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Changes in lung volumes and airway responsiveness following haematopoietic stem cell transplantation

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ABSTRACT: Changes in lung volume occur following haematopoietic stem cell transplantation (HSCT); airway hyperresponsiveness was occasionally reported, without mechanistic explanation. The present authors studied 17 patients by standard methacholine (MCh) challenge before and then 3 and 12 months after HSCT (n=16 and n=13, respectively). Another 6 patients were challenged before and 3 months after HSCT using a modified challenge to investigate the effect of deep inhalations.

No patient developed bronchiolitis obliterans or bronchiolitis obliterans organising pneumonia. At 3 months, forced vital capacity (FVC) was significantly reduced by 0.33±0.55 L, forced expiratory volume in one second (FEV_1) by 0.31±0.50 L, total lung capacity (TLC) by 0.39±0.37 L and single-breath diffusing capacity of the lung for carbon monoxide ($D_{L,CO}$) by 15±12%. At 12 months, TLC decreased by 0.43±0.36 L and $D_{L,CO}$ by 8±8%. With standard challenge, no significant changes in FEV_1 response to MCh were observed after HSCT but FVC decreased significantly less after HSCT compared with prior to HSCT, suggesting less air trapping. With modified challenge, deep inhalations reversed the MCh-induced decrease in partial expiratory flow more after HSCT compared with before HSCT and this correlated with TLC decrements.

In conclusion, an increase in airway responsiveness is unlikely after haematopoietic stem cell transplantation, at least in patients without pulmonary complications, and mechanisms opposing airway narrowing may blunt the bronchoconstrictor response.

KEYWORDS: Deep inhalations, haematological malignancies, lung restriction, methacholine

Patients undergoing allogeneic haematopoietic stem cell transplantation (HSCT) are susceptible to developing severe pulmonary complications [1, 2], including bronchiolitis obliterans and bronchiolitis obliterans organising pneumonia (BOOP). The former has a reported incidence of 0–48% and results in a purely obstructive functional abnormality at late onset (around 1 yr post-HSCT), whereas the latter is rare (<2%) and characterised by an early (usually within the first 100 days) restrictive abnormality associated with a reduction of single-breath diffusing capacity of the lung for carbon monoxide ($D_{L,CO}$) [3].

Prospective studies of patients undergoing HSCT have shown that lung function changes also occur independently of the development of BOOP or bronchiolitis obliterans [4–6]. Collectively, these studies have shown consistent reductions of forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), total lung capacity (TLC) and $D_{L,CO}$, thus suggesting the development of a restrictive disorder possibly due to the concomitant treatments. An increase in airway responsiveness to methacholine (MCh) was occasionally reported either before [7, 8] or after [8] HSCT. The clinical relevance of airway hyperresponsiveness in transplant recipients may vary depending on its underlying mechanism. In lung transplant recipients it occurs frequently [9–12] and has been regarded as a risk factor for the development of bronchiolitis obliterans [11, 12], possibly reflecting an early derangement of airway mechanics. Alternatively, airway hyperresponsiveness may be the consequence of breathing at low lung volume, thus reflecting a reduced elastic load on a normally behaving airway smooth muscle.

The present prospective study aimed to investigate whether changes in airway responsiveness occur in patients undergoing HSCT. Moreover, as bronchial responsiveness is the result of both

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airway smooth muscle contractility and mechanical modulation of airway narrowing [13, 14], a standard MCh challenge was first used to assess the airway response by FEV1 and FVC followed by a modified challenge to evaluate the bronchodilator effect of deep inhalations using a parameter of airway calibre not preceded by full lung inflation.

METHODS

Subjects characteristics

Between 2004 and 2007, 23 Caucasian patients undergoing allogeneic HSCT (sourcing from bone marrow) for haematological malignancies were studied (table 1). All patients were in stable clinical conditions at the time of study and none had a history of bronchial asthma, chronic obstructive pulmonary disease, and/or other significant respiratory disease. The study protocol was approved by the local Ethics Committee (Genova, Italy) and all patients gave written informed consent.

