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Stable Long-Term Pulmonary Function after Fludarabine, antithymocyte globulin, and intravenous Busulfan for reduced-intensity conditioning allogeneic stem cell transplantation

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ABSTRACT

Lung function decline is a well-recognized complication following allogeneic stem cell transplantation (allo-SCT). Reduced-intensity conditioning (RIC) and in vivo T-cell depletion by administration of antithymocyte globulin (ATG) may play a protective role in the occurrence of late pulmonary complications. This retrospective study reported the evolution of lung function parameters within the first 2 years after allo-SCT, in a population receiving the same RIC regimen including fludarabine and i.v. busulfan in combination with low dose ATG. The median follow-up was 35.2 months. With a median age of 59 years at time of transplant, at 2 years, the cumulative incidences of non-relapse mortality was as low as 9.7%. The cumulative incidence of relapse was 33%. At 2 years, the cumulative incidences of extensive chronic graft-versus-host disease (cGVHD) and of pulmonary cGVHD were 23.1% and 1.9%, respectively. The cumulative incidences of airflow obstruction and restrictive pattern were 3.8% and 9.6%, respectively. Moreover, FEV1, FVC and FEV1/FVC ratio remained stable from baseline up to 2 years post transplantation (p=0.26, p=0.27 and p=0.07, respectively). These results compare favorably with results obtained with other RIC regimens not incorporating ATG, and suggest that ATG may play a protective pulmonary role after allo-SCT.

Key Words: pulmonary function, reduced intensity conditioning, ATG

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-SCT) is a well-established therapy for several hematological diseases. Unfortunately, allo-SCT is limited by the high incidence of non-relapse mortality (NRM), mainly due to acute and chronic graft versus host disease (aGVHD, cGVHD) [1]. Importantly, pulmonary complications, which occur in 30 to 60% of patients after allo-SCT [2], account for meaningful mortality and morbidity, and up to 50% of transplant-related deaths.[3] Multiple factors can contribute to pulmonary complications, notably the type of conditioning regimen used. Reduced-intensity conditioning (RIC) regimens are being used with the aim of decreasing NRM in elderly patients, in heavily pretreated patients or in patients with medical comorbidities precluding the use of standard myeloablative conditioning (MAC). One study already showed that the use of RIC regimen can reduce the risk of developing late pulmonary complications, with protection against declining lung function and a reduced incidence of bronchiolitis obliterans syndrome (BOS) [4]. Beyond the degree of myeloablation of the preparative regimen itself (RIC or standard myeloablative conditioning), it is well admitted that the use of total body irradiation (TBI) in the conditioning is also associated with pulmonary toxicity [5]. In contrast, in vivo T cell depletion seems to reduce the risk of developing pulmonary complications after allo-SCT [6]. Also, cGHVD has been identified as a risk factor for late onset non-infectious pulmonary complications and in particular BOS [7-10]. On the other hand, cGVHD is usually significantly reduced in patients receiving *in vivo* T cell depletion by administration of antithymocyte globulins (ATG) [11]. However, the onset of long term pulmonary complication in patients receiving an ATG-based RIC regimen, without TBI, has not yet been investigated. This study evaluated the evolution of lung function parameters within the first 2 years following allo-SCT, in a population undergoing the same RIC regimen including fludarabine, i.v. busulfan in combination with low dose ATG.

PATIENTS AND METHODS

Patients' characteristics

This retrospective single-center study included 52 consecutive patients who received allo-SCT between January 2007 and September 2010 at the University Hospital of Nantes (CHU de Nantes, Nantes, France). Per study inclusion criteria, all patients with available pre- and post-transplant pulmonary function tests (PFT) and who received RIC regimen including 30 mg/m^2 fludarabine for 5 or 4 consecutive days, 6.4 mg/kg total dose IV busulfan and 5 mg/kg total dose ATG (Thymoglobulin; Genzyme/Sanofi, Lyon, France), were included. In our transplant program, eligibility criteria for RIC allo-SCT that preclude the use of standard myeloablative conditioning (MAC) allo-SCT include: (i) patient age older than 50 years; (ii) heavily pretreated patients who received auto-SCT or with more than 2 lines of chemotherapy before allo-SCT; and (iii) patients with poor performance status because of significant medical comorbidities as described by Sorror et al [12]. Patients were treated as part of different prospective clinical trials, and written informed consent was obtained from each patient and donor. All clinical data were prospectively collected.

