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

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RUNNING HEAD

Airway responsiveness and haematopoietic stem cell transplantation

ABSTRACT

Changes in lung volumes occur following haematopoietic stem cell transplantation (HSCT). Airway hyperresponsiveness was occasionally reported, without mechanistic explanation.

We studied 17 patients by standard methacholine (MCh) challenge before, and then 3 (n=16) and 12 (n=13) months after HSCT. 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 (BOOP). At 3 months, forced vital capacity (FVC) was significantly reduced by 0.33±0.55 L, 1-s forced expiratory volume (FEV_1) by 0.31±0.50 L, total lung capacity (TLC) by 0.39±0.37 L and single-breath diffusing capacity ($D_{L,CO}$) 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 than before HSCT, suggesting less air trapping. With modified challenge, deep inhalations reversed MCh-induced decrease in partial expiratory flow more after than before HSCT and this correlated (r=0.88) with TLC decrements.

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

KEY WORDS: haematological malignancies, lung restriction, deep inhalations, methacholine

INTRODUCTION

Patients undergoing allogeneic haematopoietic stem-cell transplantation (HSCT) are susceptible to develop severe pulmonary complications [1, 2], including bronchiolitis obliterans and bronchiolitis obliterans organising pneumonia (BOOP). The former has a reported incidence range of 0-48% and results in a purely obstructive functional abnormality at late onset (around 1 year 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 carbon monoxide lung diffusing capacity ($D_{L,CO}$) [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 showed consistent reductions of forced vital capacity (FVC), 1-s forced expiratory volume (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 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.

This prospective study was aimed at investigating whether changes in airway responsiveness occur in patients undergoing HSCT. Moreover, as bronchial responsiveness is the result of both airway smooth muscle contractility and mechanical modulation of airway narrowing [13, 14], we first used a standard MCh challenge in which the airway response was assessed by FEV_1 and FVC and then a

modified challenge in which the bronchodilator effect of deep inhalations was evaluated by using a parameter of airway calibre not preceded by full lung inflation.

METHODS

Subjects characteristics

Between 2004 and 2007, twenty-three Caucasian patients undergoing allogeneic HSCT (sourcing from bone marrow) for haematological malignancies were studied (Table 1). They were in stable clinical conditions at the time of study and none had history of bronchial asthma, chronic obstructive pulmonary disease, and/or other significant respiratory disease. The study protocol was approved by the local Ethics Committee 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) ≤12 Gy or a non-TBI-based regimen. They were prepared with conventional conditioning regimen including cyclophosphamide, cyclosporine A, methotrexate, and appropriate antibiotic prophylaxis. To prevent graft-versus-host disease (GvHD), cyclosporine A (6 to 10 $mg \cdot kg^{-1}$ daily) was continued for at least 1 year 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 by using clinical, histological, and laboratory criteria [16]. Patients diagnosed with acute GvHD were treated with prednisolone (2 $mg \cdot kg^{-1}$ daily) for 5 consecutive days.

Lung function measurements

Standard spirometry and flow-volume curves (FVC and FEV_1) were obtained by using a mass flowmeter (VIASYS-SensorMedics Inc., Yorba Linda, CA) and numerical integration of the flow signal, according to the ATS/ERS recommendations [17]. Airway resistance (Raw) was measured by whole body plethysmograph (V62J, VIASYS-SensorMedics Inc., Yorba Linda, CA, USA), while the subject was panting at a frequency slightly >1.5 Hz. Immediately after each Raw 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 (sGaw) was calculated as 1/(TGV·Raw). 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 of which performed a maximum slow expiration soon followed 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., Yorba Linda, CA, USA) and the predicted values were adjusted for the effective blood Hb concentration ($g \cdot dL^{-1}$) 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 those from QUANJER *et al.* [20] for 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 ($\dot{V}_{part}$) was always measured at the same absolute lung volume between 30 and 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 $lb \cdot in^{-2}$) 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 one week before, and 3-12 months after HSCT, seventeen 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 $FEV_1 \geq 20\%$ 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/mL and 10 mg/mL) with appropriate numbers of tidal breaths (from 1 to a maximum of 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% ($PD_{20}FEV_1$) 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%, we also used as an index of response the slope of the relationship of the percentage reduction in FEV_1 on the incremental log-transformed doses of the MCh [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 one week before and 3 months after HSCT, six patients who did not participate in standard study were challenged with three increasing MCh doses (600, 1,200, and 2,400 μg) inhaled during quiet tidal breathing (Figure 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 lasting from 10-min before the first measurement of $\dot{V}_{part}$ through the end of the challenge. All measurements of $\dot{V}_{part}$ were then taken once at 1-min after the final MCh dose. Following the last dose (2,400 μg) of MCh and $\dot{V}_{part}$ measurement, subjects were asked to take five deep inhalations from FRC to TLC during a 30-s period with measurements of $\dot{V}_{part}$ taken again 1-min later. The effects of both MCh and deep inhalations (DIs) on airway calibre were inferred from changes in $\dot{V}_{part}$ and quantified using a *relaxation index,* $RI = [(\dot{V}_{part\,DIs} - \dot{V}_{part\,MCh})/\dot{V}_{part\,Bas}]$, where $\dot{V}_{part\,DI}$, $\dot{V}_{part\,MCh}$, and $\dot{V}_{part\,Bas}$ 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 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. $PD_{20}FEV_1$ values were log-transformed before analysis. When an FEV_1 fall <20% was recorded after the last 2,400 MCh dose, this value was retained