Clinical data and conditioning regimen

The patient's underlying disease state included acute myeloid leukaemia, non-Hodgkin's malignant lymphoma and other conditions. All patients received a myeloablative treatment including either total body irradiation (TBI) $\leqslant$12 Gy or a non-TBI-based regimen. They were prepared with a conventional conditioning regimen including cyclophosphamide, cyclosporine A, methotrexate, and appropriate antibiotic prophylaxis. To prevent graft-*versus*-host disease (GvHD), cyclosporine A (6–10 mg·kg^{-1} daily) was continued for $\geqslant$1 yr and 17 patients also received antithymocyte globulin [15]. High-resolution computed tomography (HRCT) of the chest was used to assess for signs of bronchiolitis obliterans and/or BOOP. The diagnosis and staging of acute and chronic GvHD were established using clinical, histological and laboratory criteria [16]. Patients diagnosed with acute GvHD were treated with prednisolone (2 mg·kg^{-1} daily) for five consecutive days.

TABLE 1 Main anthropometric and clinical characteristics of study subjects

Male/female	16/7
Age yrs	39±11
Height m	1.73±0.09
BMI kg·m^{-2}	25.5±4.7
Smoking status current/former/never	8/2/13
Haematological disease	
Acute myeloid leukaemia	9
Non-Hodgkin's malignant lymphoma	5
Other	9
Haematological treatments	
TBI during myeloablative conditioning period yes/no	12/11
CsA level at 3 months post-HSCT ng·mL^{-1}	152±128
HRCT signs of BO or BOOP	0
GvHD acute/chronic	4/6

Data are presented as absolute numbers or mean±SD. BMI: body mass index; TBI: total body irradiation ($\leqslant$12 Gy); CsA: cyclosporine A; HSCT: haematopoietic stem cell transplantation; HRCT: high-resolution computed tomography; BO: bronchiolitis obliterans; BOOP: bronchiolitis obliterans organising pneumonia; GvHD: graft-*versus*-host disease.

Lung function measurements

Standard spirometry and flow-volume curves (FEV1 and FVC) were obtained using a mass flowmeter (VIASYS-SensorMedics Inc., Yorba Linda, CA, USA) and numerical integration of the flow signal, according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations [17]. Airway resistance (R_{aw}) was measured by whole body plethysmograph (V62J; VIASYS-SensorMedics Inc.), while the subject was panting at a frequency slightly >1.5 Hz. Immediately after each R_{aw} measurement, thoracic gas volume (TGV) was obtained by panting against a closed shutter at a frequency ranging from 0.5 to slightly <1.0 Hz, and specific airway conductance was calculated as $1/(TGV \cdot R_{aw})$. Functional residual capacity (FRC) was corrected for the difference between TGV and the end-expiratory volume of the four to six preceding tidal breaths. After the opening of the shutter, the subject resumed tidal breaths and at the end of one tidal breath performed a maximum slow expiration followed closely by a maximum inspiration allowing measurement of TLC and residual volume (RV). TLC was obtained by adding the inspiratory vital capacity to RV. The measurements were performed according to the ATS/ERS recommendations [18]. The $D_{L,CO}$ was measured (Vmax22D; VIASYS-SensorMedics Inc.) and the predicted values were adjusted for the effective blood haemoglobin concentration (g·dL^{-1}) obtained closest to the time the measurement of $D_{L,CO}$ was performed [19]. Quality control of lung function measurements was regularly made according to the ATS/ERS recommendations [17–19]. All predicted values for spirometry, lung volumes and $D_{L,CO}$ were obtained from QUANJER *et al.* [20] for the Caucasian European population.

Partial flow-volume curves were obtained and superimposed at constant absolute lung volume by measuring TGV and then asking the subject to expire forcefully from end-tidal inspiration to RV immediately after the reopening of the shutter. In each subject, partial forced expiratory flow (V'_{part}) was always measured at the same absolute lung volume of 30–40% of the pre-HSCT baseline FVC, depending on the largest change in RV after MCh inhalation [21].