All patients received the preparative regimen as inpatients in private rooms, and remained hospitalized until hematopoietic and clinical recovery. 29 donors (56%) were HLA-identical sibling donors, while 20 (38%) were HLA-unrelated donors (MUD) and 3 (6%) were HLA unrelated donors with one locus mismatch. The stem cell source was bone marrow in 2 cases (4%) and G-CSF-mobilized peripheral blood stem cells in 50 cases (96%). In this series, the median age was 59 (range, 23-70) years. Twenty-two patients were treated for acute myeloid leukemia (42%), 10 patients had a myelodysplastic syndrome (19%), 13 patients had non Hodgkin lymphoma (25%), 3 patients had Hodgkin disease (6%), 3 patients had multiple myeloma (6%) and 1 patient had acute lymphoblastic leukemia (2%).

Supportive care and antimicrobial prophylaxis were given as reported previously [13]. For

GVHD prophylaxis, patients received either CSA alone in case of an HLA-sibling donor or CSA and mycophenolate mofetil (MMF) in case of an HLA-matched unrelated donor [14]. In this series, CSA was administered at a dose of 3 mg/kg/day by continuous intravenous infusion starting from day -3 or -2, and changed to twice daily oral dosing as soon as tolerated [15]. MMF was given at a fixed oral dose of 2 g/day. No treatment adjustment was performed for MMF. MMF was decreased progressively over 4 weeks starting from day 60 and CSA from day 90 if no GVHD appeared. Of note, during the whole study period supportive care was the same. CMV infection management was also homogeneous. All blood products were filtered irradiated and CMV screened. In the first 100 days post allo-SCT, patients were assessed at least once per week for CMV reactivation by PCR assay in order to initiate preemptive ganciclovir therapy. Acute GVHD was evaluated according to the Seattle standard criteria [16].

Pulmonary Function Testing

PFT were performed before allo-SCT and then repeated 100 days, one and two years after transplant. All PFT were performed in the same laboratory in accordance with the American Thoracic Society and European Respiratory Society criteria [17]. Lung volumes and spirometry were measured with a Jaeger constant volume body plethysmograph with a pneumotachograph connected to an Epson PC-AT (Epson, Suwa, Japan). All PFT values, except FEV_1/FVC ratio were expressed as a percentage of predicted values in healthy controls with corresponding age and gender. Published equations were used to calculate predicted values of forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), total lung capacity (TLC) and lung carbon monoxide diffusing capacity (DLCO) [18]. DLCO was measured using the single breath technique of Ogilvie et al. and corrected for the most recent hemoglobin concentration but not corrected for the alveolar volume [19]. In those patients who

received a bronchodilator challenge, the prebronchodilator values were used. A lung function score (LuFS) was calculated before transplantation, and at day 100, 1 year and 2 years after transplant in order to grade the extent of lung function impairment (if any). A separate score was assigned to relative values of FEV_1 and DLCO (>80% = 1, 70-79% = 2, 60-69% = 3, 50-59% = 4, 40-49%=5, <40%=6), these scores were then summed and divided into four categories as LuFS (LuFS score 2 = category 0 [normal]; LuFS score 3-5 = category 1 [mildly decreased]; LuFS score 6-9 = category 2 [moderately decreased]; LuFS score 10-12 = category 3 [severely abnormal]), according to NIH recommendations [20]. Restrictive lung disease (RLD) was defined as TLC <80% of the predicted value, and was graded as mild at 70–79%, moderate at 60–69%, moderately severe at 50–59%, and severe at <50%. Diffusion impairment was defined as DLCO <80% of the predicted value and was graded as mild at 60–79%, moderate at 40–59%, and severe at <40% [21]. Airflow obstruction (AFO) was defined by a FEV1/FVC < 70. Criteria from the NIH consensus guidelines were used for diagnosis of BOS: 1) FEV1/ FVC < 70% and FEV1 < 75% of predicted value, 2) radiological, histological or lung volume evidence of air trapping and 3) absence of respiratory tract infection [20].