as the $PD_{20}FEV_1$. Values of $p<0.05$ were considered statistically significant. Data are presented as means ± SD.

RESULTS

None of the patients participating in either study showed radiological signs of bronchiolitis obliterans and/or BOOP on chest HRCT. Nine patients demonstrated at 3 months 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 (10±8%) than in those not receiving (5±6%) TBI-based conditioning regimen.

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 showed a reduction of similar magnitude (0.43±0.36 L; $p=0.007$) also 12 months post-HSCT. A significant percent reduction of $D_{L,CO}$ was observed at 3 (15±12%; $p=0.0002$) and at 12 months (8±8%; $p=0.048$) post-HSCT.

Before HSCT, five patients showed a cumulative $PD_{20}FEV_1$ <800 μg (ranging from 148 to 720 μg) indicating mild-to-borderline airway hyperresponsiveness to MCh, three subjects responded to doses ranging from 800 to 2,400 μg, and nine subjects did not respond to 2,400 μg. Mean $PD_{20}FEV_1$ tended to increase from baseline to 12 months ($p=0.064$) and in five subjects $PD_{20}FEV_1$ 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) (Figure 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 patients participating in the standard challenge study (0.85±0.57 L *versus* 0.39±0.37 L, respectively; p=0.23), though it failed to achieve the pre-set level of statistical significance with respect to pre-HSCT values (p=0.053). $D_{L,CO}$ 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 $\dot{V}_{part}$ 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 $\dot{V}_{part}$ 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) (Figure 3). The relaxant effect of deep inhalations after HSCT was significantly correlated (r=0.88, p=0.021) with the percent reduction of TLC (Figure 4).

DISCUSSION

The main findings of this study are that 1) TLC and $D_{L,CO}$ 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 $D_{L,CO}$ [4-6, 8] developing within 3-6 months after HSCT, even in the absence of BOOP. In line with the above studies, we have found a mild reduction of TLC and $D_{L,CO}$ 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 5 of 25 patients by RODRIGUEZ-ROISIN *et al.* [8] and 11 of 53 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 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. We recognise that the sample size of this study is rather small, which could have resulted in a Type II statistical error (acceptance of a false "null hypothesis"). However, as $PD_{20}FEV_1$ tended to increase after HSCT while changes in FEV_1 *versus* MCh log-dose were far from 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].

Apart from the different challenge protocols, other factors may explain the discrepancies between our and the previous study [8]. About 3/4 of our patients received antithymocyte globulin for GvHD prophylaxis and a 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 our patients but in the vast majority of those of 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 non-sensitised rat bronchial smooth muscle [27]. In vascular smooth muscle cells, cyclosporine A decreases proliferation and increases apoptosis [28]. Whether 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 our 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 non-infectious lung complications. In RODRIGUEZ-ROISIN *et al.* [8] study, six patients had pneumonia of different aetiology and 20 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]. We have found that, for any given decrease of FEV_1, the FVC decreased less after than 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, a reduced air trapping may reflect a greater stability of peripheral airways, possibly due to an increased load on their walls. The results of our 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 this study suggest that an increase of airway responsiveness is unlikely to occur after HSCT, at least in patients without pulmonary complications, and mechanisms opposing airway narrowing may blunt the response to constrictor agents.

