Impact of Cotransplantation of Mesenchymal Stem Cells on Lung Function After Unrelated Allogeneic Hematopoietic Stem Cell Transplantation Following Non-Myeloablative Conditioning

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Background. In the context of hematopoietic stem cell transplantation (HSCT), mesenchymal stem cells (MSC) have been used to promote engraftment and prevent graft-versus-host disease. However, in animal models, MSC were shown to cause pulmonary alterations after systemic administration. The impact of MSC infusion on lung function has not been studied in humans. The objective of the study was to investigate the impact of MSC co-infusion on lung function and airway inflammation as well as on the incidence of pulmonary infections and cytomegalovirus (CMV) reactivation after HSCT.

Methods. We have prospectively followed 30 patients who underwent unrelated HSCT with MSC co-infusion after non-myeloablative conditioning (NMA). Each patient underwent detailed lung function testing (FEV_1, FVC, FEV_1/FVC, RV, TLC, DLCO, and KCO) and measurement of exhaled nitric oxide before HSCT and 3, 6, and 12 months posttransplant. The incidence of pulmonary infections and CMV reactivation were also monitored. This group was compared with another group of 28 patients who underwent the same type of transplantation but without MSC co-infusion.

Results. Lung function tests did not show important modifications over time and did not differ between the MSC and control groups. There was a higher 1-year incidence of infection, particularly of fungal infections, in patients having received a MSC co-infusion. There was no difference between groups regarding the 1-year incidence of CMV reactivation.

Conclusions. MSC co-infusion does not induce pulmonary deterioration 1 year after HSCT with NMA conditioning. MSC appear to be safe for the lung, but close monitoring of pulmonary infections remains essential.

Keywords: MSC, Stem cell transplantation, Lung function, Exhaled nitric oxide.

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Mesenchymal stem cells (MSC) are pluripotent cells derived from the bone marrow or other sources, which have stimulated a high level of enthusiasm in recent years for their potential therapeutic use.

In the context of allogeneic hematopoietic stem cell transplantation (HSCT), MSC cotransplantation appears to be safe (*1, 2*). These cells could have a role in engraftment promotion (*3*) and graft-versus-host disease (GVHD) prevention (*4*) through their immunosuppressive activity. Because

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of their lack of major histocompatibility complex molecule expression, MSC are weakly immunogenic in humans, allowing administration to patients without HLA matching (*5*).

However, in some animal models, MSC infusion has been shown to cause pulmonary alterations. Indeed, after IV injection in mice, the cells are trapped within the pulmonary capillaries, thereby causing embolism (*6*). Moreover, mouse MSC could differentiate into tumor cells in lungs after systemic administration (*7*) and could promote tumor development (*8*). Another study in mice disclosed differentiation of MSC in the lung after irradiation, depending on the infusion timing (*9*): MSC differentiated into functional lung cells if injected at an early stage or into cells involved in fibrosis if injected once chronic inflammation and fibrosis had started. Furthermore, Salazar et al. showed evidence of a mitogenic potential of human and mouse MSC on lung fibroblasts in vitro (*10*). In contrast, other papers have shown that, in response to injury caused by endotoxin or bleomycin, MSC migrated to the lung, decreased tissue damage, and improved lung repair (*11, 12*). In addition, in rats with chronic obstructive lung disease, intra-tracheal MSC administration restored lung function (*13*). These observations may result from the ability of MSC to secrete paracrine growth factors and cytokines able to decrease inflammation. Moreover, the ability of MSC to differentiate into functional cells may be a key in promoting adequate lung repair. MSC are also suggested to be able to attenuate oxidative stress in inflammatory lung diseases induced by previous irradiation or by subclinical pathogen colonization in a context of immunosuppression (*14*).

The impact on the lung of MSC co-infusion after HSCT has not been studied so far in humans. In this study, we monitored the evolution of lung function, the value of exhaled nitric oxide (FeNO), and the occurrence of pulmonary infections and cytomegalovirus (CMV) reactivation in patients who underwent HSCT with MSC co-infusion. We also investigated the impact of MSC co-infusion on CMV reactivation and pulmonary infections in univariate and multivariate Cox models adjusted for competing risks.