The occurrence of infectious respiratory complications during the follow-up period of interest was collected in medical records. Expiration scans to evaluate air trapping, chest high resolution computed tomography and bronchoalveolar lavage with bacterial, fungal and virological searches were left to the discretion of the attending physician, according to symptoms presented by the patient.

Statistical Methods

Continuous variables were presented as mean ± standard deviation (SD) or median (range), categorical variables as count and percent. Mixed models were used to analyze PFT data during time (before transplantation until 2 years post transplantation). Comparison

between LuFS scores before transplantation and at 100 days, 1 year and 2 years post transplantation was performed with the *McNemar's test*. Overall survival (OS) and progression-free survival (PFS) were calculated by the Kaplan-Meier method. Probabilities of relapse, NRM and GVHD were calculated using the cumulative incidence procedure. The risk of respiratory failure, RP and AFO were also calculated using the cumulative incidence procedure with death considered as the competing event. Data were computed using SAS software version 9. 3, the R package (R Development Core Team, 2006. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org) and GraphPad Prism 5.0 (GraphPad Software, San Diego, CA).

RESULTS

Patients' characteristics

In this series, the overall median follow-up was 35.2 months (range, 20.0-59.8) among surviving patients. Patients, donors and transplant characteristics are summarized in **Table 1**. The median age was 59 (range 23-70) years. Patients engrafted at a median of 17 (range, 0-48) days after allo-SCT. The cumulative incidence of severe grade III-IV aGVHD at day 100 was 15.4%. At 2 years, the cumulative incidence of extensive cGVHD was 23.1%. In this series, 23 (44%) patients were active or former smokers while 21 (40%) never smoked. Smoker status data were missing for 8 patients (15%).

PFT and development of airflow obstruction and restrictive pattern

Pretransplantation PFT were performed at a median of 17 (range, 10-76) days before allo-SCT. Subsequently, PFT were performed at a median of 101 (range, 77-119), 365 (range, 251-490) and 717 (range, 588-846) days after transplantation. The proportion of surviving patients with available PFT was 85%, 89% and 82% at day 100, 1 year and 2 years, respectively. PFT data before transplantation and at day 100, 1 year and 2 years postransplantation are summarized in **Table 2.** Before stem cell transplantation, lung function was normal in 83% (n=43) of patients, while 6 patients met the criteria for obstructive lung disease defined as FEV1/FVC <70, and 3 patients had restrictive lung disease, defined as TLC <80%. Restrictive lung disease was graded as mild for 2 patients and moderate for one. Among patients with a pretransplantation obstructive pattern, only one patient had a FEV1 inferior to 75% predicted. DLCO was impaired (mean value <80%) in 83% of patients before transplantation, and remained altered but stable from baseline up to 2 years post transplantation (p=0.84). Furthermore, 60% (n=31) had a mild diffusion impairment, 19% (n=10) a moderate diffusion impairment, and 4% (n=2) a severe diffusion impairment of DLCO (**Figure 1 E**). FEV1, FEV1/FVC ratio, FVC and TLC remained stable from baseline

up to 2 years post transplantation (p= 0.26, p=0.07, p=0.27, p=0.44, respectively) (**Figure 1 A-D**). Evaluation of LuFS showed a mildly abnormal lung function (category 1) in 44 patients (77%) at baseline. From baseline up to 2 years, LuFS score remained stable with most of patients with normal or mildly abnormal lung function, and no patient had a severely abnormal lung function (**Figure 2**). There was no statistical difference when comparing time-points: before allo-SCT versus 100 days (p=0.53), before allo-SCT vs 1 year (p=0.29), and before allo-SCT versus 2 years (p=0.54).

