



Small airways function declines after allogeneic haematopoietic stem cell transplantation

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ABSTRACT: Bronchiolitis obliterans (BO) following allogeneic haematopoietic 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. The following measurements were performed: lung function, exhaled nitric oxide, forced oscillatory respiratory system resistance and reactance, acinar (Sacin) and conductive airways ventilation heterogeneity and lung clearance index (LCI) measured by multiple breath nitrogen washout. 22 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 Sacin and forced expiratory volume in 1 s % predicted. 20 patients had abnormal Sacin with normal spirometry, whereas none had airflow obstruction with normal Sacin. Sacin and LCI were the only measures to change significantly between two visits, with both worsening. Change in Sacin 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. Sacin may be more sensitive than spirometry in detecting BO at an early stage, which needs confirmation in a prospective study.

KEYWORDS: Airway physiology, airway resistance, exhaled nitric oxide, lung function in disease, obliterative bronchiolitis, stem cell transplantation

Allogeneic haematopoietic 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 noninfectious cause (2–40% of all recipients) [1–3]. BO is a progressive fibrous 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 ≥ 3 months after transplantation, as evidenced by spirometry.

Gold standard diagnosis requires transbronchial, thorascopic or open lung biopsy, which is 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 to detect low-attenuation areas, which suggest gas trapping as a manifestation of airways disease are also useful [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 enable 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 BO syndrome (BOS) in lung transplant recipients [10]. Similarly, changes in single-breath washouts were reported to precede changes in spirometry

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in a similar cohort [11]. There are no published studies on peripheral airway function in HSCT recipients.

The multiple-breath nitrogen washout (MBNW) test 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 the MBNW test in this setting is based on the notion that ventilation becomes more uneven and heterogeneous early in peripheral airways disease [17]. Respiratory system resistance (R_{rs}) and reactance (X_{rs}) measured using the forced oscillation technique (FOT) have also been used as measures of peripheral airway function when using an oscillation frequency of ≤ 8 Hz [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 X_{rs} in absolute value [21, 22]. In a lung transplant recipient with suspected acute rejection, HAMAKAWA *et al.* [23] reported a good correlation between FOT measurements and clinical symptoms before and after steroid therapy.

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 cGVHD [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 whether ventilation heterogeneity, R_{rs} , X_{rs} and eNO, as measurements of peripheral airway function, are 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 ≥ 3 months previously. Anthropometric characteristics, smoking history and clinical data related to transplantation were collected. Patients had measurements of spirometry, lung volumes, diffusing capacity of the lung for carbon monoxide (DL_{CO}) and eNO at enrolment and again ≥ 3 months later, depending on their haematological follow-up. Peripheral airway function was measured by the MBNW test 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 allogeneic HSCT recipients being managed at the Royal North Shore Hospital, Sydney,

Australia. Subjects had to be clinically stable for ≥ 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 human leukocyte antigen 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 cGVHD was performed by a haematologist, according to the International Bone Marrow Transplant Registry (IBMTR) staging system [26] and National Institutes of Health (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, as follows: 1) forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) < 0.7 and $FEV_1 < 75\%$ of predicted; 2) radiological, histological or lung volume evidence of air trapping; and 3) absence of respiratory tract infection.

Pulmonary function testing

Spirometry, lung volumes by plethysmography and DL_{CO} by single-breath technique were measured according to the American Thoracic Society (ATS) guidelines, using a SensorMedics V-Max Autobox 6200 (SensorMedics Co., Yorba Linda, CA, USA). DL_{CO} measurements were corrected for haemoglobin concentration. Predicted values were determined from the equations developed by the European Coal and Steel Community (ECSC) [28] for spirometry, and CRAPO *et al.* [29] for lung volumes and DL_{CO} . Airflow obstruction was defined by FEV_1/FVC lower limit of normal according to the ATS/European Respiratory Society (ERS) consensus [30].