It is widely accepted that performing pulmonary function tests (PFT) before and after transplantation is crucial to detect early signs of pulmonary complications. Lung function assessment before transplantation usually serves as baseline reference to evaluate changes after HSCT (*15*). We followed 30 patients who received HSCT for hematological malignancies from unrelated donors and a co-infusion of MSC after non-myeloablative conditioning (NMA). The results were compared with those of 28 patients who received the same type of transplant but without MSC. Pulmonary function parameters, including airway flow rates, lung volumes, and diffusing capacity (DLCO), as well as FeNO value, were measured before HSCT as well as 3, 6, and 12 months posttransplantation. FeNO is a noninvasive marker of airway inflammation used to monitor rejection after lung transplantation (*16*), but its utility has not been really assessed after HSCT.

RESULTS

At baseline, all patients displayed normal spirometric and lung volume values but a slight impairment of diffusing capacity. The median FeNO value was within the accepted normal range for both patient groups (*17*).

MSC co-infusion had no detrimental effect on lung function indices when expressed as predicted percentages (see **Table S1, SDC,** http://links.lww.com/TP/A951). When expressed as absolute value (data not shown), MSC co-infusion was even associated with a slight improvement in FEV1 (from 2.7±0.9 L at baseline to 2.9±0.9 L at 3 months, $P<0.05$) and FVC (from 3.6±1.1 L at baseline to 3.8±1.1 at 6 months, $P<0.05$) at some time points, which contrasted with the decrease in DLCO seen at 1 year in the group without MSC co-infusion (from 6.3±1.8 at baseline to 5.8±1.1 at 1 year, $P<0.05$).

The other lung parameters (FEV1/FVC, KCO, TLC, RV, and FeNO) were not significantly changed after 3, 6, or 12 months in any of the patient groups and did not show differences between groups when expressed as predicted percentages or absolute values.

The 1-year cumulative incidence of pulmonary infection appeared higher in the MSC group compared to the control group ($P<0.01$; see **Table 1** and **Figure S1A, SDC,** http://links.lww.com/TP/A951). Infection etiologies showed a trend for a higher rate of fungal infections in the MSC group (6 vs. 1; $P=0.06$). In contrast, the cumulative incidence of CMV reactivation did not show any difference between groups (see **Figure S1B, SDC,** http://links.lww.com/TP/A951). It should be emphasized that patients in the MSC group were all

TABLE 1. Cumulative incidence of aGVHD, cGVHD, CMV reactivation, secondary neutropenia, and pulmonary infection (including etiology)

	MSC group n=30	Controls n=28	*P*
1-Yr cumulative incidence of grade II–IV acute GVHD (%)	30	36	0.6
1-Yr cumulative incidence of chronic GVHD (%)	67	52	0.14
Moderate-severe GVHD (%)	46	33	0.3
1-Yr cumulative incidence of CMV reactivation (%)	40	43	0.76
1-Yr cumulative incidence of neutropenia (%)	14	4	0.17
1-Yr cumulative incidence of pulmonary infection (%)	48	15	0.0074
Unknown origin (n=)	9	3	0.12
Fungal (n=)	6	1	0.06
Viral (n=)	1	0	0.35
Bacterial (n=)	3	2	0.71
Total (n=)	19	6	0.02

GVHD, graft versus host disease; CMV, cytomegalovirus.

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TABLE 2. Patient characteristics

	MSC group n=30	Controls n=28	*P*
Age, yr	54±13	57±11	0.40
BMI	25±4	26±4	0.54
Gender (M/F)	21/9	12/16	0.04
Tobacco habits (n/ex/cs)	12/8/10	8/13/7	0.29
Comorbidities (HSCT-CI score)	2 (0–9)	3 (0–7)	0.16
Underlying malignancy			
HL	2	2	0.83
NHL	6	4	
CLL	2	3	
ALL	1	2	
CML	0	1	
AML	11	10	
MPD	0	1	
MM	7	5	
PLL	1	0	
Disease risk (*42*): low/standard/high	7/17/6	7/16/5	0.98
No. cells transplanted ($\times 10^6$/kg)			
$CD34^+$ cells	4.3 (1.0–11.7)	6.1 (2.6–14.5)	0.05
$CD3^+$ cells	271 (92–540)	320 (140–598)	0.24
Patient/donor compatibility			
10/10 HLA identical (allelic level)	0	28	<0.0001
Other	30	0	
Female donor-to-male recipient	9	2	0.03
Other sex combinations	21	26	
Aspergillosis before HSCT	5	3	0.51
CMV serologic status, recipient-donor			
Negative-negative	5	9	0.19
Negative-positive	3	6	
Positive-negative	12	6	
Positive-positive	8	5	

Results are expressed as mean±SD except for HCT-CI score and number of $CD3^+$ and $CD34^+$ cells transplanted expressed as median (range).