Mortality, infectious and non-infectious pulmonary complications

Of the 52 patients included in this study, a total of 19 patients died (36.5%) during the follow-up period. Overall survival, cumulative incidences of relapse and NRM rates at 2 years after allo-SCT were 65% (95% CI, 51-76%), 33% and 9.7% respectively. Four deaths (21%) were related to GVHD, 1 (5%) death was directly related to a pulmonary cause (pleuro-pericarditis), and 1 (5%) due to a secondary malignancy. The remaining 13 (69%) deaths were caused by relapse or progression of original disease. In this series, 8 patients presented a possible or probable invasive aspergillosis before transplantation (1 to 8 months before). After transplantation, two patients presented a possible aspergillosis (3 months and one year after transplantation) and one patient presented a probable aspergillosis 4 months after allo-SCT [22]. None of the patients was diagnosed with a CMV disease within 2 years after transplantation. At 2 years, the cumulative incidence of AFO and RP was 3.8% and 9.6% respectively. RP was graded as mild for the majority of patients. The cumulative incidence of pulmonary cGVHD at 2 years was 1.9%, and only one patient met the criteria for BOS diagnosis according to the NIH consensus guidelines [20] during the follow-up period of interest.

DISCUSSION

In this study, we reported a stable pulmonary function with a low rate of pulmonary complications including airflow obstruction, restrictive lung disease and BOS in the two years following allo-SCT conditioned by fludarabine, i.v. busulfan and ATG. It is well established that pulmonary complications are a major cause of morbidity and mortality after allo-SCT and occur in 30 to 60% of cases, and up to 80% in autopsy studies [2, 23]. Infectious complications are the more frequent, especially in the early phase after allo-SCT, however, many non-infectious pulmonary complications can occur after allo-SCT: restrictive lung disease, impaired gas exchange or obstructive lung disease in particular in patients with concomitant cGVHD [21].

The advent of RIC regimen allowed to perform allo-SCT in older patients or young patients with severe comorbidities, including pulmonary comorbidities. The type of agents used as part of the RIC regimens is a crucial determinant for the occurrence of pulmonary complications. In the current study, we analyzed the results of a well-established RIC regimen combining fludarabine, intermediate dose of i.v. busulfan and ATG (Thymoglobulin®) used at a total dose of 5 mg/Kg. Such regimen combines an effective disease control with low NRM and an acceptable toxicity profile [24-26]. In our study, RP and AFO cumulative incidences remained very low, at 3.8% and 9.6% respectively, suggesting that such regimen does not induce a significant pulmonary toxicity. Indeed, fludarabine is known to be less toxic to the pulmonary system than the traditional cyclophosphamide [27]. On the other hand, high dose busulfan, along with cGVHD are the main risk factors identified for spirometric obstruction [21, 28-30]. Furthermore, low dose TBI which is another component of RIC protocols, remains an important risk factor of airflow decline, despite changes in TBI techniques aiming to limit pulmonary toxicity [31]. Regarding ATG, previous data from the standard myeloablative setting already suggested a potential protective role of this agent on lung

function. The GITMO randomized trials [32] showed that in patients receiving ATG, FEV1 and FVC values remained stable at 2 years after allo-SCT (ΔFEV1, -3% and ΔFVC, +3%), whereas there was a significant decrease of FEV1 and FVC and an increase of cGVHD in the non-ATG group. Our results in the RIC setting are in accordance with these findings, further supporting the value of ATG towards reducing the incidence and severity of GVHD [33]. Here, we reported a low cumulative incidence of extensive cGVHD at 2 years (23.1%). Such protective role of ATG on lung function is likely mediated (at least in part) by its effectiveness in reducing overall cGVHD. Indeed, pulmonary damage occurs during cGVHD, and BOS is well linked to cGVHD. According to previous studies, BOS incidence ranges from 6 to 20% in long term survivors [34]. This important variability is probably related to many parameters, including the nature of conditioning regimen, stem cell source, type of donor etc. Furthermore, different definitions of airflow obstruction have been used over the years to define BOS, contributing to this variability. We chose to apply the most recent NIH criteria [20], and reported results according to the cumulative incidence procedure which is more appropriate. In our cohort, despite the use of busulfan, the cumulative incidence of BOS was very low (1.9%), supporting a protective role of ATG in the occurrence of cGVHD and BOS. In addition, cGVHD might be responsible for indirect lung damage: severe chronic scleroderma GVHD can lead to true RP. Thus, one may reasonably conclude that ATG can contribute to the low cumulative incidence of RP (9.6%) observed in our study. One classical argument against the use of ATG is the higher risk of opportunistic infections and disease relapse. However, in our study, the use of ATG did not result in an increased incidence of relapse, in contrast to the study by Soiffer et al [35]. This difference may be explained by the lower dose of ATG we used (5 mg/Kg total dose). Likewise, whereas higher doses of ATG are known to favor infections, there were very few infectious pulmonary complications in our cohort, in line with our previously published findings[36]. Furthermore, low cumulative

