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Lung function and airway inflammation monitoring after hematopoietic stem cell transplantation

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Summary
Background: Induced sputum is a non-invasive method to investigate airway inflammation, which has been used to assess pulmonary inflammatory diseases. However, this procedure has not been studied in the context of hematopoietic stem cell transplantation (HSCT).
Methods: We monitored lung function in 182 patients who underwent HSCT and measured airway inflammation by sputum induction in 80 of them. We prospectively measured FEV1, FVC, DLCO, KCO, TLC, RV, exhaled nitric oxide (FeNO) as well as sputum cell counts before and 3, 6, 12, 24 and 36 months after HSCT.
Results: For the whole cohort there was a progressive decrease in TLC, which was significant after 3 years ($p < 0.01$). By contrast, there was no change in other lung functions parameters or in FeNO. Baseline sputum analysis revealed increased neutrophil counts in patients {Median (IQR): 63% (38–79)} compared to healthy subjects matched for age {Median (IQR): 49% (17–67), $p < 0.001$} but there was no significant change in any type of sputum cell counts over the three years. When comparing myeloablative (MA) vs non-myeloablative (NMA) conditioning, falls in FEV1, FVC and DLCO, and rise in RV and sputum neutrophils were more pronounced over the first year of observation in those receiving MA.
Conclusions: There was a progressive loss in lung function after HSCT, featuring a restrictive pattern. Myeloablative conditioning was associated with early rise of sputum neutrophils

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and greater alteration in lung function over the first year.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) has been proposed to treat haematological malignancies since the 1960s. This type of transplantation requires the administration of high doses of chemotherapy and/or total body irradiation (TBI) with the aim of eradicating malignant cells [1]. In the late 1970, it has been recognized that the allograft itself confers immune-mediated antileukaemic effect termed graft-versus-tumour effects. This observation led to the development of allogeneic HSCT following non-myeloablative conditioning, in which the burden for tumour eradication has been shifted from high-dose radio/chemotherapy towards graft-versus-tumour effects [2].

Unfortunately, pulmonary complications are frequent after HSCT and are a major cause of post-transplant mortality [3]. They can be classified as infectious or non-infectious and as early or late depending on whether they occur before or after 100 days post-transplantation. Among late and non-infectious complications, bronchiolitis obliterans (BO) is characterized by a rapid and progressive decrease in expiratory flow rates and a rise in residual volume. It is admitted that less than 10% of patients develop BO after HSCT [4—7]. Follow-up of pulmonary function tests is critical in recognizing its early stages. The lung function assessment prior to transplantation usually serves as baseline reference to evaluate the changes in pulmonary function after HSCT [8].

Induced sputum is a safe method for recovering bronchial inflammatory cells. It was shown to be useful in the assessment of chronic lung rejection after lung transplantation [9] but it has never been studied in patients undergoing HSCT. A previous study has shown that bronchoalveolar lavage neutrophil counts increased during the first 6 months after HSCT in patients without overt pulmonary complications [10]. Similarly, fraction of exhaled nitric oxide (FeNO), a non-invasive marker of airway inflammation, has been used to monitor lung transplantation rejection [11] but its utility has been poorly assessed after HSCT.

In an attempt to detect modifications at airway level in patients who underwent allogeneic HSCT, we performed pulmonary function tests (PFTs), measured FeNO value, and induced sputum to look at airway cell composition prior to HSCT as well as 3 and 6 months, 1, 2 and 3 years after transplantation.

Material and methods

Subjects

We assessed 182 patients who underwent HSCT for haematological diseases at the University Hospital Center of Liege between January 2006 and October 2011, and who were reassessed 3 and 6 months, 1, 2 and 3 years later for their PFTs and FeNO measurement (Fig. 1). Over 1 year post-transplant, patients were essentially assessed if they had signs of Graft-Versus-Host-Disease (GVHD). The characteristics of these patients are presented in Table 1.