Aerosol generation and delivery

Airway responsiveness was tested by MCh challenge using a dosimeter method. Solutions of MCh of 0.2 and 1% were prepared by adding 3 mL of distilled water to dry powder MCh chloride (Laboratorio Farmaceutico Lofarma, Milano, Italy). Aerosols were generated and delivered *via* a DeVilbiss 646 nebuliser (DeVilbiss Health Care Inc., Somerset, PA, USA) attached to a KoKo (Rosenthal-French) breath-activated dosimeter (Ferraris, Louisville, CO, USA), driven by compressed air (30 lb·in^{-2}) with 1-s actuations. Aerosol output at the mouth was 10 μL per actuation. Aerosols were inhaled during quiet tidal breathing in a sitting position.

Experimental procedures

All pulmonary function tests (PFTs) and bronchial challenges pre-HSCT were obtained before each conditioning regimen was started and out of acute GvHD episodes.

Standard MCh challenge study

Approximately 1 week before, and 3–12 months after HSCT, 17 patients were challenged with a standard incremental MCh

protocol. After 20 tidal inhalations of saline as a control, subjects inhaled increasing doses of MCh until a decrease of $FEV_1 \geqslant 20\%$ of control was achieved. Increasing MCh doses from 20, 40, 80, 160, 300, 600, 1,200 and 2,400 μg were obtained by using two MCh concentrations (2.0 $mg \cdot mL^{-1}$ and 10 $mg \cdot mL^{-1}$) with appropriate numbers of tidal breaths (one to 24). The delay time between serial inhalations (*i.e.* from the start of one dose to the start of the next) ranged between 30 and 60 s. FVC and FEV_1 were measured once at each step and the dose of MCh causing a reduction of FEV_1 by 20% (provocative concentration causing a 20% fall in FEV_1 (PD_{20})) was determined by interpolating between two adjacent points of log dose-response curve. To overcome the difficulty arising when a subject's FEV_1 failed to drop by 20%, the slope of the relationship of percentage reduction in FEV_1 on the incremental log-transformed doses of the MCh was used as an index of response [22]. The occurrence of air trapping was inferred by submitting the absolute values (L) of both FVC and FEV_1 measured at all steps of MCh challenge to a simple linear regression analysis (least square best fitting). In this approach, any decrease in slope or increase in y-intercept of FVC *versus* FEV_1 represents an attenuation of air trapping (absolute increase in RV) for a given degree of induced bronchoconstriction (absolute decrease in FEV_1) and *vice versa* [23, 24].

Modified MCh challenge study

Approximately 1 week before and 3 months after HSCT, six patients who did not participate in the standard study were challenged with three increasing MCh doses (600, 1,200 and 2,400 μg) inhaled during quiet tidal breathing (fig. 1). After each dose, each patient was asked for the occurrence of respiratory discomfort before administering the next dose. After baseline measurements of FVC and FEV_1, patients were asked to refrain from taking deep breaths or sighs for 10 min before the first measurement of V'_{part} through to the end of the challenge. All measurements of V'_{part} were then taken once 1 min after the final MCh dose. Following the last dose (2,400 μg) of MCh and V'_{part} measurement, subjects were asked to take five deep inhalations from FRC to TLC during a 30-s period with measurements of V'_{part} taken again 1 min later. The effects of both MCh and deep inhalations on airway calibre were inferred from changes in V'_{part} and quantified using a relaxation index (RI).

$$RI = ((V'part_{DIs} - V'part_{MCh})/V'part_{Bas}) \quad (1)$$

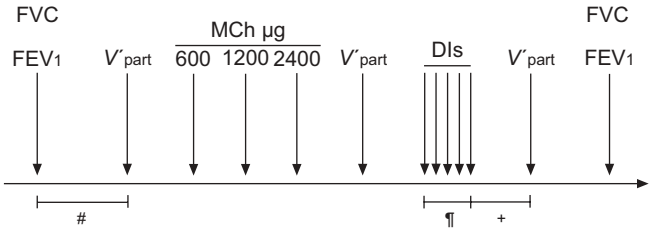

FIGURE 1. Design of modified methacholine (MCh) challenge study. FVC: forced vital capacity; FEV_1: forced expiratory volume in one second; V'_{part}: partial forced expiratory flow; DIs: deep inhalations. #: 10-min deep breath prohibition; ¶: 30 s; +: 1 min.