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REFERENCES

1. Kotloff RM, Ahya VN, Crawford SW. Pulmonary complications of solid organ and haematopoietic stem cell transplantation. *Am J Respir Crit Care Med* 2004; 170: 22-48.
2. Parimon T, Madtes DK, Au DH, Clark JG, Chien JW. Pretransplant lung function, respiratory failure, and mortality after stem cell transplantation. *Am J Respir Crit Care Med* 2005; 172: 384-390.
3. Soubani AO, Uberti JP. Bronchiolitis obliterans following haematopoietic stem cell transplantation. *Eur Respir J* 2007; 29: 1007-1019.
4. Gore EM, Lawton CA, Ash RC, Lipchik RJ. Pulmonary function changes in long-term survivors of bone marrow transplantation. *Int J Radiation Oncology Biol Phys* 1996; 36: 67-75.
5. Fanfulla F, Locatelli F, Zoia MC, *et al.* Pulmonary complications and respiratory function changes after bone marrow transplantation in children. *Eur Respir J* 1997; 10: 2301-2306.
6. Cerveri I, Fulgoni P, Giorgiani G, *et al.* Lung function abnormalities after bone marrow transplantation in children. *Chest* 2001; 120: 1900-1906.
7. Krowka MJ, Staats BA, Hoagland HC. A prospective study of airway reactivity before bone marrow transplantation. *Mayo Clinic Proc* 1990; 65: 5-12.
8. Rodriguez-Roisin R, Roca J, Grañena A, Agustí AGN, Marin P, Rozman C. Lung function in allogeneic bone marrow transplantation recipients. *Eur Respir J* 1989; 2: 359-365.
9. Glanville AR, Burke CM, Theodore J, *et al.* Bronchial hyperresponsiveness after human cardiopulmonary transplantation. *Clin Sci* 1987; 73: 299-303.
10. Maurer JR, McLean PA, Cooper JD, *et al.* Airway hyperreactivity in patients undergoing lung and heart/lung transplantation. *Am Rev Respir Dis* 1989; 139: 1038-1041.
11. Stanbrook MB, Kesten S. Bronchial hyperreactivity after lung transplantation predicts early bronchiolitis obliterans. *Am J Respir Crit Care Med* 1999; 160: 2034-2039.

12. Reid DW, Walters EH, Johns DP, *et al.* Bronchial hyperresponsiveness and the bronchiolitis obliterans syndrome after lung transplantation. *J Heart Lung Transplant* 2005; 24: 489-492.
13. Skloot G, Permutt S, Togias A. Airway hyperresponsiveness in asthma: a problem of limited smooth muscle relaxation with inspiration. *J Clin Invest* 1995; 96: 2393-2403.
14. Brusasco V, Crimi E, Barisione G, Spanevello A, Rodarte JR, Pellegrino R. Airway responsiveness to methacholine: effects of deep inhalations and airway inflammation. *J Appl Physiol* 1999; 87: 567-573.
15. Bacigalupo A, Lamparelli T, Bruzzi P, *et al.* Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomised studies from Gruppo Italiano Trapianti Midollo Osseo (GIMTO). *Blood* 2001; 98: 2942-2947.
16. Koc S, Leisenring W, Flowers MED, *et al.* Therapy for chronic graft-versus-host disease: a randomised trial comparing cyclosporine plus prednisone versus prednisone alone. *Blood* 2002; 100: 48-51.
17. Miller M, Hankinson J, Brusasco V, *et al.* Standardisation of spirometry. *Eur Respir J* 2005; 26: 319-338.
18. Wanger J, Clausen JC, Coates A, *et al.* Standardisation of the measurement of lung volumes. *Eur Respir J* 2005; 26: 511-522.
19. MacIntyre N, Crapo RO, Viegi G, *et al.* Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J* 2005; 26: 720-735.
20. Quanjer PhH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Standardised lung function testing. *Eur Respir J* 1993; 6: 1-99.
21. Crimi E, Pellegrino R, Milanese M, Brusasco V. Deep breaths, methacholine, and airway narrowing in healthy and mild asthmatic subjects. *J Appl Physiol* 2002; 93: 1384-1390.

22. O'Connor G, Sparrow D, Taylor D, *et al.* Analysis of dose response curves to methacholine. *Am Rev Respir Dis* 1987; 136: 1412-1417.