incidence of extensive cGVHD and NRM at 2 years (23.1% and 9.7% respectively), compare favorably with long term follow-up of the German randomized ATG study[37]: at 3 years, the cumulative incidence of extensive cGVHD and NRM were 12.2% and 19.4% respectively in the ATG group and 45.0% and 33.5% respectively in the non ATG group. Thus ATG seems to exert a strong protective effect against severe cGVHD, leading to a low NRM incidence, indicating a good quality of life, since extensive cGVHD is well known to be responsible for a worsened quality of life[38].

In the current series, a significant percentage of patients had DLCO impairment before allo-SCT. After allo-SCT, DLCO remained impaired, but without evidence of worsening, as previously described in the paediatric setting [39]. The baseline impairment of DLCO in these patients is likely multifactorial reflecting previous bacterial and viral infections, or toxicities related to prior chemotherapy.[40]. The impact of baseline DLCO impairment on outcome is still controversial. However, a few studies have suggested a significant impact of DLCO values on transplant outcome [41, 42 43].

Unfortunately, in the current study, we were not able to compare the results obtained using the fludarabine, i.v. busulfan and ATG-based RIC regimen with a similar regimen not containing ATG, because this is not common practice in our centre. Likewise, one may argue that lung function measurements performed during the first 2 years after allo-SCT may not be sufficient as lung function decline could appear after 2 years, and up to 18 years after allo-SCT [1, 21, 28, 44]. However, one must acknowledge that reduction in lung volumes and diffusing capacity often occurred early within 12 months after allo-SCT, followed by an incomplete recovery within the next two years [21, 45].

In conclusion, we report a low rate of pulmonary complications and lung function impairment in patients undergoing a RIC regimen including fludarabine, i.v. busulfan and low dose of ATG. These results further support the use of ATG as part of the so-called reduced-toxicity

regimens aiming to decrease long-term toxicities and to improve patients' quality of life.

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Author contributions

S. Dirou: collected, assembled and analyzed PFT data and wrote the manuscript;

F. Malard: helped in collecting, assembling and analyzing data, performed statistical analyses and wrote the manuscript;

A Chambellan: performed and analyzed PFT data, helped in collecting, assembling and analyzing PFT data;

P Chevallier, T Guillaume, J Delaunay, P Moreau, and S. Le Gouill: recruited patients, and commented on the manuscript;

P Germaud: helped in collecting PFT data, and commented on the manuscript;

B. Delasalle: helped in analyzing PFT data and performed statistical analysis;

P Lemarchand: analyzed data and wrote the manuscript;

M Mohty: recruited patients, supervised research, analyzed data, and wrote the manuscript;

All authors approved submission of the manuscript for publication purposes.

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FIGURE LEGENDS

Figure 1. Changes in PFT parameters from baseline up to 2years post allo-SCT. Data are represented by boxplot. FEV1, FEV1/FVC, FVC, TLC, DLCO are expressed as percentage of predicted value.