Where $V'part_{DIs}$, $V'part_{MCh}$ and $V'part_{Bas}$ are the forced expiratory partial flows measured at the end of MCh challenge following DIs, at the end of MCh challenge, and at baseline, respectively.

Statistical analysis

Differences between groups were assessed for significance by an unpaired t-test. Changes within groups were tested by one- or two-factor repeated measures ANOVA with Duncan's *post-hoc* comparisons and Pearson's correlation coefficient. PD_{20} values were log-transformed before analysis. When an FEV_1 fall of <20% was recorded after the last 2,400 μg MCh dose, this value was retained as the PD_{20}. Values of $p<0.05$ were considered statistically significant. Data are presented as means±SD.

RESULTS

None of the patients participating in the present study showed radiological signs of bronchiolitis obliterans and/or BOOP on chest HRCT. At 3 months, nine patients showed the presence of transient mono-segmental consolidation, suggestive of Aspergillus infection. In addition, 10 patients showed the presence of acute (n=4) or chronic (n=6) GvHD, especially skin changes and sicca syndrome, with eyes and mouth dryness. At baseline, PFTs were within the predicted normal range (tables 2 and 3), without differences between studies ($p>0.10$ for all comparisons). When the patients of both groups were considered together, there was a tendency ($p=0.073$) for TLC to decrease more at 3 months in those receiving than in those not receiving the TBI-based conditioning regimen (10±8 and 5±6%, respectively).

Standard MCh challenge study

In the group of patients studied by standard MCh challenge, there was a mild yet statistically significant absolute decrement of FVC (0.33±0.55 L; $p=0.030$), FEV_1 (0.31±0.50 L; $p=0.026$) and TLC (0.39±0.37 L; $p=0.0007$) from baseline to 3 months. The latter also showed a reduction of similar magnitude (0.43±0.36 L; $p=0.007$) at 12 months post-HSCT. A significant reduction of $D_{L,CO}$ was observed at 3 and 12 months post-HSCT (15±12%; $p=0.0002$ and 8±8%; $p=0.048$, respectively).

Before HSCT, five patients showed a cumulative PD_{20} <800 μg (148–720 μg) indicating mild-to-borderline airway hyperresponsiveness to MCh, three subjects responded to doses of 800–2,400 μg, and nine subjects did not respond to 2,400 μg. Mean PD_{20} tended to increase from baseline to 12 months ($p=0.064$), and in five subjects PD_{20} increased by more than one doubling dose. Moreover, mean slopes of FEV_1 *versus* MCh log-dose at 3 or 12 months were not significantly different from before HSCT ($p=0.33$ and $p=0.21$, respectively). Nevertheless, the decrease of FVC for any given reduction of FEV_1 was less after HSCT, as pointed out by the significantly lower slopes of absolute values (L) of FVC *versus* FEV_1 ($p=0.010$ and $p=0.008$ at 3 and 12 months, respectively) and a higher *y*-intercept at 12 months ($p=0.014$; fig. 2). These results are suggestive of less air trapping for a given degree of MCh-induced bronchoconstriction.

Modified MCh challenge study

At 3 months after HSCT, the decrease of TLC was quantitatively but not significantly greater than that observed in

TABLE 2 Baseline lung function data of standard methacholine (MCh) challenge study