23. Gibbons WJ, Sharma A, Lougheed D, Macklem PT. Detection of excessive bronchoconstriction in asthma. *Am J Respir Crit Care Med* 1996; 153: 582-589.

24. Corsico A, Pellegrino R, Zoia MC, Barbano L, Brusasco V, Cerveri I. Effects of inhaled steroids on methacholine-induced bronchoconstriction and gas trapping in mild asthma. *Eur Respir J* 2000; 15: 687-692.

25. Jansen DF, Timens W, Kraan J, Rijcken B, Postma DS. (A)Symptomatic bronchial hyper-responsiveness and asthma. *Respir Med* 1997; 91: 121-134.

26. Diasio RB, LoBuglio AF. Immunomodulators: immunosuppressive agents and immunostimulants. *In:* Hardman JG, Limbird LE, eds. Goodman & Gilman's: The Pharmacological Basis of Therapeutics. 9th Edn. New York, McGraw-Hill, 1996; pp. 1297-1299.

27. Tavakoli R, Daly RC, McGregor CG, Frossard N. Cyclosporine does not affect in vitro bronchial smooth muscle contractions in treated Lewis rats. *J Heart Lung Transplant* 1994; 13: 520-524.

28. Bonnet S, Rochefort G, Sutendra G, *et al.* The nuclear factor of activated T cells in pulmonary arterial hypertension can be therapeutically targeted. *Proc Natl Acad Sci USA* 2007; 104: 11418-11423.

29. Shields GW, Sheller JR, Newman JH, *et al.* Airway responsiveness in isolated perfused rat lungs: effect of thoracic irradiation. *Respir Physiol* 1989; 78: 135-144.

30. Ding DJ, Martin JA, Macklem PT. Effect of lung volume on maximal methacholine-induced bronchoconstriction in normal humans. *J Appl Physiol* 1987; 62: 1324-1330.

31. Bates JH, Schuessler TF, Dolman C, Eidelman DH. Temporal dynamics of acute isovolume bronchoconstriction in the rat. *J Appl Physiol* 1997; 82: 55-62.

32. Noble P, McFawn PK, Mitchell HW. Responsiveness of the isolated airway during simulated deep inspirations: effect of airway smooth muscle stiffness and strain. *J Appl Physiol* 2007; 103: 787-795.

33. Salerno FG, Pellegrino R, Trocchio G, Spanevello A, Brusasco V, Crimi E. Attenuation of induced bronchoconstriction in healthy subjects: effects of breathing depth. *J Appl Physiol* 2005; 98: 817-821.

Table 1. Subjects' main anthropometric and clinical characteristics

Gender, M/F	16/7
Age, yr	39 ± 11
Stature, m	1.73 ± 0.09
BMI, $kg \cdot m^{-2}$	25.5 ± 4.7
Smoking status, C/F/N	8/2/13
Haematological disease	
Acute myeloid leukaemia	9
Non-Hodgkin's malignant lymphoma	5
Other	9
Haematological treatments	
TBI during myeloablative conditioning period (Y/N)	12/11
CsA level at 3 months post-HSCT, ng/mL	152 ± 128
HRCT signs of BO or BOOP	0
GvHD, acute/chronic	4/6

Values are absolute numbers or means ± SD. M/F: male/female; BMI: body mass index; C/F/N: current/former/never; TBI: total body irradiation (≤12 Gy); Y/N: yes/no; CsA: cyclosporine A; HRCT: high resolution computed tomography; BO: bronchiolitis obliterans; BOOP: bronchiolitis obliterans organising pneumonia; GvHD: graft-versus-host disease.