Figure 2. Lung Function Score (LuFS) before allo-SCT, 100 days, 1 year and 2 years after allo-SCT. A separate score was assigned to relative values of FEV_1 and DLCO (>80% = 1, 70-79% = 2, 60-69% = 3, 50-59% = 4, 40-49%=5, <40%=6), these scores were then summed and divided into four categories: LuFS 2 = category 0, LuFS 3-5 = category 1, LuFS 6-9 = category 2, LuFS 10-12 = category 3. No patient had severely abnormal lung function. There were no statistical differences in changes of LuFS before and after allo-SCT (Before allo-SCT vs 100 days (p=0.53), vs 1 year (p=0.29), vs 2 years (p=0.54)).

Table 1. Demographic characteristics of patients and transplant-related events

Characteristic (%)	Study population (N=52)
Patient age, median (range)	59 (23-70)
Patient gender	
Male	32 (62)
Female	20 (38)
Smoker	
Yes	23 (44%)
No	21 (40%)
Data missing	8 (15%)
CMV serologic status	
Seronegative donor-recipient pair	23 (44%)
Diagnosis	
AML	22 (42)
MDS	10 (19)
NHL	13 (25)
Other*	7 (14)
Stem cell source	
Bone marrow	2 (4)
PBSC	50 (96)
Donor type	
Matched related donor (MRD)	29
Unrelated donor (UD)	23
$CD34^+$ cell infused, $\times 10^6$/Kg, median (range)	6.22 (1.15-16.3)
GVHD prophylaxis	
CsA alone	31 (60)
CsA + MMF	21 (40)
Grade 3-4 aGVHD at day 100 (cumulative incidence)	15.4%
Extensive cGVHD at 2 years (cumulative incidence)	23.1%

Abbreviations : CMV indicates cytomegalovirus; AML acute myelogenous leukemia; MDS myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; CsA, Ciclosporine A; MMF, mycophenolate mofetil.
*Others: 3 Hodgkin's disease, 3 multiple myeloma and 1 acute lymphoblastic leukemia.

1 **Table 2.** PFT Data Obtained Pretransplantation and at 100 Days, 1 Year, and 2 Years
2 Posttransplantation (Median expressed as percentage of predicted value).
3

	FEV1, Median [range]	FVC, Median [range]	FEV1/FVC, Median [range]	TLC, Median [range]	DLCO, Median [range]
Pretransplantation (n = 52)	109.8 [68.5- 137.3]	108.2 [66.5-138.4]	80.9 [55.3-98.1]	104.5 [65.2-137.1]	69 [37.5-96.1]
Day 100 (n = 40)	105.1 [71.5-135.1]	105.8 [68.1-136.3]	78.48 [55.5-91.1]	105.8 [77.9-138.7]	69.8 [43.3-106.3]
1 year (n = 32)	103.4 [56.1-143.7]	106.7 [59-139]	76.86 [62.8-89.9]	103.7 [76.2-132.2]	70 [37.4-94.4]
2 years (n = 28)	100.6 [63.8-118.1]	102.7 [63.9-117.8]	76.2 [58.7-90.7]	104.4 [79-144.9]	68.1 [34.4-86.2]

4 Abbreviations: FEV1 indicates forced expiratory volume in 1 second; FVC, forced vital capacity;
5 TLC, total lung capacity; DLCO, lung carbon monoxide diffusing capacity.
6