Parameters	Pre-HSCT[#]	Post-HSCT	
		3 months[¶]	12 months[+]
FVC L	4.78±0.99	4.45±0.84[§]	4.55±1.21
% pred	113±15	108±16	107±14
FEV1 L	3.96±0.80	3.65±0.75[f]	3.72±0.96
% pred	111±16	106±15	104±14
FEV1/FVC	0.81±0.08	0.81±0.07	0.82±0.08
TLC L	6.44±1.10	6.04±1.0[##]	5.99±1.36[¶¶]
% pred	104±9	100±11	98±11
FRC L	2.93±0.45	3.02±0.57	3.01±0.83
% pred	94±6	97±11	97±20
RV L	1.56±0.39	1.55±0.35	1.42±0.48
% pred	90±19	87±16	82±22
sGaw L·s^{-1}·cmH$_2$O^{-1}	0.21±0.04	0.21±0.03	0.23±0.04
% pred	92±14	91±10	95±16
DL,CO mL·min^{-1}·mmHg^{-1}	26.1±6.21	19.7±4.89	23.2±6.10
% pred for Hb	90±14	75±14[++]	80±16[§§]
MCh FEV1			
PD20 log μg	3.09±0.39	3.19±0.37	3.23±0.24
Slope units	-0.17±0.10	-0.15±0.10	-0.15±0.11

Values are expressed as mean±SD. HSCT: haematopoietic stem cell transplantation; FVC: forced vital capacity; % pred: % predicted; FEV1: forced expiratory volume in one second; TLC: total lung capacity; FRC: functional residual capacity; RV: residual volume; sGaw: specific airway conductance; DL,CO: diffusing capacity of the lung for carbon monoxide; Hb: haemoglobin; PD20: provocative dose (log-transformed) of MCh causing a 20% fall in FEV1. [#]: n=17; [¶]: n=16; [+]: n=13; [§]: p=0.030; [f]: p=0.026; [##]: p=0.0007; [¶¶]: p=0.004; [++]: p=0.0002; [§§]: p=0.048 (all *versus* baseline). 1 L·s^{-1}·cmH$_2$O^{-1} =10.2 L·s^{-1}·kPa^{-1}; 1 mL·min^{-1}·mmHg^{-1}=0.335 mmol·min^{-1}·kPa^{-1}.

TABLE 3 Baseline lung function data of modified methacholine challenge study

Parameters	Pre-HSCT[#]	3 months post-HSCT[¶]
FVC L	5.26±0.94	4.71±1.04
% pred	109±5	98±17
FEV1 L	4.14±0.79	3.67±0.76
% pred	104±10	93±17
FEV1/FVC	0.79±0.04	0.78±0.05
TLC L	7.00±1.14	6.36±1.37[+]
% pred	99±5	89±11
FRC L	3.25±0.57	3.25±0.79
% pred	95±14	97±21
RV L	1.74±0.40	1.65±0.41
% pred	87±9	83±18
sGaw L·s^{-1}·cmH$_2$O^{-1}	0.22±0.02	0.21±0.04
% pred	94±13	90±16
DL,CO mL·min^{-1}·mmHg^{-1}	24.7±5.11	19.0±4.36
% pred for Hb	82±14	68±19[§]

Values are mean±SD. HSCT: haematopoietic stem cell transplantation; FVC: forced vital capacity; % pred: % predicted; FEV1: forced expiratory volume in one second; TLC: total lung capacity; FRC: functional residual capacity; RV: residual volume; sGaw: specific airway conductance; DL,CO: diffusing capacity of the lung for carbon monoxide; Hb: haemoglobin. [#]: n=6; [¶]: n=6; [+]: p=0.053; [§]: p=0.016 (all *versus* baseline).

patients participating in the standard challenge study (0.85±0.57 L *versus* 0.39±0.37 L, respectively; p=0.23), although it failed to achieve the pre-set level of statistical significance with respect to pre-HSCT values (p=0.053). DL,CO was reduced by an extent that was similar to that observed in the standard MCh challenge study (20±13%; p=0.016 *versus* pre-HSCT).

The decrease of V'_{part} induced by the cumulative MCh dose of 4,200 μg was similar before and after HSCT (p=0.95). Repeated deep inhalations taken after MCh reversed the reduction of V'_{part} significantly after (p=0.020) but not before HSCT (p=0.79); this difference in the effect of deep inhalations was statistically significant (p=0.043 for the interaction term; fig. 3). The relaxant effect of deep inhalations after HSCT was significantly correlated (r=0.88; p=0.021) with the per cent reduction of TLC (fig. 4).