BMI, body mass index; n, non-smoker; ex, ex-smoker; cs, current smoker; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; CLL, chronic lymphocytic leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; AML, acute myeloid leukemia; MPD, myeloproliferative disorder; MM, multiple myeloma; PLL, prolymphocytic leukemia; HSCT, hematopoietic stem cell transplantation; CMV, cytomegalovirus.

HLA-mismatched while controls were 10/10 HLA-matched at the allelic level.

In univariate Cox analysis of the whole cohort (n=58), MSC co-infusion showed a significant association with the occurrence of pulmonary infections (HR=2.96 [1.15–7.60], $P<0.05$) whereas aspergillosis before HSCT and female donor-to-male recipient were borderline significant ($P=0.05$ and $P=0.08$, respectively, see **Table S2, SDC,** http://links.lww.com/TP/A951). After multivariate analysis adjusting for aspergillosis before HSCT, female donor-to-male recipient, and MSC co-infusion, there was only a trend for an association between MSC and pulmonary infections ($P=0.09$). The only significant risk factor was aspergillosis before HSCT (HR: 3.19 [1.08–9.43], $P<0.05$).

In univariate analysis, recipient (HR=7.78 [2.31–26.3], $P=0.0009$) and donor (HR=2.28 [1.03–5.03], $P=0.04$) CMV serostatus, but not MSC co-infusion, predicted for CMV reactivation, whereas age ($P=0.07$) and female donor-to-male recipient ($P=0.09$) were borderline significant. In multivariate analysis, the only parameters that appeared significant were the donor and recipient CMV positive status (HR=2.54 [1.10–5.84], $P=0.03$ and HR=7.56 [2.18–26.23], $P=0.001$, respectively) and female donor-to-male recipient was borderline significant (HR=2.25 [0.87–5.81], $P=0.09$) (see **Table S2, SDC,** http://links.lww.com/TP/A951).

DISCUSSION

In this study, we investigated the impact of MSC co-infusion on several clinical and laboratory outcomes of patients who underwent HSCT. To investigate the effect of MSC on the lung, we focused our analysis on pulmonary function monitoring and pulmonary infections.

At 1 year, even if all patients in the MSC group and none in the control group received a graft from HLA-mismatched donors, we did not observe any difference between groups for the incidence of acute GVHD (aGVHD). Indeed, transplantation from HLA-mismatched donors is known to give less favorable outcome because of a higher incidence of GVHD. In addition, the higher proportion of the female-to-male combination, usually associated with a higher risk of chronic GVHD (cGHVD) (*18*), was not correlated with

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a greater incidence of cGVHD in the MSC group. These observations might attest for a protective effect of MSC against GVHD, but this remains to be studied in prospective randomized trials.

The original finding of our study is the fact that patients receiving MSC exhibited no deterioration in lung function indices over the first year of observation. There was even a slight improvement in airway flow rates and vital capacity and no change in lung diffusing capacity in the MSC group, whereas the group without MSC exhibited a significant decrease in diffusion lung capacity reaching on average 10% after 1 year. Although some animal data drew attention to the potential fibrotic effect of MSC (*10*), our observation attests that infused MSC did not result in excessive airway or lung remodeling that could potentially alter lung function.