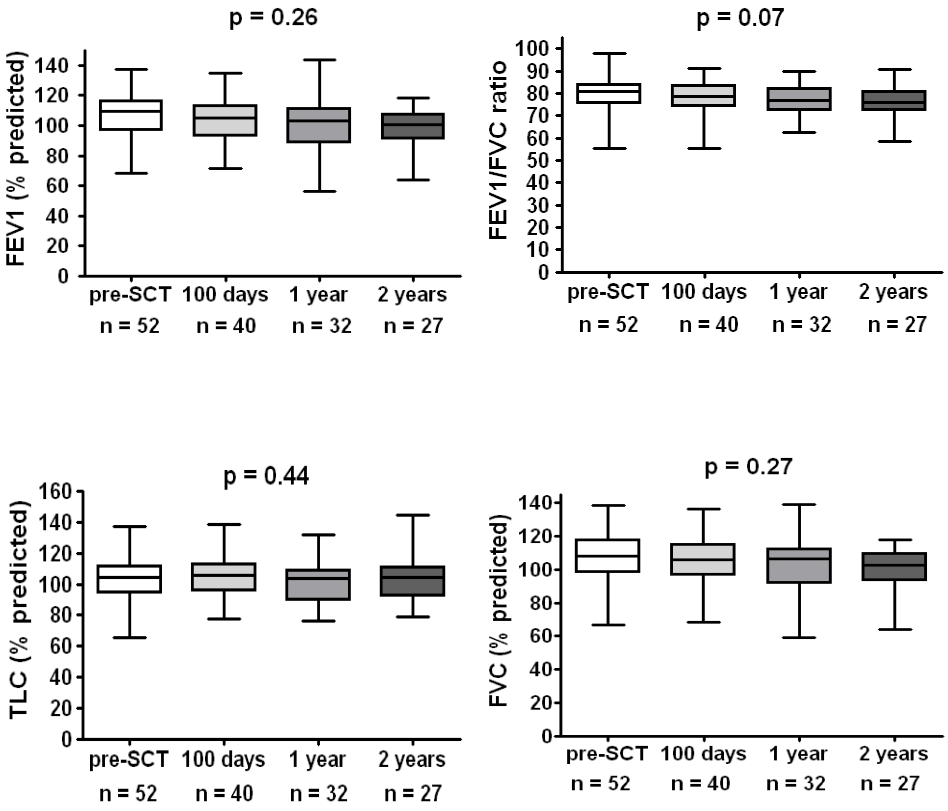
p = 0.26
FEV1 (% predicted)
pre-SCT 100 days 1 year 2 years
n = 52 n = 40 n = 32 n = 27
p = 0.07
FEV1/FVC ratio
pre-SCT 100 days 1 year 2 years
n = 52 n = 40 n = 32 n = 27
p = 0.44
TLC (% predicted)
pre-SCT 100 days 1 year 2 years
n = 52 n = 40 n = 32 n = 27
p = 0.27
FVC (% predicted)
pre-SCT 100 days 1 year 2 years
n = 52 n = 40 n = 32 n = 27
p = 0.84
DLCO (% predicted)
pre-SCT 100 days 1 year 2 years
n = 52 n = 40 n = 32 n = 25

Figure 1.

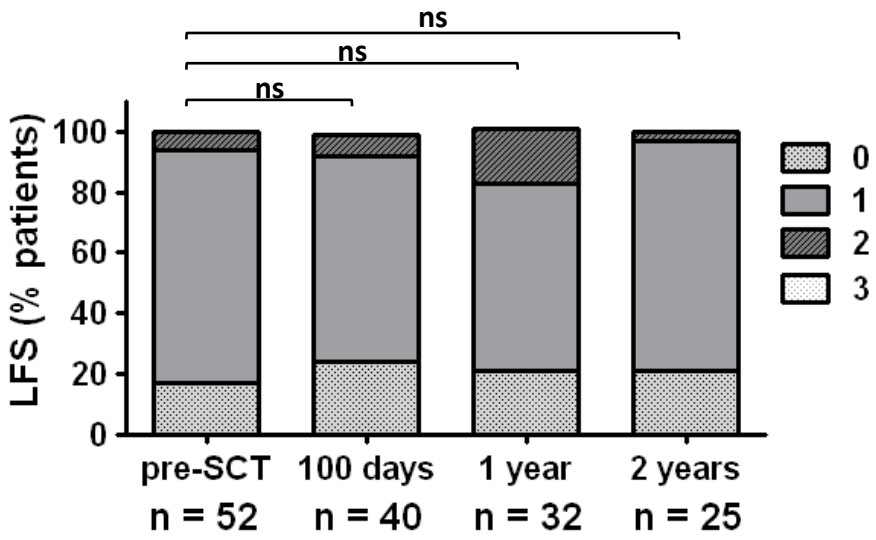
ns
ns
ns
LFS (% patients)
100
80
60
40
20
0
pre-SCT
100 days
1 year
2 years
n = 52
n = 40
n = 32
n = 25
0
1
2
3

Figure 2.