DISCUSSION

The main findings of the present study are that: 1) TLC and DL,CO decreased after HSCT, confirming previous studies; 2) airway responsiveness to MCh as assessed by standard challenge did not change; and 3) the ability of deep inhalations to reverse induced bronchoconstriction was enhanced after HSCT and this correlated with the reduction in TLC.

Previous studies have documented the occurrence of mild lung restriction [4, 8] and reduction of DL,CO [4–6, 8] developing within 3–6 months after HSCT, even in the absence of BOOP. In line with previous studies, a mild reduction of TLC and DL,CO was found at 3 months after HSCT without HRCT signs suggestive of BOOP, such as patchy consolidation, ground-glass attenuation and/or nodular opacities.

Airway hyperresponsiveness was previously investigated in two studies. Before HSCT, airway hyperresponsiveness to MCh was reported in five out of 25 patients by RODRIGUEZ-ROISIN *et al.* [8] and 11 out of 53 patients by KROWKA *et al.* [7]. In the present study, five out of 23 patients showed mild-to-borderline airway hyperresponsiveness. All these figures are within the range of reported prevalence of airway hyperresponsiveness in the general population [25]. Therefore, it cannot be concluded that airway hyperresponsiveness is a feature of haematological malignancies requiring HSCT. In the study by RODRIGUEZ-ROISIN *et al.* [8], five patients developed airway hyperresponsiveness after HSCT. This finding is not confirmed by the results of the present study. The current authors recognise that the sample size of the present study is rather small, which could have resulted in a type II statistical error (acceptance of a false ''null hypothesis''). However, as PD20 tended to increase after HSCT while changes in FEV1 *versus* MCh log-dose were far from the level of statistical significance, it is unlikely that inclusion of additional patients would have yielded results similar to those of RODRIGUEZ-ROISIN *et al.* [8].

