Transplant Infectious Disease, ISSN 1398-2273

Late-onset severe pneumonia after allogeneic hematopoietic stem cell transplantation: prognostic factors and treatments

X.-D. Mo, X.-H. Zhang, L.-P. Xu, Y. Wang, C.-H. Yan, H. Chen, Y.-H. Chen, W. Han, F.-R. Wang, J.-Z. Wang, K.-Y. Liu, X.-J. Huang. Late-onset severe pneumonia after allogeneic hematopoietic stem cell transplantation: prognostic factors and treatments.
Transpl Infect Dis 2016: **18**: 492–503. All rights reserved

Abstract: *Background.* In this study, we aimed to evaluate the prognostic factors associated with and treatments for late-onset severe pneumonia (LOSP) in patients who underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT).
Methods. Fifty consecutive patients who underwent non-T-cell-depleted allo-HSCT at the Peking University Institute of Hematology and met the criterion of LOSP after allo-HSCT were enrolled.
Results. The median time from allo-HSCT to the occurrence of LOSP was 231 (90–1487) days. Twenty-eight patients harbored 1 or more pathogens (infectious LOSP, I-LOSP), whereas 22 did not harbor any pathogens (non-infectious LOSP, NI-LOSP). The 100-day survival rate of LOSP patients was 31.1%. Patients smoking before allo-HSCT (0% vs. 35.4%, $P = 0.002$) and male gender (20.0% vs. 61.9%, $P = 0.026$) had lower 100-day survival rate. Patients with a lower bronchoalveolar lavage fluid (BALF) neutrophil percentage had higher 100-day survival rate relative to those with higher BALF neutrophil percentage (45.5% vs. 16.7%, $P = 0.012$). The 100-day survival rate of patients with I-LOSP was lower than that of patients with NI-LOSP (19.1% vs. 46.9%, $P = 0.043$). Patients given late (≥1 week after LOSP diagnosis) and low-dose methylprednisolone (MP) therapy (≤2 mg/kg/day) had the best 100-day survival rate. In the multivariate analysis, nonsmoking before allo-HSCT and late and low-dose MP therapy were significantly associated with a better survival after LOSP.
Conclusion. LOSP is a severe complication after allo-HSCT. The correct timing and corticosteroid dosage in the context of broad-spectrum antimicrobial therapy might further improve the outcomes of patients with LOSP.

X.-D. Mo[1], X.-H. Zhang[1], L.-P. Xu[1], Y. Wang[1], C.-H. Yan[1], H. Chen[1], Y.-H. Chen[1], W. Han[1], F.-R. Wang[1], J.-Z. Wang[1], K.-Y. Liu[1], X.-J. Huang[1,2,3]

[1]Peking University People's Hospital, Peking University Institute of Hematology, Beijing, China, [2]Peking-Tsinghua Center for Life Sciences, Beijing, China, [3]Beijing Key Laboratory of Hematopoietic Stem Cell Transplantation, Beijing, China

Key words: late-onset severe pneumonia; allogeneic hematopoietic stem cell transplantation; methylprednisone

Correspondence to:
Prof. Xiao-Jun Huang, Peking University People's Hospital, Peking University Institute of Hematology, No. 11 Xizhimen South Street, Xicheng District, Beijing 100044, China
Tel: 8610-8832-6006
Fax: 8610-8832-4577
E-mail: huangxiaojun@bjmu.edu.cn

Received 19 September 2015, revised 24 January 2016, accepted for publication 23 February 2016

DOI: 10.1111/tid.12553
Transpl Infect Dis 2016: **18:** 492–503

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective treatment for hematological malignancies, by which many patients are cured or achieve long-term remission. Although transplant techniques have progressed significantly, treatment-related mortality continues to seriously influence the survival of patients who have undergone allo-HSCT, and severe pulmonary complications are considered among the worst complications associated with this procedure. Infectious and non-infectious pulmonary complications are found in 68% and 29% of HSCT recipients, respectively (1). Severe pulmonary complications, therefore, account for a significant percentage of deaths after transplantation.

Pulmonary complications (infectious or non-infectious) commonly occur within 3 months after transplantation (2). As increasing numbers of patients achieve long-term survival, further attention should be given to late-onset pulmonary complications (≥3 months after transplantation). Chen et al. (3) observed that during a

median follow-up of 2 years, 25% of patients developed at least 1 episode of pneumonia after transplantation, and the cumulative incidence of a first pneumonia episode at 4 years ranged from 18% to 39%. We observed that some patients suffered from acute severe pneumonia >3 months after allo-HSCT and that these patients had some common features, such as rapid progression, an absence of pathogens, and high mortality. Liu et al. (4) reported a study of 20 patients who experienced late-onset severe pneumonia (LOSP) after allo-HSCT. The incidence of LOSP was 1.3%; among the affected patients, only 8 tested positive for pathogens, and 11 died despite receiving comprehensive therapy. It has been suggested that LOSP might be a severe complication affecting the clinical outcomes of allo-HSCT recipients. However, relatively few studies have addressed the prognostic factors associated with and treatments for LOSP after allo-HSCT.