Table 2. Baseline lung function data of standard MCh challenge study

Parameters	**Pre-HSCT** ***n*=17**	**3 months post-HSCT** ***n*=16**	**12 months post-HSCT** ***n*=13**
FVC, L	4.78 ± 0.99	4.45 ± 0.84*	4.55 ± 1.21
% predicted	113 ± 15	108 ± 16	107 ± 14
FEV_1, L	3.96 ± 0.80	3.65 ± 0.75†	3.72 ± 0.96
% predicted	111 ± 16	106 ± 15	104 ± 14
FEV_1/FVC	0.81 ± 0.08	0.81 ± 0.07	0.82 ± 0.08
TLC, L	6.44 ± 1.10	6.04 ± 1.01‡	5.99 ± 1.36 §
% predicted	104 ± 9	100 ± 11	98 ± 11
FRC, L	2.93 ± 0.45	3.02 ± 0.57	3.01 ± 0.83
% predicted	94 ± 6	97 ± 11	97 ± 20
RV, L	1.56 ± 0.39	1.55 ± 0.35	1.42 ± 0.48
% predicted	90 ± 19	87 ± 16	82 ± 22
sGaw, $L \cdot s^{-1} \cdot cmH_2O^{-1}$	0.21 ± 0.04	0.21 ± 0.03	0.23 ± 0.04
% predicted	92 ± 14	91 ± 10	95 ± 16
$D_{L,CO}$, $mL \cdot min^{-1} \cdot mmHg^{-1}$	26.1 ± 6.21	19.7 ± 4.89	23.2 ± 6.10
% predicted for Hb	90 ± 14	75 ± 14#	80 ± 16¶
MCh FEV_1			
PD_{20}, 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 means ± SD. FVC: forced vital capacity; FEV_1: 1-s forced expiratory volume; TLC: total lung capacity; FRC: functional residual capacity; RV: residual volume; sGaw: specific airway conductance; $D_{L,CO}$: carbon monoxide diffusing capacity of the lung; MCh: methacholine; PD_{20}: dose (log-transformed) of MCh causing a 20% decrease of FEV_1 from control. To convert sGaw to $L \cdot s^{-1} \cdot kPa^{-1}$ and $D_{L,CO}$ to $mmol \cdot min^{-1} \cdot kPa^{-1}$ multiply the relevant term by 10.2 and 0.335, respectively. *: p=0.030; †: p=0.026; ‡: p=0.0007; §: p=0.004; #: p=0.0002; ¶: p=0.048 (all *versus* baseline).

Table 3. Baseline lung function data of modified MCh challenge study

Parameters	**Pre-HSCT** *n*=6	**3 months post-HSCT** *n*=6
FVC, L	5.26 ± 0.94	4.71 ± 1.04
% predicted	109 ± 5	98 ± 17
FEV_1, L	4.14 ± 0.79	3.67 ± 0.76
% predicted	104 ± 10	93 ± 17
FEV_1/FVC	0.79 ± 0.04	0.78 ± 0.05
TLC, L	7.00 ± 1.14	6.36 ± 1.37*
% predicted	99 ± 5	89 ± 11
FRC, L	3.25 ± 0.57	3.25 ± 0.79
% predicted	95 ± 14	97 ± 21
RV, L	1.74 ± 0.40	1.65 ± 0.41
% predicted	87 ± 9	83 ± 18
sGaw, $L \cdot s^{-1} \cdot cmH_2O^{-1}$	0.22 ± 0.02	0.21 ± 0.04
% predicted	94 ± 13	90 ± 16
$D_{L,CO}$, $mL \cdot min^{-1} \cdot mmHg^{-1}$	24.7 ± 5.11	19.0 ± 4.36
% predicted for Hb	82 ± 14	68 ± 19†

Values are means ± SD. Abbreviations are the same as those in Table 3. *: p=0.053; †: p=0.016 (all *versus* baseline).

LEGENDS

Figure 1. Design of modified methacholine (MCh) challenge study. FVC: forced vital capacity; FEV_1: 1-s forced expiratory volume; $\dot{V}_{part}$: partial forced expiratory flow; DIs: deep inhalations.

Figure 2. Individual (*thin*) and mean (*thick*) regression lines of absolute values (L) of forced vital capacity (FVC) *versus* 1-s forced expiratory volume (FEV_1) during standard methacholine challenge before (*top*) and after 3 (*middle*) and 12 months (*bottom*) from haematopoietic stem cell transplantation (HSCT). The equations show the mean slopes and y-intercepts with standard deviations (in brackets). For each regression line, the *right* and *left* ends correspond to the FEV_1 and FVC values at baseline and at maximum response, respectively. *: p=0.010; †: p=0.008; ‡p=0.014 *versus* pre-HSCT values.

Figure 3. Relaxant effect of deep inhalations (DIs) during modified methacholine (MCh) challenge study before and 3 months after haematopoietic stem cell transplantation (HSCT). On y-axis is displayed the percent decrease (%) of partial forced expiratory flow ($\dot{V}_{part}$) from baseline after both MCh (4,200 μg, cumulative) and MCh plus 5 DIs.