MBNW test

A closed circuit, bag-in-box breathing system to deliver 100% oxygen during inspiration with separate capture of exhaled breath was used to perform the MBNW test, 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 1-L breaths of pure oxygen at 8–12 breaths·min⁻¹ until mean nitrogen concentration reached 1/40th of baseline. The washout was performed at least three times. The phase III slope of each breath is normalised to the mean nitrogen concentration (normalised phase III slope; Sn_{III}). Functional residual capacity (FRC) 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 3-L FRC, one turnover has occurred after three breaths of 1-L size. S_{cond} was the least squares slope of points between turnovers 1.5 and 6, from the plot of Sn_{III} versus turnover (fig. 1). For quality control, any washouts in which an asymptote occurred before turnover 6 and had $r^2 < 0.85$ were rejected. An Sn_{III} asymptote can occur when conductive ventilation heterogeneity is such that the nitrogen from one part of the lung is completely washed out before

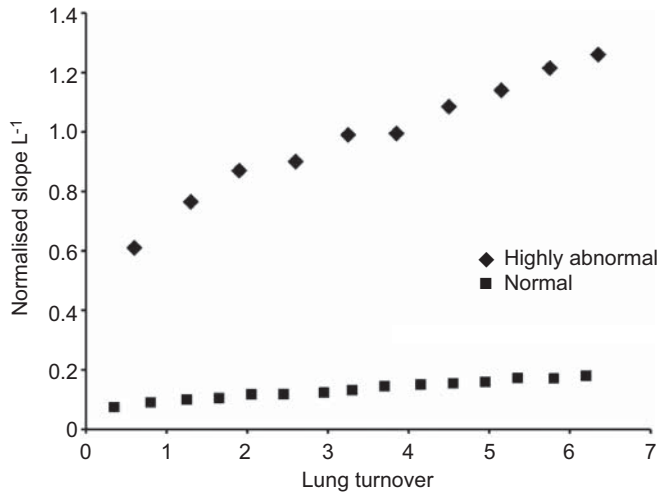


FIGURE 1. Multiple breath nitrogen washout normalised slope curves of two haematopoietic stem cell transplantation recipients with normal and highly abnormal ventilation heterogeneity.

turnover 6 and does not contribute further to gas mixing. This may then confound the S_{cond} parameter.

FOT

R_{rs} was measured continuously with FOT at 6 Hz during 1 min of tidal breathing, with the 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 6-Hz oscillation was applied. Flow, differential pressure and mouth pressure were measured as described previously [20]. Mean X_{rs} and R_{rs} were calculated as previously reported [31] and expressed as predicted values using the equations of PASKER *et al.* [32].

eNO

eNO was measured according to ATS guidelines, using an offline technique. Patients exhaled over 5–15 s at a flow rate of 200 L·min⁻¹ monitored by a 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; Thermo Environmental Instruments Inc., Franklin, MA, USA) was used to analyse the exhaled gas. Our laboratory upper limit of normal is 13 ppb.

Data analysis

Results are expressed as mean \pm 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, sex 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 who had abnormal S_{acin} 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 two 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 extrapulmonary restrictive syndrome due to severe sclerodermatous GVHD. 33 patients were included in the analyses; their anthropometric characteristics, smoking history and clinical data related to HSCT are presented in table 1. All but four patients were receiving immunosuppression in various combinations of prednisone, cyclosporine, mycophenolate mofetil, tacrolimus and azathioprine. Based on NIH consensus criteria [27], eight (24%) patients were clinically diagnosed as having BO. The pre-transplant FEV₁ was $96 \pm 10\%$ pred compared with $87 \pm 20\%$ pred 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 test, FOT and eNO measurements are shown in table 2. One patient could not perform the MBNW test 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, normalised slope curves of two patients with normal and highly abnormal ventilation heterogeneity are shown in 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. FEV₁, FVC and residual volume (RV)/total lung capacity (TLC) in % pred, as well as LCI and S_{acin} were significantly correlated with time post-transplant in univariate analysis. Forced expiratory volume at 25–75% of FVC % pred, X_{rs} % pred and S_{cond} had borderline correlations with time post-transplant since they were no longer significant after removing the two patients with highest measured values or longest time post-transplant. FEV₁/FVC as absolute values and as % pred, TLC % pred, DL_{CO} % pred, R_{rs} % pred and eNO were not related to time post-transplant.