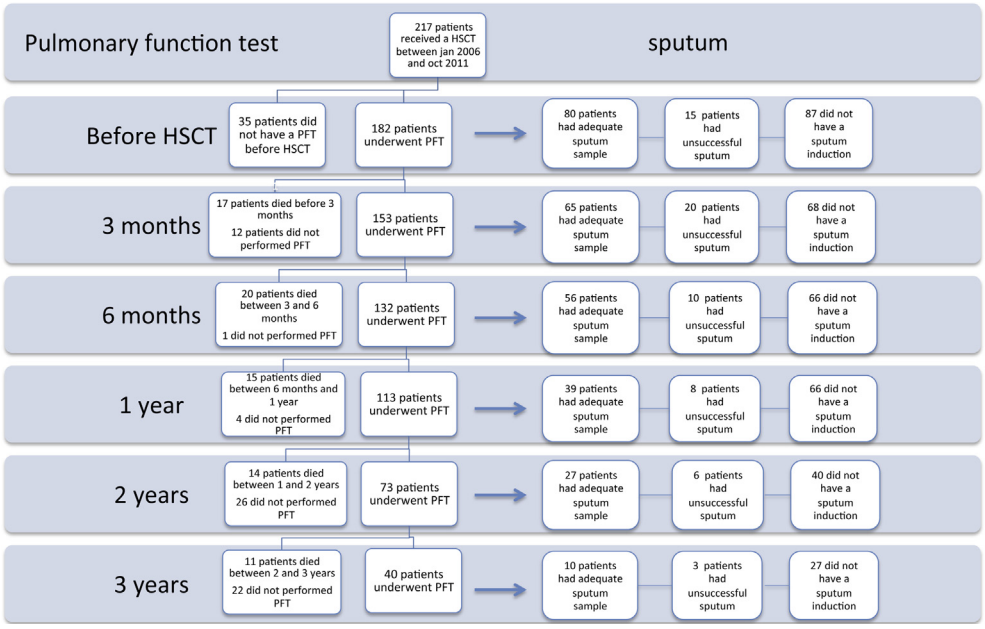

Figure 1 Flow chart of the study population PFT: pulmonary function test, HSCT: hematopoietic stem cell transplantation; unsuccessful sputum means too little material quantity or poor quality sample (squamous cell fraction $>$ 80%) or sputum induction failure. PFT and Sputum were not performed in case of technical staff unavailability or patient poor health status.

Table 1 Demographic characteristics of the patients.

Age (years)	52 ± 13
BMI	25 ± 4
Gender (M/F)	108/74
Tobacco habits (n/ex/cs)	79/65/38
MA/NMA conditioning	49/133
Underlying malignancy	
AA	3
AIHA	1
AML	60
MPD	4
CML	3
MDS	20
ALL	9
HL	6
NHL	35
MM	29
CLL	10
PLL	2
Disease risk: low/standard/high	43/92/47
Donor: Unrelated/related	125/57
Patient/donor compatibility	
10/10 HLA-identical (allelic level)	80
Other	102
Comorbidities (HSCT-CI score)	2 (0–9)
No. of cells transplanted ($\times 10^6$/kg)	
$CD34^+$ cells	5.13 (0.04–15.88)
$CD3^+$ cells	318 (0–1216)

Results are expressed as mean ± SD except for HSCT-CI score and number of $CD3^+$ and $CD34^+$ transplanted expressed as Median (range); BMI = body mass index, *n* = non-smoker, ex = ex-smoker, cs = current smoker, MA = myeloablative conditioning, NMA = non-myeloablative conditioning, AA = aplastic anaemia, AIHA = autoimmune hemolytic anaemia, AML = acute myeloid leukaemia, MPD = myeloproliferative disease, CML = chronic myeloid leukaemia, MDS = myelodysplastic syndrome, ALL = acute lymphoblastic leukaemia, HL = Hodgkin's lymphoma, NHL = non-Hodgkin's lymphoma, MM = multiple myeloma,, CLL = chronic lymphocytic leukaemia, PLL = prolymphocytic leukaemia.

Forty-nine patients underwent a myeloablative-conditioning regimen (MA), with either 8 gray (Gy) single dose or 12 Gy fractionated TBI and high-dose chemotherapy ($n = 43$), or high-dose chemotherapy alone ($n = 6$). GVHD prevention consisted in cyclosporine A or tacrolimus with ($n = 28$) or without ($n = 21$) short methotrexate (15 mg/m^2 on day 1 and 10 mg/m^2 on days 3, 6 and 11), with additional anti-thymocyte globulin (ATG; 45 mg/kg) in 22 of them. One hundred thirty-three patients underwent non-myeloablative conditioning (NMA). Two patients did not receive TBI but a chemotherapy associating fludarabine (90 mg/m^2) and cyclophosphamide (3000 mg/m^2). The others received a conditioning consisting in low-dose TBI (2 Gy) with ($n = 107$) or without ($n = 24$) fludarabine (90 mg/m^2). Their immunosuppressive regimen associated tacrolimus and mycophenolate mofetil (45 mg/kg from day 0 to day 28 in case of HLA-identical sibling donor or day 42 in case of alternative donor).

Healthy controls ($n = 116$) were recruited by local advertisement in the hospital. None of them exhibited respiratory symptoms and all had normal spirometric results (FEV1 > 80% predicted value) and none had airways hyperresponsiveness (provocative concentration of methacholine causing a fall in FEV1 of 20% > 16 mg/ml). They were well matched with patients undergoing HSCT according to age (53 ± 8 years) and tobacco habits (non-smokers: $n = 60$; ex-smokers: $n = 38$ and current smokers: $n = 18$).

This study was approved by the Ethics Committee of the Faculty of Medicine at the University of Liege and all subjects gave written informed consent for participation.