Figure 4. Relationship between percent decrease of total lung capacity (TLC) and increase in relaxant effect of deep inhalations (DIs) during modified methacholine (MCh) challenge after haematopoietic stem cell transplantation (HSCT). RI: relaxation index (see text).

Figure 1.

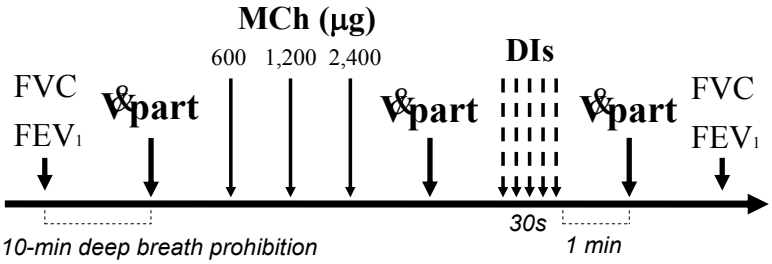
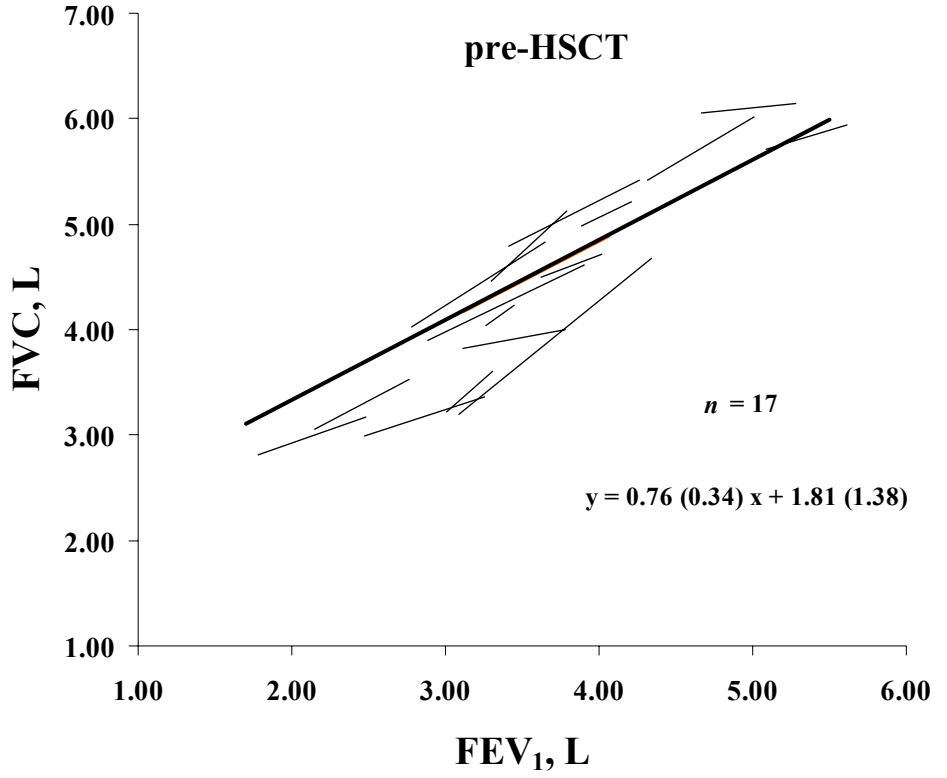
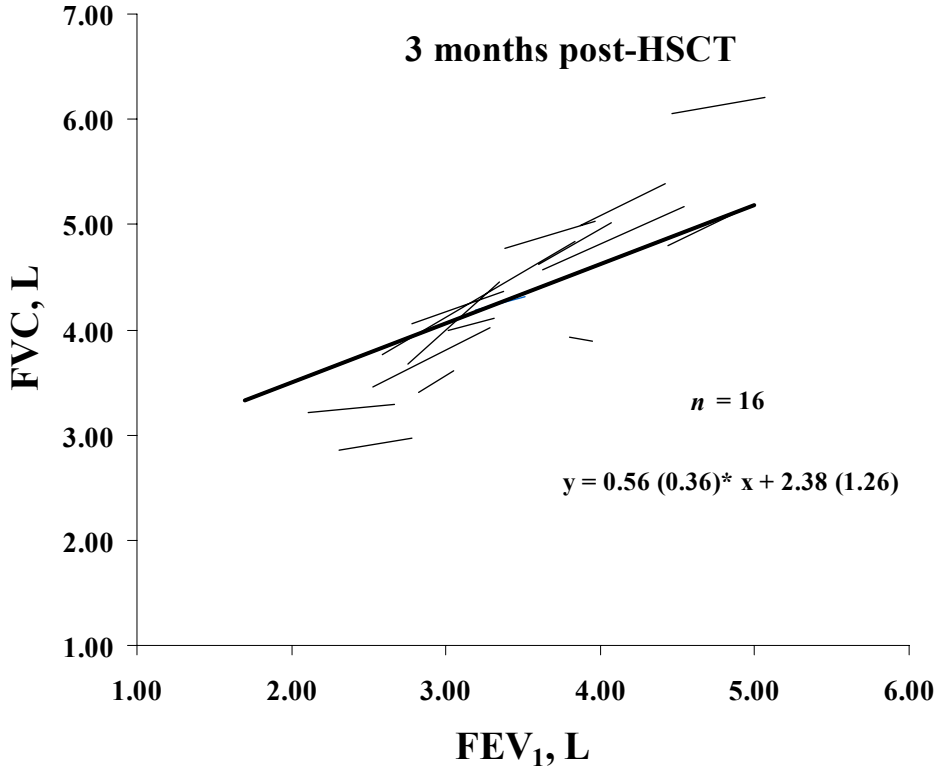

