ORIGINAL ARTICLE

Pulmonary complications after T-cell-depleted allogeneic stem cell transplantation: low incidence and strong association with acute graft-versus-host disease

C Huisman¹, HM van der Straaten¹, MR Canninga-van Dijk², R Fijnheer¹ and LF Verdonck¹

¹Department of Hematology, University Medical Center Utrecht, Utrecht, The Netherlands and ²Department of Pathology, University Medical Center Utrecht, Utrecht, The Netherlands

Lung injury limits the success of allogeneic stem cell transplantation (SCT). The overall incidence varies from 30 to 50% and non-infectious causes occur in one-third to one-half of these. We reviewed pulmonary complications in 369 consecutive patients who received a partially T-cell-depleted myeloablative allogeneic hematopoietic SCT at our institution between 1993 and 2003. All patients were treated uniformly with cyclophosphamide followed by total body irradiation. Control subjects were matched on sex, underlying diagnosis, age, type of transplantation and cytomegalovirus (CMV)-serostatus. Sixty-one patients (16.5%) developed pulmonary complications. Twenty-one patients (5.7%) developed infectious pneumonia. Forty patients developed non-infectious complications which were further subclassified as bronchiolitis obliterans (3.5%), bronchiolitis obliterans-organizing pneumonia (0.5%), diffuse alveolar hemorrhage (0.8%), idiopathic pneumonia syndrome (5.5%) or mixed etiology (0.5%). Acute graft-versus-host disease (GVHD) grade II was significantly more common in pulmonary patients than in the controls (36/61 versus 22/61 patients, \( P = 0.02 \)). There was no significant difference in the incidence of chronic GVHD (\( P = 0.09 \)). CMV reactivation was significantly more frequent in patients with lung injury (\( P = 0.02 \)). Median survival was 41 weeks for the pulmonary patients and 350 weeks for the controls (\( P = 0.001 \)). Altogether, the incidence of pulmonary complications is low after T-cell-depleted SCT and we analyze the relationship between this complication and acute GVHD and CMV reactivation.

Bone Marrow Transplantation (2006) 38, 561–566. doi:10.1038/sj.bmt.1705484; published online 4 September 2006

Keywords: stem cell transplantation; T-cell depletion; pulmonary complications; graft-versus-host disease

Introduction

Pulmonary complications significantly contribute to treatment-related morbidity and mortality after allogeneic stem cell transplantation (SCT). Whereas infectious diseases of the lung predominated in previous years, the increased use of prophylactic antibiotics has shifted the spectrum towards non-infectious causes. Traditionally, pulmonary toxicities were observed to range from 30 to 50%3 but more recent studies report a diverse and generally lower incidence.4–14

Unfortunately, these trials did not apply a uniform definition of non-infectious pulmonary complications. The best-characterized early onset lung injury after SCT is diffuse alveolar hemorrhage (DAH), whereas bronchiolitis obliterans (BO) and bronchiolitis obliterans-organizing pneumonia (BOOP) occur later in the post transplant period. The most common complication is the idiopathic pneumonia syndrome (IPS), which is defined as evidence of widespread alveolar injury in the absence of lower respiratory tract infection15 and may develop in the early but more commonly in a later setting. Additional, less frequently encountered forms of pulmonary toxicity are the periengraftment respiratory distress syndrome and delayed pulmonary toxicity syndrome.

Among the variables analyzed for their possible influence on the development of pulmonary complications, graft-versus-host disease (GVHD) emerges as a consistent risk factor9,16,17 suggesting a role for inflammatory mediators and alloreactive donor lymphocytes. However, experimental data have not yet been able to show a mechanistic relationship.18 Moreover, the association with GVHD varies among the different types of lung injury. Whereas BO and BOOP occur almost exclusively in patients with chronic GVHD,11,19,20 GVHD is among the many risk factors but not a prerequisite for the development of IPS.21

The pathogenesis of DAH is unclear but it is usually diagnosed early post transplant and GVHD has not been identified as a critical factor.22

In the current study, we characterize the spectrum and incidence of pulmonary toxicities in a large cohort of patients uniformly treated with cyclophosphamide and total body irradiation (TBI) followed by a partially T-cell-depleted SCT and we analyze the relationship between this lung injury and GVHD.
Patients and methods