Age, sex, body mass index (BMI), conditioning regimen (myeloablative *versus* nonmyeloablative) 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 ($r=0.55$, $p=0.001$; fig. 2). Corrected S_{acin} was used in subsequent analyses. None of the other measurements were related to any of these potential confounders.

TABLE 1 Clinical characteristics of haematopoietic stem cell transplantation (HSCT) recipients at time of testing

Subjects n	33
Age yrs	47 ± 15
Sex male	22 (67)
BMI kg·m⁻²	24.7 ± 4.5
Smoking history pack-yrs	1 (0–60)
Time post HSCT months	12 (3–73)
Diagnosis	
AML	11 (33)
CML	1 (3)
ALL	5 (15)
CLL	1 (3)
Lymphoma	6 (18)
Multiple myeloma	3 (9)
Aplastic anaemia	3 (9)
Myelofibrosis	1 (3)
Myelodysplasia	2 (6)
Donor HLA status	
HLA-matched related	30 (91)
HLA-mismatched related	2 (6)
HLA-matched unrelated	1 (3)
Conditioning regimen	
Myeloablative	14 (42)
Nonmyeloablative	19 (58)
Total body irradiation	6 (18)
Hypogammaglobulinaemia	14 (42)
Acute GVHD	
Past episode	15 (45)
Active	0 (0)
Unknown	3 (9)
Chronic GVHD grade	
0	12 (36)
1	10 (30)
2	6 (18)
3	2 (6)
Unknown	3 (9)

Data are presented as mean ± SD, n (%), or median (range), unless otherwise stated. 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.

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 Sacin (partial $r^2=0.18$, $p=0.04$) and FEV1 % pred (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. Sacin 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

TABLE 2 Pulmonary and peripheral airway function of 33 haematopoietic stem cell transplantation recipients

Pulmonary functions	
FEV1 % pred	87 ± 20
FVC % pred	94 ± 17
FEV1/FVC %	80 (45–89)
FEF _{25–75%} % pred	68 ± 29
TLC % pred	96 ± 10
RV/TLC % pred	100 (79–165)
DLCO % pred	73 ± 19
MBNW	
LCI	12.1 (8.0–22.2)
S _{cond} L ⁻¹ (N<0.04)	0.07 ± 0.03
S _{acin} L ⁻¹ (N<0.13)	0.24 (0.08–1.72)
FOT	
R _{rs} % pred	118 ± 31
X _{rs} % pred	153 (9–997)
eNO ppb	7.2 (5.1–14.1)
SGRQ total score	
Symptoms score	14 (0–64)
Activity score	35 (0–93)
Impact score	6 (0–44)

Data are presented as mean ± SD or median (range). FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; FEF_{25–75%}: forced expiratory volume at 25–75% of FVC; TLC: total lung capacity; RV: residual volume; DLCO: diffusing capacity of the lung for carbon monoxide; MBNW: multiple breath nitrogen washout; FOT: forced oscillation technique; LCI: lung clearance index; S_{cond}: index of conductive airways heterogeneity; S_{acin}: index of diffusive airways heterogeneity; N: upper limit of normal; R_{rs}: respiratory system resistance; X_{rs}: respiratory system reactance; eNO: exhaled nitric oxide; SGRQ: St George's Respiratory Questionnaire.

significant ($r_s=0.41$, $p=0.02$). However, in a multivariate analysis with time post-transplant and age at transplant, time post-transplant was the sole independent predictor of Sacin.