MCh (μg)
600
1,200
2,400
DIs
FVC
FEV1
V̇part
V̇part
V̇part
FVC
FEV1
30s
10-min deep breath prohibition
1 min

Figure 2.


pre-HSCT
FVC, L
FEV1, L
n = 17
y = 0.76 (0.34) x + 1.81 (1.38)
7.00
6.00
5.00
4.00
3.00
2.00
1.00
1.00
2.00
3.00
4.00
5.00
6.00


3 months post-HSCT
FVC, L
FEV1, L
n = 16
y = 0.56 (0.36)* x + 2.38 (1.26)
7.00
6.00
5.00
4.00
3.00
2.00
1.00
1.00
2.00
3.00
4.00
5.00
6.00

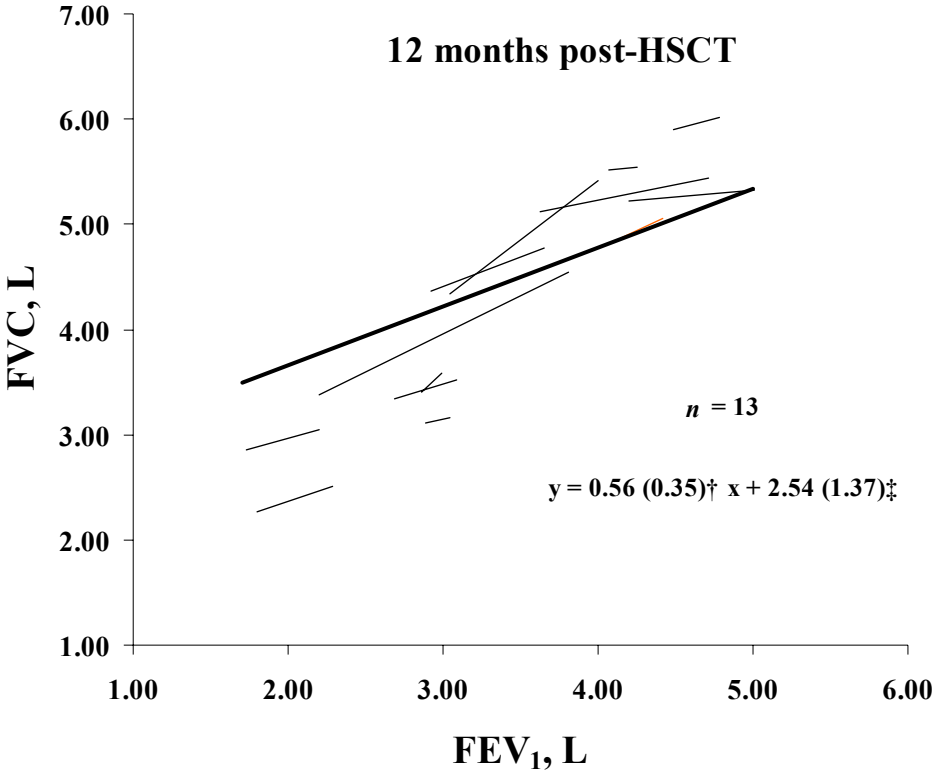

12 months post-HSCT
7.00
6.00
5.00
4.00
3.00
2.00
1.00
FVC, L
1.00
2.00
3.00
4.00
5.00
6.00
FEV1, L
n = 13
y = 0.56 (0.35)† x + 2.54 (1.37)‡

