r_s=0.41, p=0.03), and was not related to the conditioning regimen (myeloablative vs non myeloablative), presence of hypogammaglobulinemia, or previous episode of acute GVHD.

Longitudinal results (repeat visits)

A repeat visit was obtained in 22 of the 33 HSCT recipients and was performed 10±6 months after the first visit. The remainder could not be contacted or were unable to attend for lung function, while two had died. Changes in measured parameters between the initial and second tests are shown in table 4. S_{acin} and LCI were the only parameters to change significantly between the 2 tests, both worsening over time. Change in S_{acin} (in absolute value and in % change) was the only parameter to correlate with change in cGVHD grade over the period of follow-up (r_s=0.63, p=0.003 and r_s=0.65, p=0.002 respectively) (Figure 4). Change in S_{acin} was not correlated with change in FEV_1 % predicted (r_s=0.11, p=0.65), change in RV/TLC % predicted (r_s= -0.06, p=0.80), nor with change in any other measured parameters. Change in

SGRQ total score between tests was not correlated with change in any physiological measurement.

DISCUSSION

In this prospective observational study of allogeneic HSCT recipients, we showed that peripheral airway function as measured by S_{acin}, an index of ventilation heterogeneity in the peripheral airways where gas movement is by diffusion, is worse, the longer the time post transplant. S_{acin} and FEV_1 % predicted were independently related to the time since transplant. Ventilation heterogeneity also correlated with clinical parameters in that S_{acin} was the sole predictor of cGVHD grade, and LCI independently correlated with SGRQ. A significant number of patients had abnormal S_{acin} but had normal spirometry. The longitudinal data confirmed that S_{acin} and LCI change over time, with the change in S_{acin} correlating with change in cGVHD grade. We speculate that there may be an ongoing process in HSCT survivors affecting peripheral airway function even when spirometry is normal. BO is believed to be a lung manifestation of cGVHD, and given the relationship between S_{acin} and GVHD stage in the present study, changes in S_{acin} may in part, be due to BO.

Spirometric obstruction may develop in up to 26% of patients after allogeneic HSCT [1 2 25 34 35] with the main risk factors for obstruction being cGVHD and busulfan [3 25] and myeloablative preconditioning [7]. Although most of the obstruction occurs within the first year, there is an annual incidence of approximately 1-3% [3 24]. Although biopsy proof of BO may be obtained in only 50% of patients for various clinical reasons [3], the development of obstruction is often assumed to be due to BO in the absence of other identifiable causes [2 3 7 24 25]. Our findings of worse small airway function over time following HSCT suggest that future studies should be done to examine the time course of changes in small airway function from pre-transplantation onwards. If the changes in small airway function prove to be predictive of subsequent spirometric obstruction, this would have clinical implications because of the strong association between spirometric obstruction and mortality [1 2 6 24].

The present study is the first systematic analysis of peripheral airways function using both MBNW and FOT in an unselected cohort of HSCT recipients, with and without respiratory symptoms. The MBNW parameters LCI, S_{cond} and S_{acin} reflect ventilation heterogeneity in specific zones of the lung. Lung clearance index is a global measure, while S_{cond} and S_{acin} represent the small conducting airways zone and the diffusive gas transport zone respectively [15 16]. Histologically, BO is a process affecting membranous and respiratory bronchioles [36 37] which theoretically is in the region of diffusive gas mixing represented by S_{acin}. Using heliox maximal expiratory flow-volume curves in five allogeneic bone marrow transplant recipients with severe obstruction, Chan et al. suggested that the site of obstruction was in the peripheral airways [38]. However, histopathologic correlation is absent from the present study and is therefore an area requiring future study.

It is probable that processes other than BO might have affected small airways function, given the myriad of complications that commonly occur in these patients. To minimize this risk, all the patients were tested beyond 3 months post transplant (when chronic BO also starts to occur), were clinically stable and had no current respiratory infection. Moreover, correlations between peripheral airway parameters were corrected for potential confounding factors such as age, smoking history and conditioning regimen. We found that age at transplant correlated with S_{acin}. This suggests that S_{acin} could be increased due to the normal ageing process and/or

due to an increased risk of GVHD related peripheral airway damage with increasing age [24]. It was therefore necessary to correct for age, to determine the effects of processes occurring after the transplant alone. In terms of immunosuppression, all but 4 of the 33 patients were receiving it and in varying combinations, which meant that we were unable to examine its potential effects on S_{acin}.