Methods

Lung function tests

Each subject underwent a global lung function assessment using a body box plethysmography (Sensormedics, Vmax series 22, Viasyhealthcare, Yorba Linda, California, USA) allowing to measure flow rates, lung volumes and diffusion capacity according to ATS/ERS standard criteria [12–14]. Spirometry (measure of Forced Expiratory Volume in 1 s: FEV_1 and Forced Vital Capacity: FVC) was performed before and after 400 μg inhaled salbutamol MDI administered through a Volumatic. Diffusion for carbon monoxide was measured by the single breath wash-out technique and corrected for the blood haemoglobin value. FeNO was measured using a chemoluminescence analyser (NIOX, Aerocrine, Stockholm, Sweden) at a flow rate of 50 ml/s, in accordance with the recommendation of the ATS/ERS task force [15].

Bronchiolitis obliterans syndrome

The international Society for Heart and Lung Transplantation (ISHLT) proposed a clinical description of BO termed *bronchiolitis obliterans syndrome (BOS)* and defined it by pulmonary function changes (fall of FEV1) rather than by histology [16]. In our series we assessed the proportion of patients who satisfied the criteria of BOS stage > 1 according to this classification (fall of FEV1 > 20% from baseline).

Sputum induction and processing

The sputum was induced on the same day after completion of lung function tests by inhalation of hypertonic (4.5%) or isotonic (0.9%) saline solution according to the post-bronchodilation FEV_1 value, as previously described [17]. Ninety-five patients had a sputum induction. Only 80 patients out of 95 produced an adequate sample suitable for cell count analysis (Fig. 1). Failure to get sputum cell count was explained either by unsuccessful sputum induction or by poor quality sample (squamous cell fraction greater than 80%). Cell viability was assessed by trypan blue exclusion and the differential leucocyte count performed on cytospins stained with May-Grünwald-Giemsa on 500 non-squamous cells.

Statistical analysis

Survival analyses were made using the Kaplan–Meier method. The assessment of the distribution normality was made with the Kolmogorov–Smirnov test. Lung function parameters were normally distributed, expressed as mean ± SD (tables) or ± SEM (figures) and compared to baseline using "*t*" tests while sputum cell counts and FeNO, which were not normally distributed, were expressed as median (IQR) and compared to baseline using Wilcoxon rank test or Mann–Whitney test for paired or unpaired data respectively. Bonferroni correction was applied to take into account multiple comparisons, so that only p value < 0.01 at each time point was considered as significant vs baseline. Statistical analyses were performed with Graph Pad Prism 5.0.

Results

Survival analysis

Overall survival at 1, 2 and 3 years after HSCT were 71%, 63% and 56% respectively (Fig. 2).

BMI

The body mass index (BMI) decreased significantly at day 100 compared to before HSCT (25 ± 4; 22 ± 4; $p < 0.0001$) and then begun to increase progressively to return to values close to baseline at 3 years (24 ± 5). Overall, the BMI remained within the normal range at each time point.

Lung function

Unpaired comparisons on the whole cohort shows that, compared to baseline, TLC decreased significantly after 3 years ($p < 0.01$) (Table 2). In contrast, there was no significant change in FEV1, FVC, FEV1/FVC, DLCO, KCO and FeNO over time compared to baseline, this latter remaining in accepted normal range [18]. For patients who provided paired data TLC and RV decreased over time (3 years vs baseline $p < 0.01$ for both) while there was no change in FEV1, FVC, FEV1/FVC, DLCO, KCO and FeNO (Fig. 3). Patients who underwent MA conditioning generally exhibited greater falls in FEV1, FVC and corrected DLCO compared to those receiving NMA conditioning (Fig. 3) (NMA vs MA: FEV1-D100, $p < 0.01$; FVC-D100, $p < 0.01$; D200: $p < 0.01$; DLCO: $p < 0.01$ for D100, D200 and 1 year; RV-D100, $p < 0.01$). Among the one hundred thirteen patients who were followed up for at least one year, only 4 developed BOS based on functional criteria (3.5%). Among them, only 3 had a consistent and persistent fall in lung function indices (2.6%) (Fig. 4).

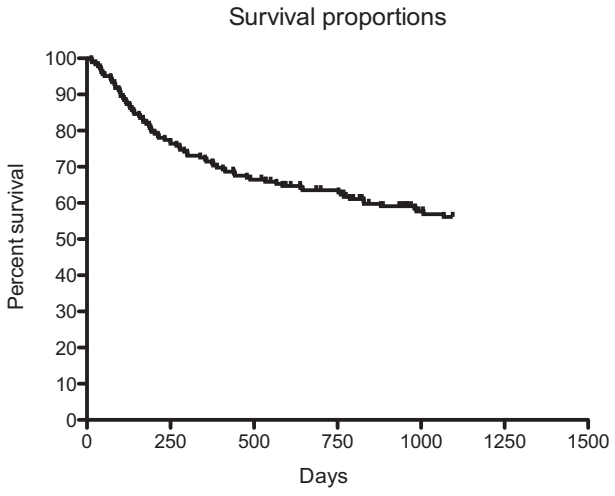

Figure 2 Survival analysis over 3 years of the 182 patients who underwent HSCT.