Study group
Pulmonary complications were reviewed in a computerized database of 369 consecutive patients who received either allogeneic bone marrow (allo-BMT) or peripheral blood SCTs (allo-PBSCTs) between January 1993 and July 2003 at our institution. Controls were selected from the same database. Controls were matched to each pulmonary case on sex, underlying diagnosis, age, type of transplantation (sib or matched-unrelated donor (MUD)), BMT or PBSCT and cytomegalovirus (CMV)-serostatus to the best possible extent. Patients who underwent previous allogeneic or autologous SCT were excluded from the analysis.

Transplantation procedure
Patients were treated according to clinical protocols approved by the local investigation review board after informed consent was obtained. For all patients, the conditioning myeloablative regimen consisted of cyclophosphamide (60 mg/kg/day for 2 days) followed by TBI (600 cGy/day for 2 days) with partial shielding of the lungs (total lung dose 850 cGy). The graft was partially T-cell-depleted consisting of 1–2 × 10^5 T cells/kg and was infused after the second TBI fraction (day 0). CD34 dose was only measured for PBSCT and the median dose was 1.2 × 10^6/kg. Antithymocyte globulin (Thymoglobulin, Sangstat, Amstelveen, The Netherlands) was given to MUD patients before cyclophosphamide was infused, at a total dose of 20 mg/kg until April 1999 and 8 mg/kg thereafter.

Post transplant immunosuppression consisted of cyclosporine, which was discontinued within 3 months after transplantation when no active GVHD was present. GVHD was diagnosed according to the Seattle criteria and treated with 1–2 mg/kg/day prednisolone and resumption of full dose cyclosporine if applicable. Donor lymphocyte infusions were administered in case of residual disease or relapse at a dose of 0.01–1.0 × 10^6 T cells/kg to eight patients with pulmonary disease and to 20 controls.

Infection prevention consisted of ciprofloxacin, fluconazole plus amphotericin B until granulocyte counts exceed 500 cells/μl. Cefalothrin was given for 10 days from day +3. Furthermore, co-trimoxazole and (val)acyclovir were given orally from day +1 until 12 months post transplantation or longer in the case of active GVHD, in a dose of 480 and 500 mg b.i.d., respectively. CMV-seropositive patient/donor combinations were monitored twice a week during the first 120 days post transplant. Until April 2001, CMV monitoring was based on pp65 antigenemia. Since then CMV viral load was determined by a CMV DNA PCR technique. Pre-emptive treatment with ganciclovir was started when tests became positive.

Pulmonary complications
The established etiologies of the pulmonary disease were reviewed by two of the authors (CH and LFV) on the basis of clinical, radiological, microbiologic and histological findings. Pneumonia was classified as infectious based on the pathogen reported. According to distinguishing features described previously, non-infectious complications were defined as BO (absence of fever and pulmonary infiltrates, presence of airway obstruction), BOOP (fever, patchy pulmonary consolidation and typical histology), DAH (diffuse pulmonary infiltrates and progressively bloodier aliquots of lavage return and/or ≥20% hemosiderin-laden macrophages during bronchoscopy) and IPS (evidence of widespread alveolar injury in the absence of lower respiratory tract infection). Uncomplicated upper airway infections or exacerbations of chronic obstructive pulmonary disease were excluded from analysis.

Baseline pulmonary function tests were not obtained in all patients. Post transplant pulmonary function tests were performed in patients presenting with pulmonary complaints. Imaging studies (CT scan and/or X-ray of the chest) as well as cultures and viral screening of sputum and blood were performed on all patients presenting with fever, cough, dyspnea and/or pulmonary infiltrates post transplant. From October 1997 to March 2001, routine nasopharyngeal and throat swabs were analyzed by PCR for the detection of respiratory viruses (adenovirus, parainfluenza virus, RS virus, influenza virus, rhinovirus, coronavirus, enterovirus), as described previously. Treatment was initiated with broad-spectrum antibiotics given intravenously in case of neutropenia, and amphotericin B was added if the patient remained febrile. Fiber-optic bronchoscopy with sampling of bronchoalveolar lavage (BAL) fluid was performed whenever possible if patients did not respond to empirical antibiotic therapy. Histological biopsies were taken according to the clinical situation and the condition of the patient.