Figure 3

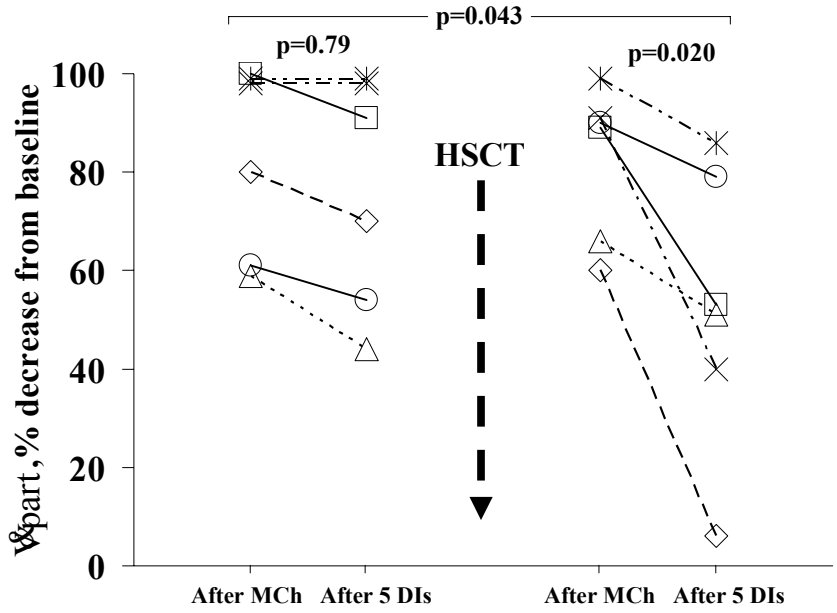
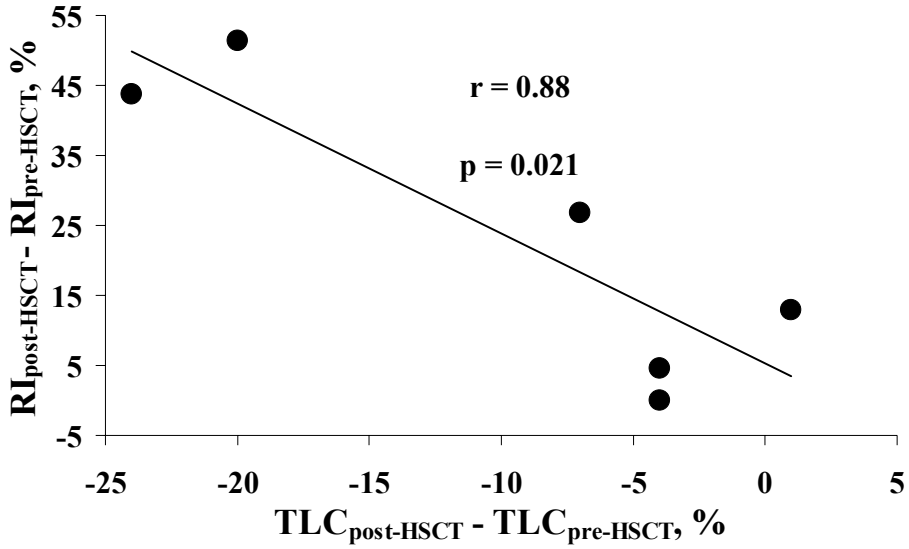

p=0.043
p=0.79
p=0.020
HSCT
100
80
60
40
20
0
FVC, % decrease from baseline
After MCh
After 5 DIs
After MCh
After 5 DIs

Figure 4.


55
45
35
25
15
5
-5
-25
-20
-15
-10
-5
0
5
r = 0.88
p = 0.021
RIpost-HSCT - RIpre-HSCT, %
TLCpost-HSCT - TLCpre-HSCT, %