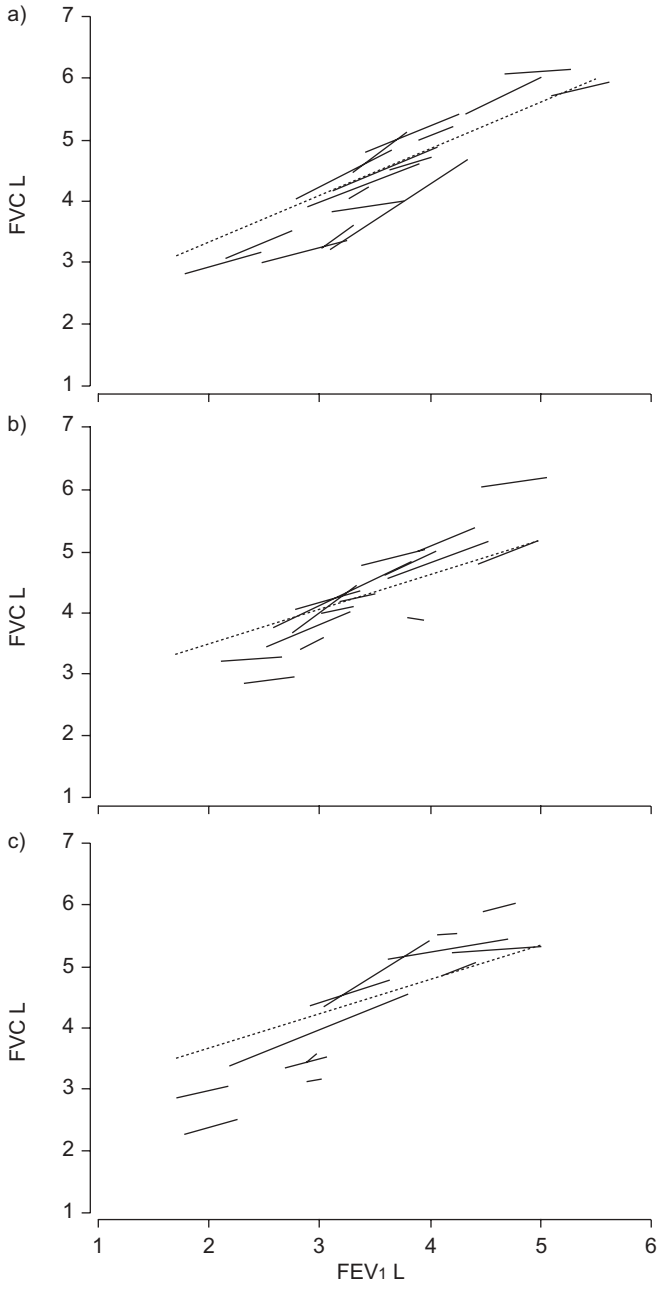

FIGURE 2. Individual (——) and mean (· · · · ·) regression lines of absolute values (L) of forced vital capacity (FVC) *versus* forced expiratory volume in one second (FEV_1) during standard methacholine challenge a) before and b) after 3 and c) 12 months from haematopoietic stem cell transplantation (HSCT). The following equations show the mean slopes and *y*-intercepts ± SD. For the regression line, the right and left ends correspond to the FEV_1 and FVC values at baseline and at maximum response, respectively. a) $y=(0.76\pm0.34)\ x + 1.81\pm1.38$; n=17. b) $y=(0.56\pm0.36)\ x + 2.38\pm1.26$; n=16; p=0.010 for the *y*-intercept *versus* pre-HSCT values. c) $y=(0.56\pm0.35)\ x + 2.54\pm1.37$; n=13; p=0.008 for the slope and p=0.014 for the *y*-intercept both *versus* pre-HSCT.

Apart from the different challenge protocols, other factors may explain the discrepancies between the current and the previous study [8]. Approximately three quarters of the present patients received antithymocyte globulin for GvHD prophylaxis and a

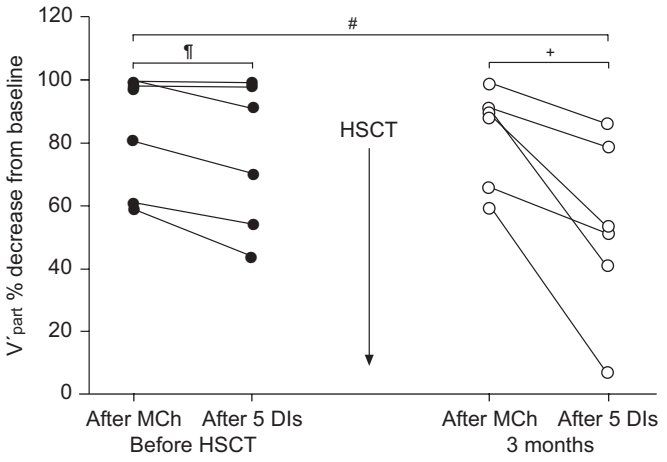

FIGURE 3. Relaxant effect of deep inhalations (DIs) during modified methacholine (MCh) challenge study before and 3 months after haematopoietic stem cell transplantation (HSCT). Per cent decrease of partial forced expiratory flow (V'_{part}) from baseline after both MCh (4,200 μg, cumulative) and MCh plus 5 DIs. #: p=0.043; ¶: p=0.79; +: p=0.020.

myeloablative conditioning regimen consisting of cyclophosphamide was used and followed by cyclosporine A/methotrexate after HSCT in all patients, whereas in the previous study [8] only two patients received cyclosporine A after HSCT. Moreover, TBI was included in the myeloablative conditioning regimen in about half of the present patients but in the vast majority of those in the study by RODRIGUEZ-ROISIN *et al.* [8]. Whether these different treatments may have affected airway hyperresponsiveness is a matter of speculation. The effects of cyclosporine A on airway smooth muscle are controversial. In animal models of asthma, cyclosporine A ablated hyperresponsiveness [26], but did not affect the contraction of isolated nonsensitised rat bronchial smooth muscle [27]. In vascular smooth muscle cells, cyclosporine A decreases proliferation and increases apoptosis [28]. Whether