Management of GVHD following HSCT requires continuous monitoring of all organ systems. Sufficient suppression of cGVHD to protect organ systems has also to be balanced against the beneficial anti-tumour effects. BO may result from cGVHD and other potential causes. The significant correlation between S_{acin} and cGVHD grade is consistent with this relationship. However, because of the low sensitivity of spirometry, BO is presently detected late in the evolution of the disease, when airway obstruction may be less likely to be reversible. Since a significant number of patients had abnormal S_{acin} with normal spirometry, MBNW may be useful for monitoring lung function in HSCT recipients. Similarly ventilation heterogeneity measured by single breath gas washout in lung transplant recipients became abnormal before obstruction was evident on spirometry, suggesting possible utility for early detection of BOS [10 39]. Given the identical pathology of BO in HSCT and lung transplant recipients [40], single breath washout may also have clinical utility in HSCT follow-up.

Measurements of lung mechanics appear to be insensitive to peripheral airways disease in this population. Respiratory system resistance is a measurement of frictional pressure loss in airways, while reactance represents the compliance of the respiratory system. Although Rrs is increased and Xrs is decreased at low frequencies in small airways disease, neither are specific markers [18 41]. We found no correlation between time post transplantation and Rrs, while the correlation with Xrs was driven by the two highest values. The very large range of Xrs values in our cohort could reflect high inter-individual variability related to unidentified factors, which might have been responsible for its lack of significant correlations with clinical parameters. The area under the reactance versus frequency curve from multi-frequency data could be a worthwhile parameter to examine in future studies. In bilateral lung transplant recipients, BOS is associated with stiffer lungs in terms of both static compliance and the exponential constant K [39]. Interestingly, increased lung stiffness is also associated with greater ventilation heterogeneity even when spirometry is normal [39], which is consistent with the presence of sub-clinical BO.

Exhaled NO is an established marker of lung inflammation, but has never been studied systematically in allogeneic HSCT recipients. In our study, there was no correlation between eNO and time post transplant, and the vast majority of our patients had values within the normal range (< 13 ppb). In a mouse model, eNO was elevated following allogeneic transplantation [42], but its relation to BO in humans is unclear. Other factors unrelated to BO, such as atopy or conditioning-related lung injury could affect eNO and make any relationships hard to detect.

Respiratory quality of life decreased over time post HSCT due predominantly to an increase in the Symptoms score. There was no change in quality of life over the follow-up period probably because of the short interval in relation to the total time post transplant. Although Symptoms score was strongly related to FEV_1% predicted, RV/TLC % predicted and LCI, LCI was the sole predictor of SGRQ total score in multivariate analysis. This suggests that respiratory quality of life following HSCT may be affected by peripheral airway dysfunction as measured by ventilation heterogeneity in a global sense and is in keeping with similar findings with FOT [43]. The absence of correlation between S_{acin} and both SGRQ total score and Symptoms score in this longitudinal cohort with 5 of the 22 patients with clinical BO,

suggests that abnormalities in the more peripheral diffusion dependent airway compartment are too peripheral to impact on respiratory symptoms.

In conclusion, the present study shows that impairment of peripheral airway function is related to time post allogeneic HSCT, independently of spirometric signs of airflow obstruction and of respiratory symptoms, and that this impairment is related to cGVHD stage. The results of this cross-sectional study, along with findings from a limited period of follow-up testing suggest that a prospective longitudinal study is warranted to determine whether MBNW is a potentially useful non-invasive method of monitoring peripheral airways function that could contribute to the clinical management of allogeneic HSCT recipients. If it proves to be a sensitive, early method to detect BO, it would facilitate the exploration of novel therapies and strategies in HSCT related BO.

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FIGURE LEGENDS

Figure 1. MBNW normalized slope curves of 2 HSCT recipients with normal and highly abnormal ventilation heterogeneity.