Statistical methods
Differences between the groups were compared by logistic regression, accounting for the matching used in the control selection. This model included the variables of interest as well as the covariates considered a priori to be potential confounders (sex, underlying diagnosis, age, type of transplantation and CMV-serostatus). The prevalence of chronic GVHD could be calculated for patients surviving >100 days. The difference between patients with infectious and non-infectious causes of lung injury was compared by the Pearson χ^2 test. Overall survival was estimated by Cox proportional hazard analysis. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated. For all tests, a two-sided P-value of ≤0.05 was considered statistically significant. Calculations were performed using SPSS/PC + 12.0 (SPSS Inc., Chicago, IL, USA).

Results
Characterization of patients and pulmonary complications
Of the 369 patients reviewed in our study, 61 (16.5%) developed pulmonary complications after allogeneic SCT. Patient and transplantation characteristics of this group and their control subjects are shown in Table 1. A minority of the pulmonary diseases was represented by infectious
causes (21 patients). In four cases, the clinical course was fully compatible with infectious pneumonia but the pathogen could not be determined. Forty patients developed non-infectious complications. These were further subclassified as BO (13 patients), BOOP (two patients), DAH (three patients) or IPS (20 patients). Bronchoscopy with BAL was performed in 71% of patients classified as IPS, yielding negative culture results. A mixed etiology was established in two patients (Table 2). The pulmonary complications were diagnosed at a median of 22 weeks after transplantation (range 2–263 weeks). The median time of onset did not differ between patients with infectious problems and those with IPS (19 and 18 weeks, respectively), whereas BO occurred at a later stage (median 28 weeks).

Clinical course and outcome

Table 3 shows the prevalence and severity of acute and chronic GVHD in the group of pulmonary patients and the controls. As a consequence of the T-cell-depleted allografting, the numbers of grades III and IV GVHD were very small. Overall, the incidence of acute GVHD ≥ grade II was significantly higher among patients with pulmonary complications ($P = 0.02$). In this group, no difference was seen between patients with infectious and non-infectious causes of lung injury ($P = 0.15$). The prevalence of chronic GVHD did not differ significantly between the groups, although there was a tendency towards a higher overall incidence in pulmonary patients ($P = 0.09$ for limited and extensive chronic GVHD). The ORs for acute and chronic GVHD were 2.5 (95% CI, 1.2–5.3) and 2.0 (95% CI, 0.89–4.7), respectively. None of the other variables assessed in the logistic regression model could be identified as correlated factors. However, CMV reactivation was significantly more frequent among patients with pulmonary complications ($P = 0.02$, OR 3.4, 95% CI, 1.2–9.5).

In 19 patients, pulmonary complications occurred during the period when all viral upper and lower respiratory tract infections were monitored. Viral airway infections were detected in 12 patients but the upper respiratory tract infection preceded subsequent lung injury (BO) in only one patient.

### Table 1  Characteristics of pulmonary patients and controls

| Characteristics              | Pulmonary patients | Control subjects |
|------------------------------|--------------------|------------------|
| Total no. of patients        | 61                 | 61               |
| Male                         | 41                 | 41               |
| Female                       | 20                 | 20               |
| Age (years)                  |                    |                  |
| Median                       | 44                 | 44               |
| Range                        | 18–57              | 21–55            |
| Underlying diagnosis         |                    |                  |
| Acute myeloid leukemia       | 8                  | 9                |
| Acute lymphoid leukemia      | 7                  | 7                |
| Chronic myeloid leukemia     | 14                 | 14               |
| Othera                       | 32                 | 31               |
| Type of transplantation      |                    |                  |
| Allo-BMT                     | 42                 | 44               |
| MUD-BMT                      | 5                  | 2                |
| Allo-PBSCCT                   | 12                 | 14               |
| MUD-PBSCCT                   | 2                  | 1                |
| CMV serostatus               |                    |                  |
| Positive                     | 34                 | 34               |
| Negative                     | 27                 | 27               |
| CMV reactivation             |                    |                  |
| Yes                          | 17                 | 6                |
| No                           | 44                 | 55               |
| History of pulmonary disease |                    |                  |
| No                           | 53                 | 57               |
| Asthma                       | 5                  | 3                |
| COPD                         | 2                  | 0                |
| Tuberculosis                 | 1                  | 1                |
| Smoking                      |                    |                  |
| No                           | 33                 | 30               |
| Yes/quit for <1 year         | 16                 | 13               |
| Unknown                      | 12                 | 18               |