Sputum cell counts

Baseline sputum cells counts showed a dominant neutrophilic inflammation, which was greater than that seen in healthy subjects matched for age (Fig. 5, $p < 0.001$). Based on unpaired comparisons on the whole cohort, there was no change in total cell counts or in the percentages of different cell types over time (Table 3). Likewise, for patients who provided paired data, there was no significant change in total or in differential cell counts (Fig. 6). However, when examining the variations according to the conditioning regimen, those receiving MA conditioning showed a sharp and early rise in the percentage of neutrophils at 3 months compared to baseline ($P < 0.01$) an observation not found in those receiving NMA conditioning (Fig. 6). In addition, the increase in sputum neutrophils at 3 months was significantly greater after MA compared to NMA ($p < 0.001$) and mirrored by a greater fall in macrophages ($p < 0.01$).

There was no significant correlation between the change in lung function indices and the rise in sputum neutrophil count over the first 12 months even if there was a trend for an inverse correlation between the change in DLCO and the change in sputum neutrophil count at 3 months ($r = -0.31$; $p = 0.052$).

Predictive factors for survival at one year

There was no significant difference in baseline demographic and functional features between those who survive at one year and those who died in the first year of observation (Table 4). Likewise there was no difference with respect to sputum cells counts.

Discussion

The originality of our study is the combination of lung function assessment, including FeNO, with airway inflammation by measuring sputum cell counts in patients who underwent HSCT. Our main findings are the intense neutrophilic airway inflammation at baseline and the progressive appearance of a restrictive lung function defect associated with a persistent airway neutrophilic inflammation after HSCT. By contrast, occurrence of BOS was rare and only found in 3.5% of patients in our series.

Table 2 Lung function test results over time.

	Before HSCT $N = 182$	3 months $N = 153$	6 months $N = 132$	12 months $N = 113$	24 months $N = 73$	36 months $N = 40$
FEV1 pre-BD (% predicted)	89 ± 18	90 ± 18	91 ± 18	89 ± 20	87 ± 17	82 ± 21
FEV1 post-BD (% predicted)	88 ± 17	91 ± 18	91 ± 17	90 ± 22	86 ± 17	83 ± 19
FVC pre-BD (% predicted)	96 ± 17	96 ± 15	98 ± 16	96 ± 19	96 ± 15	91 ± 18
FVC post-BD (% predicted)	96 ± 15	96 ± 16	98 ± 16	97 ± 18	94 ± 17	91 ± 18
FEV1/FVC (%)	76 ± 10	77 ± 10	76 ± 11	76 ± 10	75 ± 10	74 ± 13
FEV1/FVC pots BD (%)	76 ± 10	79 ± 11	77 ± 12	76 ± 12	76 ± 10	76 ± 12
Corrected DLCO (% predicted)	74 ± 25	72 ± 27	72 ± 21	69 ± 19	67 ± 18	65 ± 17
Corrected KCO (% predicted)	90 ± 33	88 ± 28	91 ± 30	88 ± 27	88 ± 24	93 ± 29
TLC (% predicted)	96 ± 14	97 ± 15	96 ± 17	96 ± 16	94 ± 18	87 ± 20*
RV (% predicted)	104 ± 38	108 ± 44	103 ± 41	104 ± 41	100 ± 43	89 ± 35
FE_{NO} (ppb)	18 (12–29)	16 (12–27)	20 (15–35)	20 (12–28)	17 (11–22)	14 (11–23)

Results are expressed as mean ± SD except for FeNO value expressed as median (IQR). FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; DLCO: diffusing capacity of the lung for carbon monoxide; KCO: gas transfer coefficient; TLC: total lung capacity; RV: residual volume; BD: bronchodilation; FeNO: exhaled nitric oxide; * <0.01 vs before HSCT; unpaired comparisons.

At baseline our patients had flow rates, lung volumes and FeNO values within the normal range. By contrast, they showed a slight impairment of diffusing capacity that could reflect peripheral mismatch between ventilation and perfusion of the lung as a consequence of past chemotherapy and radiotherapy. As far as sputum cells are concerned, patients were characterised at baseline by a neutrophilic inflammation compared to figures usually seen in healthy subjects matched for age, although it was to a lesser extent than that seen in severe COPD [19–21]. Neutrophils are cells that easily and readily migrate into the lung in response to chemoattractants released upon airway irritation or injury [22,23]. In our patients, high sputum neutrophil counts at baseline are likely to reflect the impact of heavy treatment before HSCT. The persistence of intense neutrophilic airway inflammation after HSCT is remarkable and could indicate recurrent airways micro-injuries or bacterial colonisation in fragile patients because of their immune suppressive treatment. It also might be a sign of a repair process following aggressive therapy prior to the transplantation.