Correlations between measured parameters

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

Clinical correlates

SGRQ

SGRQ total score was significantly correlated with time post-transplantation ($r=0.48$, $p=0.006$) but was unrelated to age,

TABLE 3 Correlations between measured parameters and time post haematopoietic stem cell transplantation

Parameters	Univariate correlations		Independent predictors [#]	
	r	p-value	Partial r ²	p-value
Pulmonary function % pred				
<i>FEV₁</i>	-0.58	<0.001	0.24	0.02
<i>FVC</i>	-0.44	0.01		
FEV ₁ /FVC	<i>r_s</i> = -0.30	0.10		
FEF _{25-75%}	-0.41	0.02 [*]		
TLC	0.20	0.25		
Log RV/TLC	0.41	0.02		
<i>DL_{CO}</i>	0.07	0.69		
MBNW				
<i>Log LCI</i>	0.62	<0.001		
<i>S_{cond}</i>	0.45	0.01 ⁺		
<i>Log Sacin</i>	0.62	<0.001 [§]	0.18	0.04 ^f
FOT % pred				
<i>R_{rs}</i>	<i>r_{rs}</i> = -0.01	0.97		
<i>Log X_{rs}</i>	<i>r</i> =-0.51	0.002 ⁺		
eNO (log)	<i>r</i> =0.05	0.77		

FEV₁: forced expiratory volume in 1 s; FVC: forced vital capacity; FEF_{25-75%}: forced expiratory volume at 25–75% of FVC; TLC: total lung capacity; RV: residual volume; DL_{CO}: diffusing capacity of the lung for carbon monoxide; MBNW: multiple breath nitrogen washout; LCI: lung clearance index; S_{cond}: index of conductive airways heterogeneity; Sacin: index of diffusive airways heterogeneity; FOT: forced oscillation technique; R_{rs}: respiratory system resistance; X_{rs}: respiratory system reactance; eNO: exhaled nitric oxide. [#]: independent predictors of time post transplant in multiple regression model including significant parameters (in italics) from univariate analyses; ^{*}: insignificant after removing the two patients with longest time post-transplant; ⁺: insignificant after removing the two 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); ^f: Sacin corrected for confounding factors was used in the multiple regression model. Italics indicates significant univariate correlations after correction for multiple comparisons.

sex, BMI, or smoking history. SGRQ total score was significantly correlated with FEV₁ % pred (*r*= -0.50, *p*=0.004), RV/TLC % pred (*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 Sacin. In multivariate analysis, LCI was the only independent predictor of SGRQ total score.

cGVHD grade

cGVHD 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 Sacin 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 *versus*

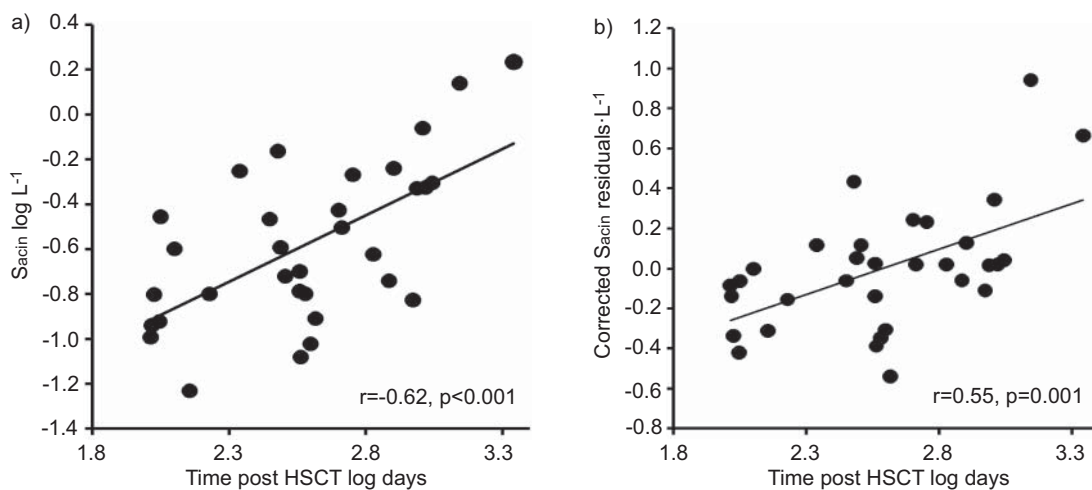


FIGURE 2. Correlation between acinar airways ventilation heterogeneity (Sacin) and time post haematopoietic stem cell transplantation (HSCT). a) Sacin uncorrected for confounding factors. b) Sacin corrected for age and smoking history.