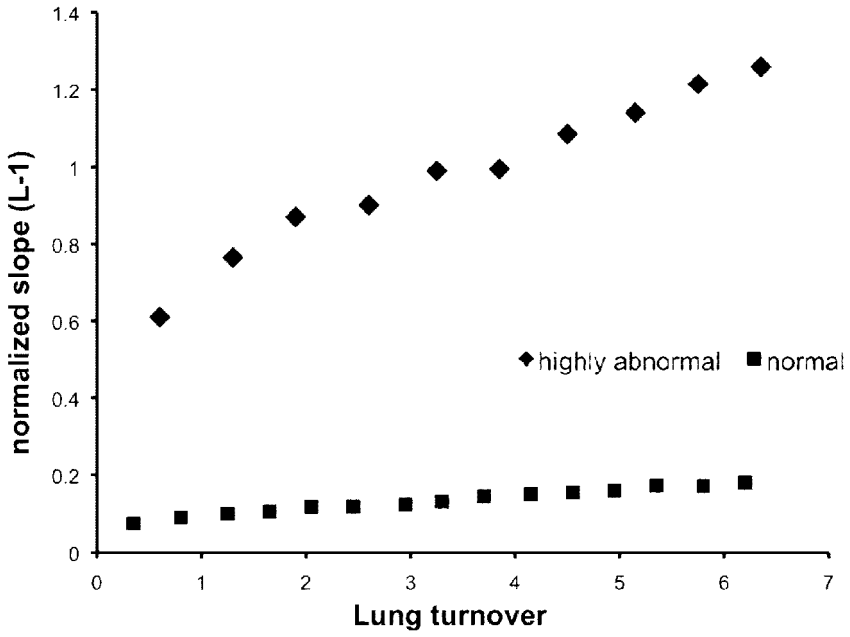

Figure 2. Correlation between S_{acin} and time post HSCT.

a) S_{acin} uncorrected for confounding factors.
b) S_{acin} corrected for age and smoking history

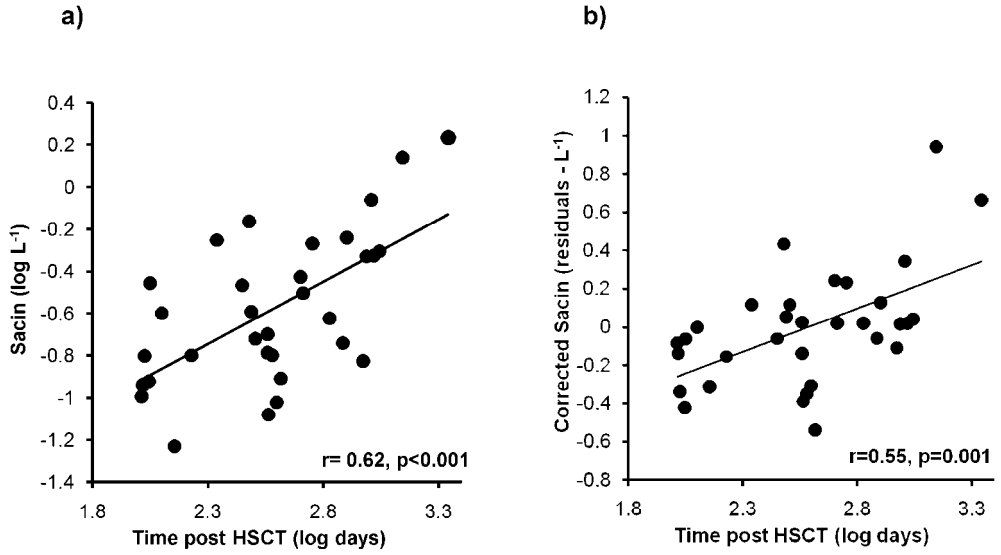

Figure 3. S_{acin} versus airflow obstruction on spirometry in HSCT recipients.
The horizontal line represents the upper limit of normal for S_{acin} [12], and vertical line represents lower limit of normal for FEV_1 % predicted. Twenty patients had an abnormal S_{acin} without airflow obstruction on spirometry, whereas no patient had a normal S_{acin} with an abnormal FEV_1/FVC.
LLN = lower limit of normal according to ATS/ERS consensus [30]