In this retrospective study, we therefore aimed to evaluate these prognostic factors and treatments in patients with LOSP after undergoing human leukocyte antigen (HLA)-related donor HSCT.

Patients and methods

Patients

Consecutive patients who underwent non-T-cell-depleted allo-HSCT at the Peking University Institute of Hematology from June 1, 2012 to June 30, 2015 were enrolled if they met the criteria used to define LOSP after allo-HSCT and underwent chest computerized tomography (CT) and bronchoalveolar lavage (BAL). Patients who experienced severe pneumonia within 3 months after HSCT and those who did not receive CT or BAL were excluded. A total of 50 patients were enrolled; 16 and 34 had undergone HLA-identical sibling donor HSCT and HLA-haploidentical related donor (haplo-RD) HSCT, respectively (Table 1). The final follow-up visits for the endpoint analysis were conducted in September 1, 2015. Informed consent was obtained from all patients, and the study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of Peking University People's Hospital. Five included patients were previously reported by Liu et al. (4).

Transplantation regimen

Preconditioning comprised cytarabine, busulfan (3.2 mg/kg/day, days −8 to −6), cyclophosphamide (1.8 g/m^2/day, days −5 to −4), and simustine (250 mg/m^2, day −3). Cytarabine was administered at 4 g/m^2/day (days −10 to −9) in the haplo-RD group and 2 g/m^2/day (day −9) in the identical sibling donor group, and rabbit anti-thymocyte globulin (2.5 mg/kg/day, days −5 to −2; Thymoglobulin, Sanofi, France) was administered in the haplo-RD group (5, 6). Granulocyte colony-stimulating factor-mobilized, fresh, and unmanipulated harvested bone marrow and peripheral blood were infused into the recipients on the same day as collection. Granulocyte colony-stimulating factor (5 μg/kg/day subcutaneously) was administered to all haplo-RD HSCT recipients from day 6 after transplantation until their white blood cell counts exceeded 2×10^9 cells/L for 3 consecutive days. In addition, patients received cyclosporine, mycophenolate mofetil, and short-term methotrexate for graft-versus-host disease (GVHD) prophylaxis (7, 8). Comorbidities in HSCT recipients were assessed according to the hematopoietic cell transplantation-specific comorbidity index (9). The donor selection, HLA typing, and stem cell harvesting procedures have been described elsewhere (10).

Infection prevention regimen

All patients were hospitalized in rooms with high efficiency particle-air filters for 4–5 weeks, from day −10 to the time at which neutrophil recovery was achieved. All received antibiotic prophylaxis with oral trimethoprim-sulfamethoxazole to prevent *Pneumocystis jirovecii* infection from days −10 to +180. Patients also received fluconazole for *Candida albicans* from days −10 to +75, acyclovir for herpex simplex virus (HSV) and varicella zoster virus (VZV) from day +1 to the time of cyclosporine discontinuation, and ciprofloxacin for intestinal decontamination. Ganciclovir (5 mg/kg) was administered intravenously (IV) twice daily from days −9 to −2 for prophylaxis against cytomegalovirus (CMV) infection. The infection surveillance and treatment protocols used at our institute were previously described elsewhere (11–13).

Definition and management of LOSP

The following major criteria were used to define LOSP: (i) invasive mechanical ventilation and (ii) septic shock requiring vasopressors. The following minor criteria were used: (i) a respiratory rate ≥30 breaths/min; (ii) arterial oxygen pressure/fraction of inspired oxygen ratio ≤250; (iii) multilobar infiltrate; (iv) confusion/