Our finding of a restrictive pattern that appears in the months and years following HSCT is in line with what most authors reported so far as highlighted in a review article [24]. However, we have to recognise that, in our study, the changes remained quite limited in their magnitude. The restrictive pattern was characterised by a reduced lung volume including TLC with preserved FEV1/FVC ratio. The overall decline in lung volume was progressive and maximal at 3 years. Those receiving NMA conditioning did not show an early decline in the first year, but displayed a similar loss in lung volumes at 3 years compared to those receiving MA conditioning. However, the diffusion capacity was minimally altered after NMA conditioning (5% fall) even after 3 years when compared to MA conditioning where the fall in DLCO was quite marked (exceeding 25% at this time point).

The reason for the appearance of the restrictive pattern in our study is unclear. The fact that most of our patients followed beyond the first year had a GVHD is likely to contribute to this process as GVHD was shown to favour lung restriction after HSCT though the underlying mechanism remains unclear [25]. As BMI remained quite stable we cannot incriminate a cachectic status to explain the loss of lung volumes. One could potentially think of skeletal muscle weakness or sequel of recurrent lung injuries likely to occur in these immuno-compromised patients. On the other hand thoracic irradiation and cytotoxic chemotherapeutic agents are recognized factors contributing to the occurrence of lung fibrosis that may lead to restriction [24]. This probably explains the marked alteration in flow rates and diffusing capacity observed in the first year after HSCT in those patients receiving myeloablative conditioning.

Only less than 5% of patients developed clinically significant BOS, which is in line with a large review on more than 6000 patients [26], but contrasts with what is generally observed after lung transplantation where it can occur in up to 50% of patients surviving beyond 6 months [27].

FeNO has been initially validated as a marker of airway eosinophilic inflammation in asthma [28,29] with thresholds values of 40–50 ppb being indicative of sputum eosinophilia >3%. By contrast, it has been recently shown that FeNO values remained within the normal range in non-eosinophilic asthma including neutrophilic asthma [30]. This exhaled biomarker has also been shown to rise early after lung transplantation complicated by BO [31] but the value of FeNO after HSCT has been poorly investigated so far. In our subjects FeNO values, which were within the normal range [18] at baseline (median 18 ppb), did not change significantly over time. This is not surprising as the airway inflammation in patients undergoing HSCT is rather neutrophilic, a feature that amplifies after transplantation. Our finding contradicts what was recently reported by Enocson A et al. [32] who followed 68 patients for 6 months after HSCT. However, in the latter study the baseline values of FeNO were similar to ours (median 15 ppb) and the change over time, although statistically significant, was of small magnitude with a median only reaching 20 ppb at 6 months. On the other hand, Lahzami et al. did not find any change in FeNO over a one-year