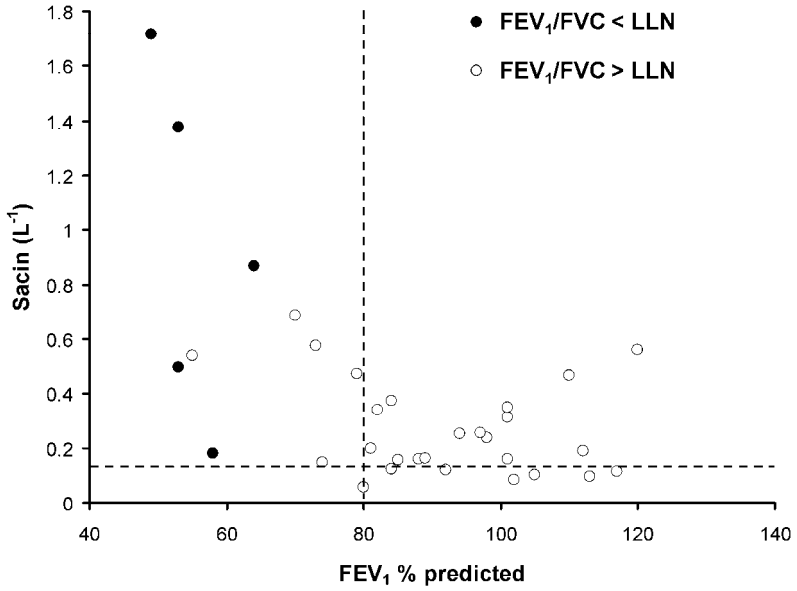

Figure 4. Repeat visits: correlation between change in Sacin and change in cGVHD grade.

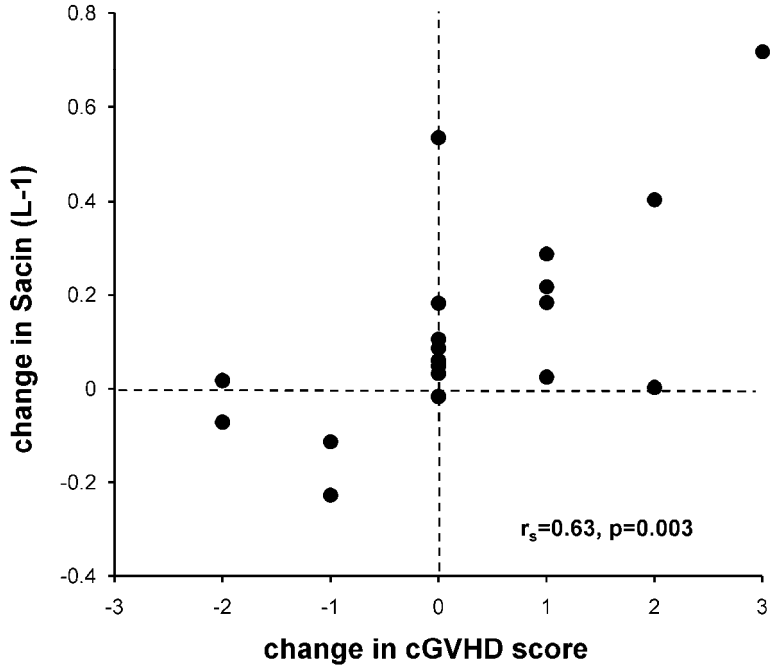

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Table 1. Clinical characteristics of HSCT recipients at time of testing