### Table 2  Characterization of pulmonary complications

| Pulmonary complications | Patients no. | %   |
|-------------------------|--------------|-----|
| Infectious              | 21           | 5.7 |
| Bacterial               | 6            | 1.6 |
| Viral                   | 3            | 0.8 |
| Aspergillus             | 8            | 2.2 |
| No pathogen established | 4            | 1.1 |
| Non-infectious          | 40           | 10.8|
| IPS                     | 20           | 5.5 |
| BO                      | 13           | 3.5 |
| BOOP                    | 2            | 0.5 |
| DAH                     | 3            | 0.8 |
| Mixed                   | 2            | 0.5 |

### Table 3  Prevalence and severity of graft-versus-host disease

| Pulmonary patients | Control subjects |
|--------------------|------------------|
| n = 61             | n = 61           |
|                   | %                | %                |
| Acute GVHD         |                  |                  |
| Grade 0            | 11               | 18               |
| Grade I            | 14               | 23               |
| Grade II           | 33               | 54               |
| Grade III–IV       | 3                | 5                |
| Chronic GVHD       |                  |                  |
| No                 | 22               | 36               |
| Limited            | 16               | 26               |
| Extensive          | 10               | 17               |
| Not applicablea    | 13               | 21               |

Abbreviations: BMT = bone marrow transplantation; CMV = cytomegalovirus; COPD = chronic obstructive pulmonary disease; MUD = matched-unrelated donor; PBSCCT = peripheral blood stem cell transplantation.

aIncluding non-Hodgkin’s lymphoma, multiple myeloma and myelodysplastic syndrome.

Abbreviations: BO = bronchiolitis obliterans; BOOP = bronchiolitis obliterans-organizing pneumonia; DAH = diffuse alveolar hemorrhage; IPS = idiopathic pneumonia syndrome.

aPatients who did not survive > 100 days.
Overall, 14 pulmonary patients were alive at the time of analysis. Forty-seven patients died, mainly (42 patients) owing to pulmonary complications. Of the control subjects, 32 were alive and 29 died. Median survival was 41 weeks (range 4–583 weeks) for the pulmonary patients and 350 weeks (range 8–582 weeks) for the control subjects ($P = 0.001$), after a median follow-up of 41 and 175 weeks, respectively. Survival was similar in patients with infectious causes and those with IPS (median 34 weeks, range 4–484 and 6–585 weeks, respectively), whereas patients with BO had a better outcome (median 55 weeks, range 28–410 weeks).

**Discussion**

Our data confirm that partial T-cell depletion results in a low incidence of GVHD and pulmonary complications, in contrast to results of recent studies without T-cell depletion. In our study, pulmonary complications were significantly associated with acute GVHD $\geq$ grade II and CMV reactivation. The increased risk of pulmonary problems that we found among patients with chronic GVHD was not statistically significant. This is probably due to the use of partially T-cell-depleted grafts, resulting in a much lower incidence of chronic GVHD than reported in two studies which did demonstrate a relationship between chronic GVHD and late non-infectious pulmonary complications. Moreover, in a subset of patients presenting between 1997 and 2001, we could not identify viral respiratory tract infections as a risk factor for progression to lung injury.

The suggestion that non-infectious pulmonary complications after SCT are associated with GVHD initially came from the observation that their incidence was lower after autologous than after allogeneic SCT. In line with these findings, T-cell depletion lowered the incidence of both GVHD and lung injury. The association between chronic GVHD and BO is well accepted, mainly on the basis of consistent epidemiological data. However, experimental and epidemiological data on the role of GVHD in the development of IPS are more contradictory, underlining the lack of a clear mechanistic basis. Epithelial cell apoptosis, the classical GVHD histopathology finding, is not a consistent finding in IPS. However, epithelial cell apoptosis is not a requirement of GVHD pathology, and lung biopsies may be difficult to interpret because of nonspecific changes occurring, for example, during mechanical ventilation and suboptimal biopsy specimens owing to the risks associated with the procedure.