By contrast, the incidence of pulmonary infections appeared to be higher in the MSC group, which was also HLA-mismatched, compared to the control group that was HLA-matched. This observation could theoretically be linked to the immunosuppressive effects of MSC, GVHD, or CMV reactivation (*19*). However, GVHD and CMV reactivation were not predictive of lung infections, neither in univariate nor in multivariate analyses, but we have no detailed data on immune reconstitution. Likewise, neutropenia, aspergillosis before HSCT, and tobacco status, all recognized risk factors for pulmonary infections, in general (*20–22*) were evaluated, but only a previous episode of aspergillosis before HSCT was significantly predictive. It should be emphasized that these analyses did not investigate the impact of MSC alone but also the combined effect of HLA mismatch and MSC co-infusion, as the two factors were systematically associated. HLA mismatching could have caused a higher incidence of severe GHVD in the MSC group and hence a greater risk of fungal infections (*23*). However, we did not observe any difference between the groups for the incidence of aGVHD and cGVHD. Nevertheless, we cannot exclude that immune reconstitution may have been relatively impaired or delayed in the MSC group because patients were all HLA-mismatched with their donors.

Although MSC have been shown to possess antimicrobial properties, this ability has been essentially observed in vitro or in animal models and in ex vivo models of human lung tissues (*24*). Moreover, this property was mainly described against bacterial, viral, and parasitic infections (*25–27*). Interestingly, we encountered a higher incidence of fungal infections in the MSC group. Although not statistically significant, the impact of MSC could have been significant if we did not have to exclude from the study two cases with aspergillosis because they could not undergo PFT. However, patients receiving MSC were also HLA-mismatched with their donors, which could also favor infection. Forslow et al. also found an association between MSC co-infusion and pneumonia-related death (*28*). The majority of patients had mold-related pneumonia, but authors were not able to prove the relation with MSC co-infusion because of low patient numbers in this study. However, in a further paper, the use of MSC was associated with a higher incidence of invasive fungal infections in case of severe aGVHD (*29*), which is in line with our results.

Contrary to pulmonary infections, MSC co-infusion did not have an impact on CMV reactivation, and this was attested by the fact that the cumulative incidence of CMV reactivation did not differ from the control group. A similar finding was observed in a recent paper by Lucchini et al. (*30*), which demonstrated that MSC did not interfere with antiviral responses in vivo. The only parameters found to be strongly predictive of CMV reactivation in this study were donor and recipient CMV positive status, which are widely recognized as risk factors for such reactivation (*31*).

In conclusion, the main finding of our study is the fact that patients who underwent HSCT with MSC co-infusion after NMA showed no deterioration of lung function over a period of 1 year after transplantation. Nevertheless, longer follow-up in larger groups of patients would be required to formally exclude any lung toxicity of MSC, in particular in relation with cGHVD. However, the pulmonary infection rate (mainly the occurrence of fungal infections) appeared to be increased. This indicates the need for prolonged antifungal prophylaxis and close monitoring of pulmonary infections in patients after HSCT and MSC cotransplantation.

MATERIALS AND METHODS

Subjects

Patients who underwent HSCT after NMA conditioning in the University Hospital Center of Liege between December 2006 and December 2012 were screened for the following inclusion criteria: (1) conditioning with total body irradiation (TBI) 2 Gy and fludarabine, (2) immunosuppression with mycophenolate mofetil (MMF) and tacrolimus, (3) transplantation with peripheral blood stem cells (PBSC), (4) unrelated donor, and (5) minimal follow-up of 100 days. Thirty-eight patients met the inclusion criteria in the MSC group. Among them, eight were excluded because they did not have lung function assessment 3 months after HSCT. Indeed, three patients died before day 100 (one died of graft rejection and sepsis, one of relapse, and one of GVHD and sepsis associated with organ failure). The other five patients were not able to perform lung function tests at day 100 (two had invasive lung aspergillosis, complicated by renal failure in one; two had a relapse associated with severe encephalopathy in one; one had severe GVHD and organ failure). Consequently, 30 patients remained eligible in the MSC group. They took all part in one of two consecutive clinical trials investigating the safety and efficacy of MSC co-infusion at time of HSCT from mismatched unrelated donors after NMA conditioning (*1*). In the non-MSC group, 35 patients also undergoing HSCT after NMA conditioning were included. However, three died before 100 days (two of relapse, one of GVHD associated with sepsis and organ failure), and four did not perform lung function tests because of poor health status (two had a relapse combined with sepsis, one had severe GVHD associated with organ failure, and one had GVHD combined with encephalopathy). Therefore, 28 patients remained eligible in the control group. Characteristics of the patients are presented in Table 2. All patients included in the NMA MSC trial had HLA mismatches (one allele up to two antigens), whereas all controls were 10/10 HLA-matched at allelic level.