Parameters	Patients (n= 33)
Age, years (mean ± SD)	47 ± 15
Sex, male (n,%)	22 (67)
BMI, Kg/m^2 (mean ± SD)	24.7 ± 4.5
Smoking history, pack/years (median, range)	1 (0-60)
Time post HSCT, months (median, range)	12 (3-73)
Diagnosis	
AML (n, %)	11 (33)
CML (n, %)	1 (3)
ALL (n, %)	5 (15)
CLL (n, %)	1 (3)
Lymphoma (n, %)	6 (18)
Multiple myeloma (n, %)	3 (9)
Aplastic anemia (n, %)	3 (9)
Myelofibrosis (n, %)	1 (3)
Myelodysplasia (n, %)	2 (6)
Donor HLA status	
HLA-matched related (n, %)	30 (91)
HLA-mismatched related (n, %)	2 (6)
HLA-matched unrelated (n, %)	1 (3)
Conditioning regimen	
Myeloablative (n, %)	14 (42)
Non-myeloablative (n, %)	19 (58)
Total body irradiation (n, %)	6 (18)
Hypogammaglobuminemia (n, %)	14 (42)
Acute GVHD	
Past episode (n, %)	15 (45)
Active (n, %)	0 (0)
Unknown (n, %)	3 (9)
Chronic GVHD grade	
Grade 0 (n, %)	12 (36)
Grade 1 (n, %)	10 (30)
Grade 2 (n, %)	6 (18)
Grade 3 (n, %)	2 (6)
Unknown (n, %)	3 (9)

Definition of abbreviations: HSCT = Hematopoietic stem cell transplantation; BMI = Body mass index; AML = Acute myeloid leukaemia; CML = Chronic myeloid leukaemia; ALL = Acute lymphoblastic leukaemia; CLL = Chronic lymphocytic leukaemia; HLA = Human leukocyte antigen, GVHD = Graft-versus-host disease.

Table 2. Pulmonary and peripheral airway function of 33 HSCT recipients.

Parameters	values
Pulmonary functions	
FEV_1, % predicted (mean ± SD)	87 ± 20
FVC, % predicted (mean ± SD)	94 ± 17
FEV_1/FVC, % (median, range)	80 (45-89)
FEF 25-75, % predicted (mean ± SD)	68 ± 29
TLC, % predicted (mean ± SD)	96 ± 10
RV/TLC, % predicted (median, range)	100 (79-165)
DLCO, % predicted (mean ± SD)	73 ± 19
Multiple breath nitrogen washout	
LCI (median, range)	12.1 (8.0-22.2)
S_{cond}, L^{-1} (mean ± SD) (N < 0.04)	0.07 ± 0.03
S_{acin}, L^{-1} (median, range) (N < 0.13)	0.24 (0.08-1.72)
Forced oscillation technique	
Rrs, % predicted (mean ± SD)	118 ± 31
Xrs, % predicted (median, range)	153 (9-997)
Exhaled NO, ppb (median, range)	7.2 (5.1-14.1)
SGRQ total score (median, range)	26 (0-46)
Symptoms score (median, range)	14 (0-64)
Activity score (median, range)	35 (0-93)
Impact score (median, range)	6 (0-44)

Definition of abbreviations : FEV_1 = forced expiratory volume in 1s; FVC = forced vital capacity; FEF 25-75 = forced expiratory volume between 25 and 75% of FVC; TLC = total lung capacity; RV = residual volume; DLCO = carbon monoxide diffusing capacity; LCI = Lung clearance index; S_{cond} = index of conductive airways heterogeneity; S_{acin} = index of diffusive airways heterogeneity; N = upper limit of normal; Rrs = respiratory system resistance; Xrs = respiratory system reactance; NO = nitric oxide; ppb = part per billion; SGRQ = St George respiratory questionnaire.

Table 3. Correlations between measured parameters and Time post HSCT

Parameters	Univariate correlations		Independent predictors*	
Pulmonary function				
FEV_1 % predicted	r = - 0.58	p < 0.001	partial r^2 = 0.24	p = 0.02
FVC % predicted	r = - 0.44	p = 0.01	—	—
FEV_1/FVC % predicted	r_s = - 0.30	p = 0.10	—	—
FEF 25-75 % predicted	r = - 0.41	p = 0.02†	—	—
TLC % predicted	r = - 0.20	p = 0.25	—	—
Log RV/TLC % predicted	r = 0.41	p = 0.02	—	—
DLCO % predicted	r = 0.07	p = 0.69	—	—
MBNW				
Log LCI	r = 0.62	p < 0.001	—	—
S_{cond}	r = 0.45	p = 0.01‡	—	—
Log S_{acin}	r = 0.62	p < 0.001§	partial r^2 = 0.18	p = 0.04‖
Forced oscillation technique				
Rrs % predicted	r_s = - 0.01	p = 0.97	—	—
Log Xrs % predicted	r = 0.51	p = 0.002‡	—	—
Exhaled nitric oxide (log)	r = 0.05	p = 0.77	—	—

* Independent predictors of time post transplant in multiple regression model including significant parameters (shaded) from univariate analyses.