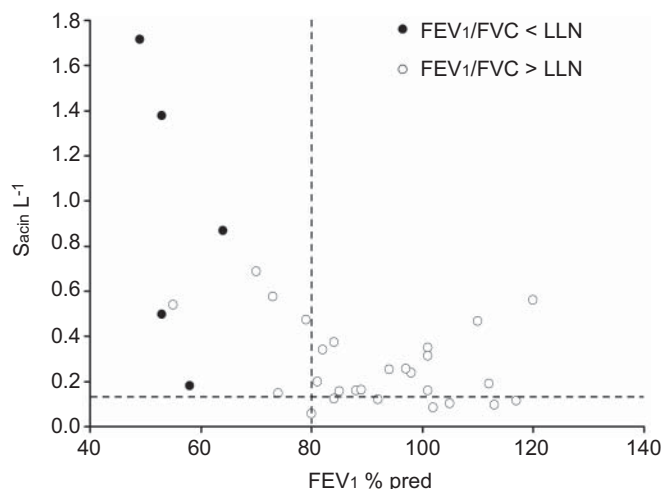


FIGURE 3. Acinar airways ventilation heterogeneity (S_{acin}) versus airflow obstruction on spirometry in haematopoietic stem cell transplantation (HSCT) recipients. The horizontal dashed line represents the upper limit of normal for S_{acin} [12], and the vertical dashed line represents lower limit of normal for forced expiratory volume in 1 s (FEV_1) % predicted. 20 patients had an abnormal S_{acin} without airflow obstruction on spirometry, whereas no patient had a normal S_{acin} with an abnormal FEV_1 /forced vital capacity (FVC). LLN: lower limit of normal according to American Thoracic Society/European Respiratory Society consensus [30].

nonmyeloablative), presence of hypogammaglobulinaemia, 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 11 patients without repeat visits either 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 two 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; fig. 4). Change in S_{acin} was neither correlated with change in FEV_1 % pred ($r_s=0.11$, $p=0.65$), change in RV/TLC % pred ($r_s=-0.06$, $p=0.80$), 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 % pred 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

TABLE 4 Repeat visits[#] in 22 haematopoietic stem cell transplantation recipients: changes in measured parameters

Parameters	1st visit	2nd visit	p-values [†]
Pulmonary function			
FEV_1 % pred	88 ± 15	85 ± 21	0.27
FVC % pred	95 ± 15	94 ± 18	0.76
FEV_1/FVC %	78 (67–87)	74 (47–85)	0.08
FEF _{25–75%} % pred	68 ± 26	62 ± 29	0.09
TLC % pred	95 ± 10	97 ± 10	0.12
RV/TLC % pred	99 (71–138)	97 (73–165)	0.62
DL_{CO} % pred	72 ± 14	73 ± 17	0.68
MBNW			
LCI	11.6 ± 2.5	13.5 ± 3.5	0.003
$S_{cond} L^{-1}$	0.06 (0.02–0.17)	0.07 (0.02–0.24)	0.21
$S_{acin} L^{-1}$	0.28 (0.06–0.69)	0.41 (0.08–1.07)	0.007
FOT % pred			
R_{rs}	113 (77–187)	103 (73–259)	0.50
X_{rs}	151 (9–792)	172 (47–788)	0.37
eNO ppb	7.5 (4.7–40.1)	6.4 (3.6–16)	0.46
SGRQ total score			
Symptoms score	21 (0–73)	17 (0–68)	0.93
Activity score	29 ± 25	33 ± 27	0.45
Impact score	7 (0–22)	6 (0–59)	0.19
cGVHD grade			
	0 (0–3)	1 (0–3)	0.38