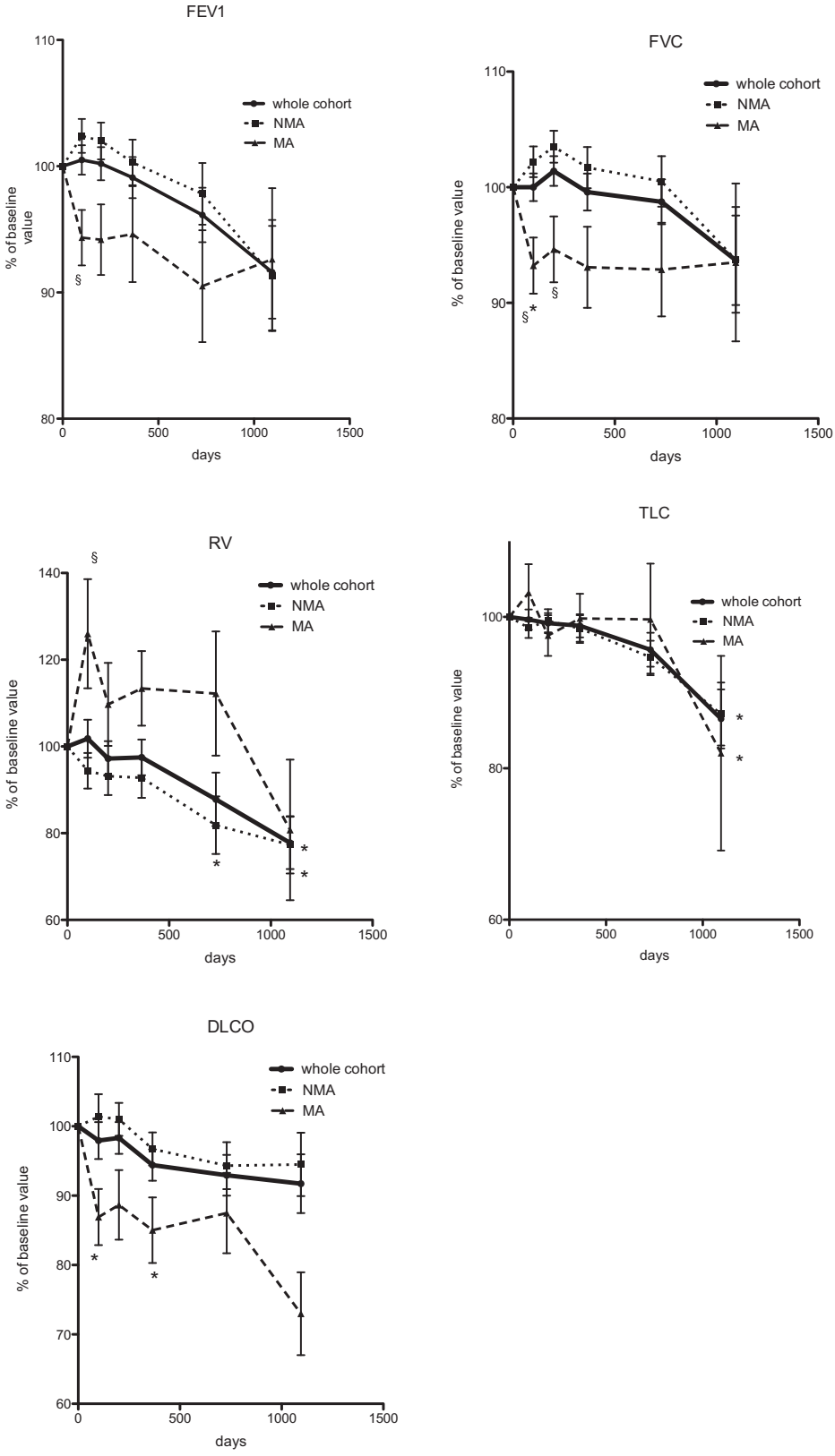

Figure 3 Changes of lung function indices over time in patients who provided paired data. Results are presented as % of baseline value ± SEM; 100% represent the baseline value. The number of patients at each time point is: 152, 133, 113, 70 and 40 for 3, 6, 12, 24 and 36 months, respectively. For NMA conditioning, the number of patients at each time point is 117, 101, 87, 54 and 32 for 3, 6, 12, 24 and 36 months, respectively. For MA conditioning, the number of patients at each time point is 35, 32, 26, 16 and 8 for 3, 6, 12, 24 and 36 months respectively. FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; TLC: total lung capacity; RV: residual volume; DLCO: diffusing capacity of the lung for carbon monoxide; NMA: non-myeloablative conditioning; MA: myeloablative conditioning. * < 0.01 vs before HSCT; paired comparisons. § < 0.01 vs NMA conditioning; unpaired comparisons.

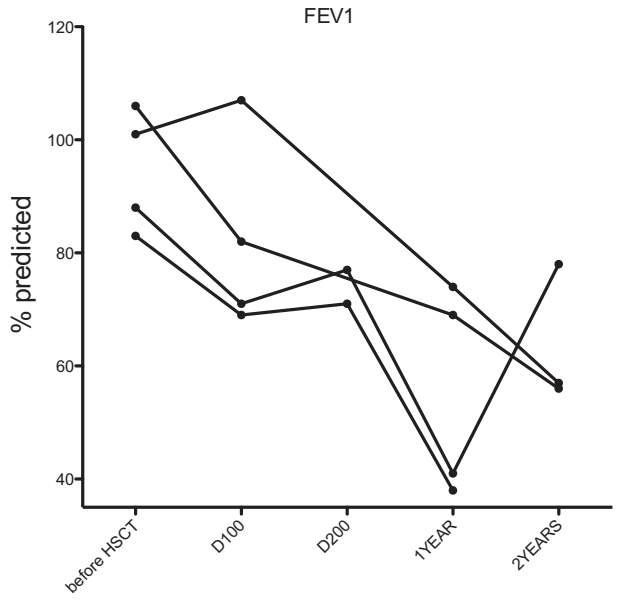

Figure 4 Individual changes in FEV1 over time in the 4 patients who had bronchiolitis obliterans.