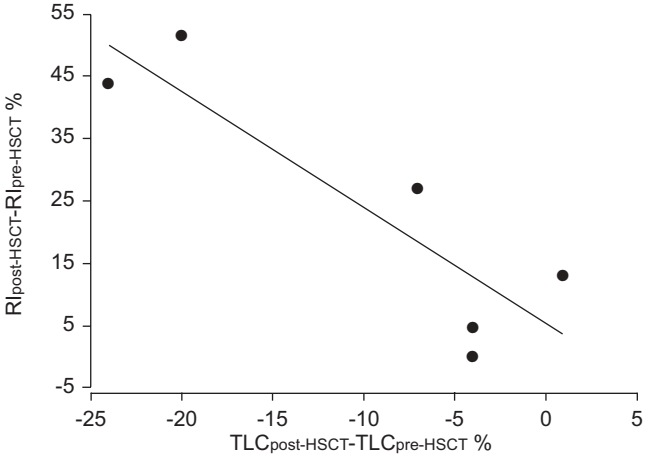

FIGURE 4. Relationship between per cent decrease of total lung capacity (TLC) and increase in relaxant effect of deep inhalations during modified methacholine challenge after haematopoietic stem cell transplantation (HSCT). RI: relaxation index. r=0.88; p=0.021.

similar effects may occur in humans is unknown. An increase of airway responsiveness after irradiation was reported in isolated perfused rat lungs [29], but no data *in vivo* are available. In the present study, no difference between before and after HSCT was observed in the response to MCh when deep inhalations were avoided (modified MCh challenge study) suggesting that the contractile response of airway smooth muscle was unmodified. Collectively, these data make it unlikely that the different changes in airway responsiveness between the current and the previous study [8] are due to different treatment regimens. Other reasons for increased airway responsiveness in transplanted patients are post-HSCT infectious and noninfectious lung complications. In the study by RODRIGUEZ-ROISIN *et al.* [8], six patients had pneumonia of different aetiology and 20 out of 25 acute and/or chronic GvHD.

Recent studies have shown that airway responsiveness is modulated by the mechanical interdependence between airways and lung parenchyma [30, 31]. The present authors have found that, for any given decrease of FEV_1, the FVC decreased less after HSCT compared with before HSCT, suggesting that, for a given level of airway smooth muscle activation, less air trapping occurred [23, 24]. A similar finding was observed in asthmatic patients with low-dose inhaled corticosteroids, which was attributed to a decrease in the thickness of peripheral airway walls [24]. Moreover, reduced air trapping may reflect a greater stability of peripheral airways, possibly due to an increased load on their walls. The results of the current modified MCh challenge showing a greater bronchodilator effect of deep inhalations, as assessed by V'_{part}, tend to support the latter mechanism.

The bronchodilator effect of deep inhalations is proportional to the magnitude of airway distension (strain) [32] and, in turn, to the magnitude of change in lung volume [33]. In the present study, the increased ability of deep inhalations to reverse bronchoconstriction was correlated with the magnitude of the decrease in TLC, suggesting that stress on airway walls was increased despite a reduced lung volume expansion. Although it must be kept in mind that a significant correlation does not prove a definite causality, this finding and the reduced air trapping might suggest that sub-clinical interstitial fibrosis with increased lung elastic recoil may have occurred in these patients thus opposing airway narrowing.

Previous studies have documented the occurrence of airway hyperresponsiveness after lung or heart-lung transplantation [9–12]. Possible explanations for post-transplant airway hyperresponsiveness included denervation hypersensitivity, epithelial damage or changes in mucus properties and clearance, decreased baseline airway calibre, disruption of lymphatic channels or lung perfusion and effects of drugs [9, 10]. In this context, the lack of increase in airway responsiveness after HSCT would suggest that transplantation by itself and the associated treatments are not a cause of airway hyperresponsiveness, whereas organ-specific mechanisms may play a major role in lung transplantation.

In conclusion, the results of the present study suggest that an increase of airway responsiveness is unlikely to occur after haematopoietic stem cell transplantation, at least in patients without pulmonary complications, and mechanisms opposing airway narrowing may blunt the response to constrictor agents.

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