Data are presented as mean \pm SD or median (range), unless otherwise stated. FEV_1 : forced expiratory volume in 1 s; FVC: forced vital capacity; FEF_{25–75%}: forced expiratory volume at 25–75% of FVC; TLC: total lung capacity; RV: residual volume; DL_{CO} : diffusing capacity of the lung for carbon monoxide; MBNW: multiple breath nitrogen washout; LCI: lung clearance index; S_{cond} : index of conductive airways heterogeneity; S_{acin} : index of diffusive airways heterogeneity; FOT: forced oscillation technique; R_{rs} : respiratory system resistance; X_{rs} : respiratory system reactance; eNO: exhaled nitric oxide; SGRQ: St George's Respiratory Questionnaire; cGVHD: chronic graft-versus-host disease. [#]: 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.

the 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 cGVHD 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 pre-conditioning [7]. Although most of the obstruction occurs within the first year, there is an annual incidence of ~ 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 airways function over time following HSCT suggest that future studies should be carried out to examine the time course of changes in small airways

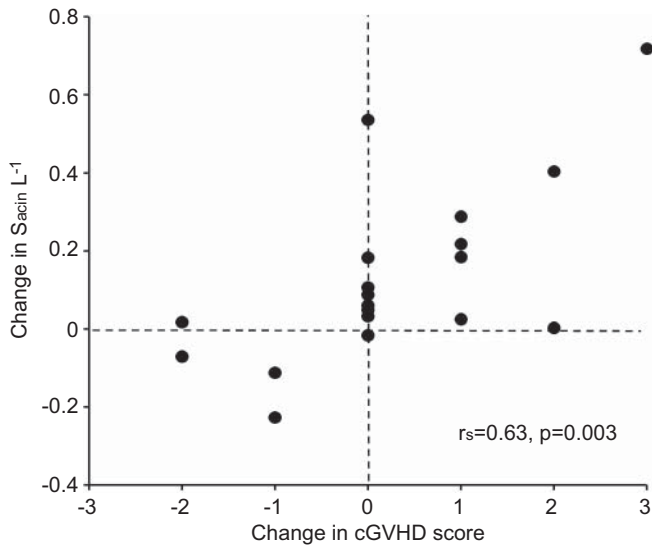


FIGURE 4. Repeat visits. Correlation between change in acinar airways ventilation heterogeneity (Sacin) and change in chronic graft-versus-host disease (cGVHD) grade.

function from pre-transplantation onwards. If the changes in small airways 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 airway function using both the MBNW test and FOT in an unselected cohort of HSCT recipients, with and without respiratory symptoms. The MBNW test parameters LCI, S_{cond} and Sacin reflect ventilation heterogeneity in specific zones of the lung. The LCI is a global measure, while S_{cond} and Sacin 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 Sacin. Using heliox maximal expiratory flow–volume curves in five allogeneic bone marrow transplant recipients with severe obstruction, CHAN *et al.* [38] suggested that the site of obstruction was in the peripheral airways. However, histopathological 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 minimise 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 Sacin. This suggests that Sacin 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 four of the

33 patients were receiving it and in varying combinations, which meant that we were unable to examine its potential effects on Sacin.

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 Sacin 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 Sacin 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. R_{rs} is a measurement of frictional pressure loss in airways, while reactance represents the compliance of the respiratory system. Although R_{rs} is increased and X_{rs} is decreased at low frequencies in small airways disease, neither are specific markers [18, 41]. We found no correlation between time post-transplantation and R_{rs} , while the correlation with X_{rs} was driven by the two highest values. The very large range of X_{rs} 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 multifrequency 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 subclinical 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 the symptoms score was strongly related to FEV1 % pred, RV/TLC % pred 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 Sacin and both SGRQ total score and the symptoms score in this longitudinal cohort with five out 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 airway function that could contribute to the clinical management of allogeneic HSCT recipients. If it proves to be a sensitive, early method with which to detect BO, it would facilitate the exploration of novel therapies and strategies in HSCT-related BO.

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STATEMENT OF INTEREST

A statement of interest for G.G. King can be found at www.ersjournals.com/site/misc/statements.xhtml

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