Patient characteristics

Characteristics	NI-LOSP group (n = 22)	I-LOSP group (n = 28)	P-value
Median age at HSCT, years (range)	43 (20–59)	37 (15–62)	0.591
Median time from HSCT to pneumonia, days (range)	234 (90–1054)	206 (91–697)	0.494
Gender, *n* (%)			
Male	17 (77.3)	19 (67.9)	0.537
Female	5 (22.7)	9 (32.1)	
Smoking pre-HSCT, *n* (%)	1 (4.5)	5 (17.9)	0.211
Diagnosis, no. (%)			
AML	6 (27.3)	13 (46.4)	0.025
ALL	6 (27.3)	11 (39.3)	
MDS	3 (13.6)	4 (14.3)	
CML	5 (22.7)	0 (0.0)	
Others	2 (9.1)	0 (0.0)	
Disease status at transplantation, *n* (%)			
Standard risk	15 (68.2)	25 (89.3)	0.084
High risk	7 (31.8)	3 (10.7)	
Immunosuppression discontinued when pneumonia occurred, *n* (%)	7 (31.8)	13 (46.4)	0.295
Donor–recipient gender match, *n* (%)			
Male–male	12 (54.5)	8 (28.6)	0.245
Male–female	4 (18.2)	5 (17.9)	
Female–male	5 (22.7)	11 (39.3)	
Female–female	1 (4.6)	4 (14.2)	
Donor–recipient relation, *n* (%)			
Identical sibling donor	9 (40.9)	6 (21.4)	0.214
Haploidentical related donor	13 (59.1)	22 (78.6)	
Number of HLA-A, HLA-B, HLA-DR mismatches, *n* (%)			
0	10 (45.5)	6 (21.4)	0.175
2	3 (13.6)	4 (14.3)	
3	9 (40.9)	18 (64.3)	
Pre-HSCT EBV status, donor/recipient, *n* (%)			
Positive/negative	0 (0.0)	2 (7.1)	0.497
Others	22 (100.0)	26 (92.9)	
Pre-HSCT CMV status, donor/recipient, *n* (%)			
Positive/negative	2 (9.1)	2 (7.1)	1.000
Others	20 (90.9)	26 (92.9)	
Lung function test before HSCT, % (range)			
FEV1%	92.7 (71.0–107.2)	96.8 (81.1–116.4)	0.172
DLco%	97.8 (76.0–157.0)	106.6 (55.3–234.3)	0.874
HCT-CI before HSCT, *n* (%)			
0	12 (54.5)	17 (60.7)	0.923
1–2	7 (31.8)	8 (28.6)	
≥3	3 (13.7)	3 (10.7)	

Table 1 Continued

Characteristics	NI-LOSP group (n = 22)	I-LOSP group (n = 28)	P-value
Acute GVHD prior to pneumonia, n (%)			
None	16 (72.7)	12 (42.9)	0.088
Grade I	1 (4.7)	4 (14.3)	
Grade II-IV	5 (22.6)	12 (42.8)	
Chronic GVHD prior to pneumonia, n (%)			
None	14 (63.6)	21 (75.0)	0.460
Mild	1 (4.6)	1 (3.6)	
Moderate	2 (9.1)	4 (14.3)	
Severe	5 (22.7)	2 (7.1)	
Survived after pneumonia, n (%)	9 (40.9)	6 (21.4)	0.214
Median duration of follow-up after pneumonia, days (range)	43 (7–2028)	33 (6–1229)	0.087

The level of statistical significance was set at $P < 0.05$.
NI-LOSP, non-infectious late-onset severe pneumonia; I-LOSP, infectious late-onset severe pneumonia; HSCT, hematopoietic stem cell transplantation; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; CML, chronic myelocytic leukemia; HLA, human leukocyte antigen; EBV, Epstein–Barr virus; CMV, cytomegalovirus; FEV1, forced expiratory volume in 1 sec; DLco, diffusion capacity of carbon monoxide; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; GVHD, graft-versus-host disease.

Table 1

disorientation; (v) uremia (blood urea nitrogen level ≥20 mg/dL); (vi) leukopenia resulting solely from infection (white blood cell count $<4.0 \times 10^9$ cells/L); (vii) thrombocytopenia (platelet count $<100 \times 10^9$ cells/L); (viii) hypothermia (core temperature, <36°C); and (ix) hypotension requiring aggressive fluid resuscitation (14). No evidence of cardiogenic pulmonary edema was observed according to clinical findings, central venous pressure measurements, or cardiac echocardiography. Patients who experienced pneumonia >3 months after allo-HSCT and exhibited 1 major criterion or 3 minor criteria were diagnosed as having LOSP.

All patients underwent CT and BAL. Blood and BAL fluid (BALF) samples were routinely subjected to the following tests: (i) Gram stain, fungal stain, Grocott-Gomori methenamine-silver stain, and acid-fast bacilli stain; (ii) cytology examination; (iii) bacterial and fungal culture; and (iv) real-time polymerase chain reaction (PCR) and reverse transcription-PCR assays for the detection of atypical bacteria (e.g., *Legionella* species, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*), herpesviruses (HSV types 1 and 2, Epstein–Barr virus [EBV], CMV, VZV, and human herpesvirus-6), respiratory viruses (respiratory syncytial virus [RSV], parainfluenza virus, influenza types A and B, human metapenumovirus, human rhinoviruses, human coronaviruses [CoVs; OC43, 229E, NL63, and HKU1], and human bocavirus), polyomaviruses (BK virus and JC virus), adenovirus, parvovirus B19, norovirus, and enterovirus (coxsackie virus and enterovirus 71) (15). For positive CMV and EBV targets, the pathogen load was determined by quantitative PCR.