Experimental models have identified a role for alloreactive T cells as well as inflammatory mediators and antigen-presenting cells in the pathogenesis of IPS, as recently reviewed. More specifically, expression of the chemokine ligand RANTES by donor T cells contributes to leukocyte recruitment to the lung. Interestingly, it has been observed in a murine model that T-cell depletion of the allograft significantly reduced but did not eliminate pulmonary toxicity. Mature donor T cells were significantly increased in the BAL fluid of mice that received allogeneic T cells as compared to syngeneic controls even in the absence of GVHD, giving weight to the theory that host reactive donor T cells play a central role rather than GVHD per se. In addition, in an allogeneic study using a low T-cell dose ($0.2–1.0 \times 10^7$/kg), 6.4% of patients died of IPS, while none of them developed significant GVHD. Yet novel insights come from the growing experience with non-myeloablative SCT. Recently, it was shown in a group of 53 patients that the rate of non-infectious pulmonary toxicity was low after non-myeloablative conditioning without irradiation: infectious pneumonia occurred in nine patients, two patients developed IPS and no cases of DAH or BO were encountered. The overall incidences of acute GVHD grade I/II, grade III/IV and extensive chronic GVHD were 19, 19 and 11%, respectively. Another report demonstrated that reduced-intensity conditioning decreased the incidence of BO, whereas chronic GVHD was not a risk factor. Moreover, non-myeloablative patients experienced a significantly lower rate of lung function decline after SCT than myeloablative patients. Both the intensity of the conditioning regimen (conventional versus non-myeloablative) and the occurrence of severe acute GVHD (grades III–IV) were prognostic for the development of IPS in a multiple regression analysis. In our large cohort, patients with pulmonary complications and controls were comparable with respect to well-known risk factors for lung injury, and acute GVHD $\geq$ grade II was significantly related with pulmonary toxicity. There was no difference in the incidence of acute GVHD between patients with infectious and those with non-infectious pulmonary complications. We could not identify viral respiratory tract infections as a risk factor for progression to lung injury.

This study has several limitations. Owing to the retrospective nature of this study, we might have missed a single patient with pulmonary problems, in spite of our detailed computerized database and extensive chart review. Moreover, we cannot rule out any undetermined relevant differences between the cases and controls for which we did not match. Nevertheless, our focus was to assess the influence of GVHD on the development of pulmonary diseases. These data illustrate that the incidence of pulmonary complications is low after partial T-cell-depleted SCT and demonstrate a clear association with acute GVHD. Still, improvement of the poor outcome of pulmonary complications is of utmost importance.

**Acknowledgements**

We thank Dr P Westers, statistician at the Department of Biostatistics, for his help in the statistical analysis of this study. We are also grateful to Dr E Meijer (Department of Hematology, University Medical Center Utrecht) and Dr MGJ van Kraaij (Department of Hematology, University Medical Center Nijmegen) for providing their data.
References