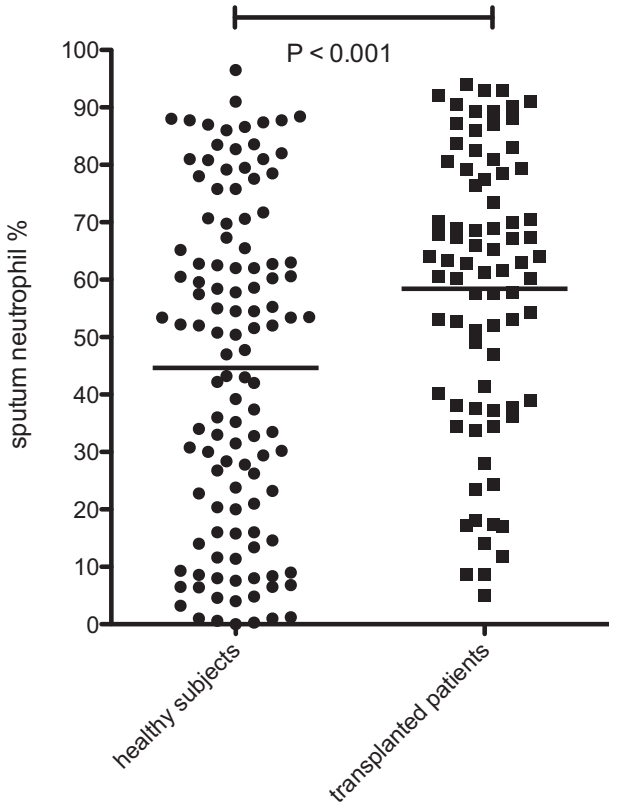

Figure 5 Sputum neutrophil percentage comparisons between patients before HSCT (n = 80) and healthy subjects (n = 116) matched for age and tobacco status. Unpaired comparisons.

follow-up after HSCT even if the patients showed signs of small airway dysfunction [33].

High sputum neutrophil counts have been linked to an irreversible airway obstruction in chronic obstructive pulmonary disease (COPD) [19] and BO following lung transplantation [34]. Neutrophilic airway inflammation is also seen in diseases with intense airway bacterial load such as bronchiectasis [35] and cystic fibrosis [36]. On the other hand, neutrophils in induced sputum were reported to be increased in lung transplant recipients even without BO [9]. To the best of our knowledge, we are the first to report on sputum cell count after HSCT. Even if baseline values of sputum neutrophil counts were already elevated prior to HSCT, we found a further rise in sputum neutrophils after HSCT in those receiving myeloablative conditioning. Recruitment of neutrophils in the airways is a major event in case of activation of innate immunity in response to bacterial and viral infections [37,38] and toxic or pollutant exposure [39]. The rise in neutrophils during the first year after MA conditioning may be related to the greater injury of respiratory mucosal system caused by the intense irradiation and chemotherapy. Even if not significant because of the limited number of subjects assessed at three years in our study, there was in our study, a trend for a new wave in sputum neutrophil increase by this time, whose underlying mechanism may differ from the first wave. Whether the rise in sputum neutrophils contributes to loss of lung function indices remains uncertain but neutrophils are a potent source of proteases endowed with remodelling capabilities. Our finding at the late time point needs certainly to be confirmed in a larger cohort as our patient number analysed at 3 years is limited.

None of the baseline functional and airway inflammation features had a prognostic value for survival at one year. This may seem discrepant from previous findings that

Table 3 Total and differential sputum cell counts over time.

	Before HSCT N = 80	3 months N = 65	6 months N = 56	12 months N = 39	24 months N = 27	36 months N = 10
Total cells (10^6/g)	0.73 (0.34–1.57)	0.94 (0.37–1.97)	0.55 (0.34–1.98)	0.73 (0.25–3.02)	1.17 (0.30–4.01)	2.91 (0.66–7.10)
Cell viability (%)	70 (58–80)	69 (57–84)	70 (53–80)	77 (66–86)	64 (54–83)	74 (65–87)
Macrophages (%)	27 (14–43)	27 (14–46)	27 (14–44)	23 (13–42)	16 (9–39)	15 (11–34)
Eosinophils (%)	0.0 (0.0–0.8)	0.0 (0.0–0.8)	0.0 (0.0–0.9)	0.0 (0.0–0.7)	0.4 (0.0–2.0)	0.0 (0.0–0.4)
Neutrophils (%)	63 (38–79)	67 (45–81)	65 (48–78)	65 (51–78)	72 (36–84)	74 (55–78)
Lymphocytes (%)	1.8 (0.6–4.4)	1.4 (0.2–4.0)	1.0 (0.5–3.0)	1.5 (0.2–5.0)	1.0 (0.5–2.2)	0.9 (0.4–2.0)
Epithelial cells (%)	2.3 (1.0–5.2)	2.7 (0.6–6.0)	2.0 (1.0–4.8)	1.8 (0.8–4.0)	2.7 (0.7–7.0)	2.7 (0.7–16.3)

Results are expressed as median (interquartile range); unpaired comparisons.