† Insignificant after removing the 2 patients with longest time post transplant

‡ Insignificant after removing the 2 patients with highest measured values

§ After correction for age and smoking history (confounding factors), the correlation was still highly significant (r= 0.55, p= 0.001)

‖ Sacin corrected for confounding factors was used in the multiple regression model

Shaded boxes indicates significant univariate correlations after correction for multiple comparisons.

Definition of abbreviations : FEV_1 = forced expiratory volume in 1s; FVC = forced vital capacity; FEF 25-75 = forced expiratory volume between 25 and 75% of FVC; TLC = total lung capacity; RV = residual volume; DLCO = carbon monoxide diffusing capacity; MBNW = Multiple Breath Nitrogen Washout; LCI = lung clearance index; S_{cond} = index of conductive airways heterogeneity; S_{acin} = index of diffusive airways heterogeneity; Rrs = respiratory system resistance; Xrs = respiratory system reactance

Table 4. Repeat visits* in 22 HSCT recipients: changes in measured parameters

Parameters	1st visit	2nd visit	p values†
Pulmonary function			
FEV_1, % predicted (mean ± SD)	88 ± 15	85 ± 21	0.27
FVC, % predicted (mean ± SD)	95 ± 15	94 ± 18	0.76
FEV_1/FVC, % (median, range)	78 (67-87)	74 (47-85)	0.08
FEF 25-75, % predicted (mean ± SD)	68 ± 26	62 ± 29	0.09
TLC, % predicted (mean ± SD)	95 ± 10	97 ± 10	0.12
RV/TLC, % predicted (median, range)	99 (71-138)	97 (73-165)	0.62
DLCO, % predicted (mean ± SD)	72 ± 14	73 ± 17	0.68
Multiple breath nitrogen washout			
LCI (mean ± SD)	11.6 ± 2.5	13.5 ± 3.5	0.003
S_{cond}, L^{-1} (median, range)	0.06 (0.02-0.17)	0.07 (0.02-0.24)	0.21
S_{acin}, L^{-1} (median, range)	0.28 (0.06-0.69)	0.41 (0.08-1.07)	0.007
Forced oscillation technique			
Rrs, % predicted (median, range)	113 (77-187)	103 (73-259)	0.50
Xrs, % predicted (median, range)	151 (9-792)	172 (47-788)	0.37
Exhaled NO, ppb (median, range)	7.5 (4.7-40.1)	6.4 (3.6-16)	0.46
SGRQ total score (median, range)	15 (0-40)	17 (1-63)	0.23
Symptoms score (median, range)	21 (0-73)	17 (0-68)	0.93
Activity score (mean ± SD)	29 ± 25	33 ± 27	0.45
Impact score (median, range)	7 (0-22)	6 (0-59)	0.19
cGVHD grade (median, range)	0 (0-3)	1 (0-3)	0.38

* The second visits were performed 10±6 months after the first visits.

† paired T test or Wilcoxon signed rank test depending on the distribution of the differences.

Definition of abbreviations : FEV_1 = forced expiratory volume in 1s; FVC = forced vital capacity; FEF 25-75 = forced expiratory volume between 25 and 75% of FVC; TLC = total lung capacity; RV = residual volume; DLCO = carbon monoxide diffusing capacity; LCI = Lung clearance index; S_{cond} = index of conductive airways heterogeneity; S_{acin} = index of diffusive airways heterogeneity; N = upper limit of normal; Rrs = respiratory system resistance; Xrs = respiratory system reactance; NO = nitric oxide; ppb = part per billion; SGRQ = St George respiratory questionnaire; cGVHD = chronic graft-versus-host disease.