MSC were cultured as described previously (*1, 32*). The conditioning regimen consisted of fludarabine 90 mg/m^2, followed by a single dose of 2 Gy TBI administered on day 0 before infusion of cells. MSC were infused first, followed by PBSC infused at least 60 to 120 min later. MMF was administered orally from day 0 through day 42 at the dose of 15 mg/kg three times a day. Tacrolimus was given orally at the dose of 0.06 mg/kg twice a day starting on day −3 until day 180 and then progressively tapered to be definitely discontinued by day 365 in the absence of GVHD. The conditioning regimen and postgrafting immunosuppression used were identical in the MSC and the control groups.

The diagnosis and clinical grading of aGVHD were performed according to standard criteria (*33, 34*). Diagnosis and grading of cGVHD were made using the National Institute of Health consensus criteria (*35*).

All subjects gave written informed consent for participation as well as for collection and analyses of posttransplant data. The MSC clinical trials were approved by the Ethics Committee of the University of Liege.

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Pulmonary Function Assessment

At each time point, subjects underwent a global lung function assessment using a body box plethysmography (Sensormedics, Vmax series 22; Viasyhealthcare, Yorba Linda, CA) allowing to measure flow rates, lung volumes, and diffusion capacity according to ATS/ERS standard criteria (*36–38*). Spirometry (measure of forced expiratory volume in 1 sec: FEV_1 and forced vital capacity) was performed before and after 400 μg inhaled salbutamol metered dose inhaler administered through a Volumatic. Diffusion for carbon monoxide (DLCO) was measured by the single breath washout technique and corrected for the hemoglobin content. FeNO was measured using a chemoluminescence analyzer (NIOX; Aerocrine, Stockholm, Sweden) at a flow rate of 50 mL/sec, in accordance with the recommendation of the ATS/ERS task force (*39*).

Pulmonary Infections and CMV Reactivation

Standard prophylaxis against infections was used (*40*), and disease evaluation was routinely carried out on days 40, 100, 180, and 365. Pulmonary infections were diagnosed based on respiratory symptoms, microbial analysis of bronchoalveolar lavage, and chest radiography or CT scan, or both. Bronchitis leading to hospitalization and pneumonia were recorded. The day of the first CMV reactivation episode, defined as the first viral load greater than 1,000 copies/mL by PCR, was also recorded and positive patients were treated preemptively with ganciclovir. Secondary neutropenia was defined as an episode of at least 2 weeks with absolute neutrophil count less than 500 cells/μL occurring at least 1 month after HSCT.

Statistical Analysis

Categorical parameters were compared using the chi-square test. Comparisons between PFT values before and after HSCT were performed using paired *t* tests or Wilcoxon matched-pair signed rank tests for the FeNO values. Intergroup comparisons were made using unpaired *t* tests or Mann-Whitney tests as appropriate. Cumulative incidences of aGVHD, cGHVD, neutropenia, CMV reactivation, and pulmonary infection were calculated as previously described (*41*), taking death as competing event. The impact of MSC co-infusion (and correlatively of HLA mismatch because of the linkage between the two characteristics) on CMV reactivation and pulmonary infections was assessed in univariate and multivariate Cox models. Factors analyzed in univariate analysis were as follows: patient age, gender, tobacco habits, acute leukemia versus other diagnoses, number of CD34+ cells transplanted, aspergillosis before HSCT, secondary neutropenia, grade II–IV aGVHD, female donor-to-male recipient versus other sex combinations, and MSC co-infusion. CMV reactivation was also assessed as risk factor for pulmonary infection, and pulmonary infection and donor and recipient CMV serostatus were evaluated as risk factors for CMV reactivation. Factors with a *P* value less than 0.10 were then introduced into multivariate analysis. Differences were considered statistically significant when a two-sided *P* value was less than 0.05. Statistical analyses were carried out with GraphPad Prism (GraphPad Software, San Diego, CA) and SAS version 9.3 (SAS Institute, Cary, NC).

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