1 Breuer R, Lososs IS, Berkman N, Or R. Pulmonary complications of bone marrow transplantation. Respir Med 1993; 87: 571–579.
2 Cordonnier C, Bernaudin JF, Bierling P, Huet Y, Vernant JP. Pulmonary complications occurring after allogeneic bone marrow transplantation. A study of 130 consecutive transplanted patients. Cancer 1986; 58: 1047–1054.
3 Krowka MJ, Rosenow III EC, Hoagland HC. Pulmonary complications of bone marrow transplantation. Chest 1985; 87: 237–246.
4 Afessa B, Tefferi A, Lizutow MR, Krowka MJ, Wyland ME, Peters SG. Diffuse alveolar hemorrhage in hematopoietic stem cell transplant recipients. Am J Respir Crit Care Med 2002; 166: 641–645.
5 Chen CS, Boeckh M, Seidel K, Clark JG, Kansu E, Madtes DK et al. Incidence, risk factors, and mortality from pneumonia developing late after hematopoietic stem cell transplantation. Bone Marrow Transplant 2003; 32: 515–522.
6 Freudenberger TD, Madtes DK, Curtis JR, Cummings P, Storer PE, Hackman RC. Association between acute and chronic graft-versus-host disease and bronchiolitis obliterans organizing pneumonia in recipients of hematopoietic stem cell transplants. Blood 2003; 102: 3822–3828.
7 Fukuda T, Hackman RC, Guthrie KA, Sandmeier BM, Boeckh M, Maris MB et al. Risks and outcomes of idiopathic pneumonia syndrome after nonmyeloablative and conventional conditioning regimens for allogeneic hematopoietic stem cell transplantation. Blood 2003; 102: 2777–2785.
8 Ho VT, Weller E, Lee SJ, Alyea EP, Antin JH, Soiffer RJ. Prognostic factors for early and severe pulmonary complications after hematopoietic stem cell transplantation. Blood 2001; 7: 223–229.
9 Kantrow SP, Hackman RC, Boeckh M, Myerson D, Crawford SW. Idiopathic pneumonia syndrome: changing spectrum of lung injury after marrow transplantation. Transplantation 1997; 63: 1079–1086.
10 Nusair S, Breuer R, Shapira MY, Berkman N, Or R. Low incidence of pulmonary complications following nonmyeloablative stem cell transplantation. Eur Respir J 2004; 23: 440–445.
11 Palmas A, Tefferi A, Myers JL, Scott JP, Swensen SJ, Chen MG et al. Late-onset noninfectious pulmonary complications after allogeneic bone marrow transplantation. Br J Haematol 1998; 100: 680–687.
12 Sakaida E, Nakaseko C, Harima A, Yokota A, Cho R, Saito Y et al. Late-onset noninfectious pulmonary complications after allogeneic stem cell transplantation are significantly associated with chronic graft-versus-host disease and with the graft-versus-leukemia effect. Blood 2003; 102: 4236–4242.
13 Savani BN, Montero A, Wu C, Nlonza N, Read E, Dunbar C et al. Prediction and prevention of transplant-related mortality from pulmonary causes after total body irradiation and allogeneic stem cell transplantation. Blood Marrow Transplant 2005; 11: 223–230.
14 Wong R, Rondon G, Saliba RM, Shannon VR, Giralt SA, Champlin RE et al. Idiopathic pneumonia syndrome after high-dose chemotherapy and autologous hematopoietic stem cell transplantation for high-risk breast cancer. Bone Marrow Transplant 2003; 31: 1157–1163.
15 Clark JG, Hansen JA, Hertz MI, Parkman R, Jensen L, Peavy HH. NHLBI workshop summary. Idiopathic pneumonia syndrome after bone marrow transplantation. Am Rev Respir Dis 1993; 147: 1601–1606.
16 Crawford SW, Hackman RC. Clinical course of idiopathic pneumonia after bone marrow transplantation. Am Rev Respir Dis 1993; 147: 1393–1400.
17 Weiner RS, Bortin MM, Gale RP, Gluckman E, Kay HE, Kolb HJ et al. Interstitial pneumonitis after bone marrow transplantation. Assessment of risk factors. Ann Intern Med 1986; 104: 168–175.
18 Cooke KR, Yanik G. Acute lung injury after allogeneic stem cell transplantation: is the lung a target of acute graft-versus-host disease? Bone Marrow Transplant 2004; 34: 753–765.
19 Crawford SW, Clark JG. Bronchiolitis associated with bone marrow transplantation. Clin Chest Med 1993; 14: 741–749.
20 Holland HK, Wingard JR, Beschorner WE, Saral R, Santos GW. Bronchiolitis obliterans in bone marrow transplantation and its relationship to chronic graft-versus-host disease and low serum IgG. Blood 1988; 72: 621–627.
21 Afessa B, Lizutow MR, Tefferi A. Bronchiolitis obliterans and other late onset non-infectious pulmonary complications in hematopoietic stem cell transplantation. Bone Marrow Transplant 2001; 28: 425–434.
22 Weisdorf DJ. Diffuse alveolar hemorrhage: an evolving problem? Leukemia 2003; 17: 1049–1050.
23 Verdonck LF, Dekker AW, de Gast GC, van Kempen ML, Lokhorst HM, Nieuwhuis HK. Allogeneic bone marrow transplantation with a fixed low number of T cells in the marrow graft. Blood 1994; 83: 3090–3096.
24 Thomas ED, Storb R, Cilié RA, Fefer A, Johnson L, Neiman PE et al. Bone-marrow transplantation (second of two parts). N Engl J Med 1975; 292: 895–902.
25 van Elden LJ, van Loon AM, van Alphen F, Hendriks KA, Hoeapelman AL, van Kraaij MG et al. Frequent detection of human coronaviruses in clinical specimens from patients with respiratory tract infection by use of a novel real-time reverse-transcriptase polymerase chain reaction. J Infect Dis 2004; 189: 652–657.
26 van Kraaij MG, van Elden LJ, van Loon AM, Hendriks KA, Laterveer L, Dekker AW et al. Frequent detection of respiratory viruses in adult recipients of stem cell transplants with the use of real-time polymerase chain reaction, compared with viral culture. Clin Infect Dis 2005; 40: 662–669.
27 Wingard JR, Sostrin MB, Vriesendorp HM, Mellits ED, Santos GW, Fuller DJ et al. Interstitial pneumonitis following autologous bone marrow transplantation. Transplantation 1988; 46: 61–65.
28 Pecego R, Hill R, Appelbaum FR, Amos D, Bruckner CD, Feder A et al. Interstitial pneumonitis following autologous bone marrow transplantation. Transplantation 1986; 42: 515–517.
29 Breuer R, Or R, Lijovetzky G, Naparstek E, Engelhard D, Lafair J et al. Interstitial pneumonitis in T cell-depleted bone marrow transplantation. Bone Marrow Transplant 1988; 3: 622–630.
30 Clark JG, Schwartz DA, Flournoy N, Sullivan KM, Crawford SW, Thomas ED. Risk factors for airflow obstruction in recipients of bone marrow transplants. Ann Intern Med 1987; 107: 648–656.
31 Curtis DJ, Smale A, Thien F, Schwarzer AP, Szer J. Chronic airflow obstruction in long-term survivors of allogeneic bone marrow transplantation. Bone Marrow Transplant 1995; 16: 169–173.
32 Urbanski SJ, Kossakowska AE, Curtis J, Chan CK, Hutcheon MA, Hyland RH et al. Idiopathic small airways pathology in patients with graft-versus-host disease following allogeneic bone marrow transplantation. Am J Surg Pathol 1987; 11: 965–971.
33 Wyatt SE, Nunn P, Hows JM, Yin J, Hayes MC, Catovsky D et al. Airways obstruction associated with graft versus host
disease after bone marrow transplantation. *Thorax* 1984; **39**: 887–894.