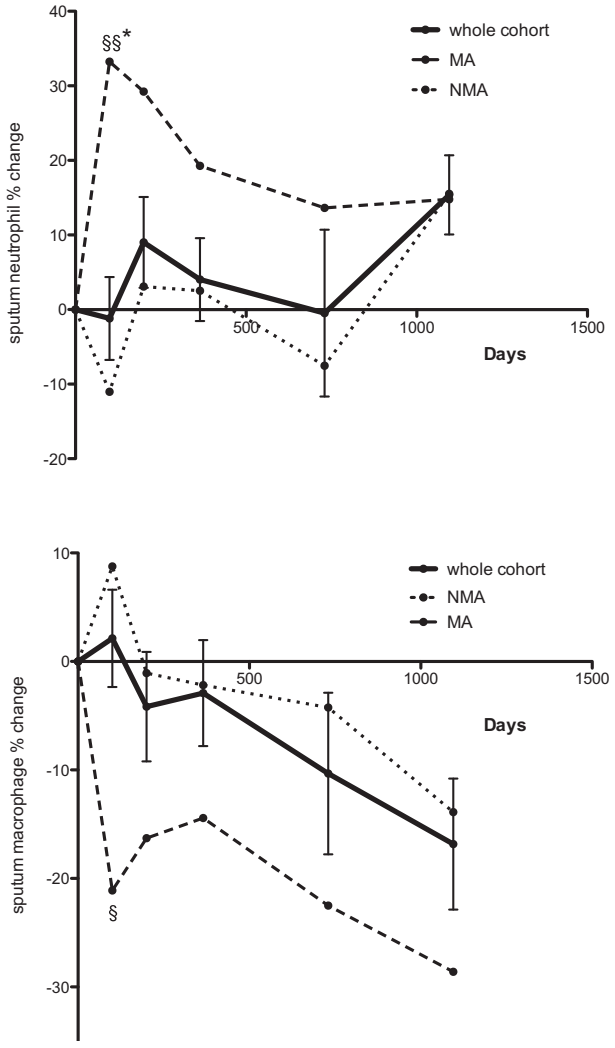

Figure 6 Sputum neutrophil and macrophage changes over time in patients who provided paired data. Results are presented as mean of % sputum neutrophils or macrophages changes ±SEM. For clarity reason, SEM were removed for NMA and MA conditioning lines; the number of patients at each time point is: 45, 35, 24, 12 and 5 for 3, 6, 12, 24 and 36 months, respectively. For NMA conditioning, the number of patients at each time point is 35, 25, 20, 8 and 4 for 3, 6, 12, 24 and 36 months, respectively. For MA conditioning, the number of patients at each time point is 10, 10, 4, 4 and 1 for 3, 6, 12, 24 and 36 months, respectively; NMA: non-myeloablative conditioning; MA: myeloablative conditioning. * < 0.01 vs before HSCT; paired comparisons. § < 0.01, §§ < 0.001 vs NMA conditioning; unpaired comparisons.

reported that low FEV1 and DLCO were associated with poorer prognosis [40,41]. However, we recognise that lung function impairment at baseline was rather mild in our cohort. On the other hand, survival after HSCT has dramatically improved over the last ten years [42] and the potential disadvantage of small lung function impairment in our series may have been compensated by the marked improvement in the management of the post-transplant period.

Table 4 Difference in baseline demographic and functional features between patients who survived at one year and those who died in the first year of observation.

Baseline parameter	Patients who died before 1 year; $N = 52$	Patients who did not die before 1 year; $N = 130$	*P* value
Age	54 ± 13	52 ± 13	0.30
BMI	24 ± 3	25 ± 5	0.52
NMA/MA conditioning	34/18	99/31	0.14
Gender: F/M	22/30	52/78	0.87
Tobacco habits (n/ex/cs)	22/20/10	57/45/28	0.87
FEV1 (% predicted)	87 ± 18	90 ± 17	0.19
FVC (% predicted)	94 ± 16	97 ± 16	0.33
FEV1/FVC	75 ± 10	76 ± 9	0.30
TLC (% predicted)	95 ± 16	97 ± 13	0.44
RV (% predicted)	103 ± 39	104 ± 37	0.94
DLCO (% predicted)	77 ± 28	72 ± 24	0.24
KCO (% predicted)	94 ± 35	88 ± 33	0.29
FeNO (ppb)	18 (14–35)	18 (12–25)	0.47
Total sputum cell number (10^6/g)	0.7 (0.4–1.4)	0.7 (0.3–1.5)	0.92
Neutrophils (%)	63 (38–82)	63 (38–75)	0.47
Macrophages (%)	26 (14–46)	28 (16–41)	0.56
Lymphocytes (%)	2.7 (1.0–4.8)	1.5 (0.5–3.8)	0.20
Eosinophils (%)	0.0 (0.0–0.6)	0.2 (0.0–1.3)	0.08
Epithelial cells (%)	2.5 (0.8–6.7)	2.2 (1.0–5.2)	0.99

Results are expressed as mean ± SD except for FeNO value, sputum cell count and cell percentages, which are expressed as median (IQR). BMI: body mass index, NMA: non-myeloablative conditioning, MA: myeloablative conditioning, *n* = non-smoker, ex = ex-smoker, cs = current smoker, FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; TLC: total lung capacity; RV: residual volume; DLCO: diffusing capacity of the lung for carbon monoxide; KCO: gas transfer coefficient; FeNO: exhaled nitric oxide.

Our study shows that patients who undergo HSCT display mild progressive loss of total lung capacity associated with sustained airway neutrophilic inflammation. By contrast bronchiolitis obliterans syndrome rarely occurs after HSCT.

Conflict of interest statement

All authors have declared the absence of potential conflict of interest related to this manuscript.

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