34. Beschorner WE, Saral R, Hutchins GM, Tutschka PJ, Santos GW. Lymphocytic bronchitis associated with graft-versus-host disease in recipients of bone-marrow transplants. *N Engl J Med* 1978; **299**: 1030–1036.

35. Sloane JP, Depledge MH, Powles RL, Morgenstern GR, Trickey BS, Dady PJ. Histopathology of the lung after bone marrow transplantation. *J Clin Pathol* 1983; **36**: 546–554.

36. Cooke KR, Krenger W, Hill G, Martin TR, Kobzik L, Brewer J *et al.* Host reactive donor T cells are associated with lung injury after experimental allogeneic bone marrow transplantation. *Blood* 1998; **92**: 2571–2580.

37. Hildebrandt GC, Olkiewicz KM, Choi S, Corrion LA, Clouthier SG, Liu C *et al.* Donor T-cell production of RANTES significantly contributes to the development of idiopathic pneumonia syndrome after allogeneic stem cell transplantation. *Blood* 2005; **105**: 2249–2257.

38. Yoshihara S, Tateishi U, Ando T, Kunitoh H, Suyama H, Onishi Y *et al.* Lower incidence of bronchiolitis obliterans in allogeneic hematopoietic stem cell transplantation with reduced-intensity conditioning compared with myeloablative conditioning. *Bone Marrow Transplant* 2005; **35**: 1195–1200.

39. Chien JW, Madtes DK, Clark JG. Pulmonary function testing prior to hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2005; **35**: 429–435.