All patients with LOSP received oxygen therapy, empirical broad-spectrum antimicrobial therapies (covering *Pseudomonas aeruginosa*, methicillin-resistant *Staphylococcus aureus*, *Aspergillus* [itraconazole: 200 mg IV, 12 hourly for 48 h then 200 mg IV daily; voriconazole: 6 mg/kg IV, 12-hourly for 24 h then 4 mg/kg IV, 12-hourly; caspofungin: 70-mg loading dose, then 50 mg IV, daily; micafungin: 100–150 mg IV, daily; liposomal amphotericin B: 1–3 mg/kg IV, daily; and amphotericin B deoxycholate: 0.5–1.0 mg/kg IV, daily], and *P. jiroveciii*), and corticosteroids. Some patients received donor lymphocyte infusions (DLIs). Patients with increased hypoxemia despite receiving high levels of supplemental nasal oxygen were transferred to the intensive care unit (ICU), where some required noninvasive ventilation or endotracheal intubation.

Definitions and assessments

High-risk patients were defined as follows: (i) acute leukemia patients in the first or second complete

remission with a cytogenetic marker of "poor-risk," including *t(4,11)* and *t(9,22);* (ii) patients in complete remission after third complete remission; (iii) patients in partial remission, nonremission, or in a state of relapse before HSCT; and (iv) patients with chronic myelogenous leukemia beyond the first chronic phase. All other patients were stratified into standard-risk categories (7). Neutrophil engraftment was defined as the first day when the absolute neutrophil count was $\geq 0.5 \times 10^9$/L for 3 consecutive days, and platelet engraftment was defined as the first day when the platelet count was $\geq 20 \times 10^9$/L for 7 consecutive days without transfusion. GVHD was diagnosed in accordance with the accepted international criteria (16, 17). Overall survival (OS) was defined as the time from pneumonia to death from any cause or the date of last contact. Infectious LOSP (I-LOSP) was defined as the identification of any pathogen in blood and/or BALF samples; non-infectious LOSP (NI-LOSP) was defined as the absence of pathogens in blood and/or BALF samples.

Statistical analysis

Data were censored at the time of death or the last available follow-up. Continuous variables were compared using the Mann–Whitney *U*-test; categorical variables were compared using the chi-squared test and Fisher's exact test. The Kaplan–Meier method was used to estimate the probability of OS. A landmark analysis was performed to assess the survival of patients with LOSP. The post-transplant day of LOSP diagnosis was defined as the landmark day. OS was calculated from the landmark day to death from any cause or the date of last contact. Potential prognostic factors for 100-day OS after LOSP were evaluated through univariate and multivariate analyses, using Cox proportional hazards regression with a backward-stepwise model selection approach. Independent variables with *P*-values >0.1 were sequentially excluded from the model, and the level of significance was set at $P < 0.05$. All reported *P*-values were based on 2-sided tests. Data analyses were conducted with SPSS software (SPSS Inc., Chicago, Illinois, USA).

Results

Patient characteristics

Table 1 summarizes the characteristics of the 50 patients with LOSP in this study (NI-LOSP, $n = 22$; I-LOSP, $n = 28$). The median value of diffusion capacity of carbon monoxide (DLco) percentage and forced expiratory volume in 1 sec (FEV1) percentage before HSCT was 102.4% (55.3–234.3%) and 96.6% (71.0–116.4%), respectively. Ten patients had prior pneumonia, caused by *Aspergillus* ($n = 8$) and *Mycobacterium tuberculosis* ($n = 2$), respectively, and 9 were given secondary prophylaxis (itraconazole: $n = 4$; caspofungin: $n = 2$; voriconazole: $n = 1$; isoniazid and ethambutol: $n = 2$). A chimerism analysis indicated that all patients achieved full donor chimerism by day 30. In addition, all patients achieved neutrophil engraftment within 30 days after HSCT, with a median time to neutrophil engraftment of 15 (range, 10–23) days. During the follow-up period, 47 patients exhibited platelet engraftment, with a median time to platelet engraftment of 17 (range, 8–120) days.

The median time from transplantation to the occurrence of LOSP was 231 (90–1487) days. All patients exhibited cough and dyspnea. The median temperature at LOSP diagnosis was 38.0 (36.4–40.0)°C. The median respiratory rate was 23 (14–45) breaths/min. One patient exhibited confusion, and 2 patients experienced septic shock. Thirty-six patients were transferred to the ICU; 21 required noninvasive ventilation, and 31 required endotracheal intubation. Thirty-four of the 35 patients who died after treatment, died of LOSP, and the median duration from LOSP diagnosis to death was 29 (6–82) days. The remaining patient was cured after treatment but died of relapse 275 days after LOSP. The OS rate at 100 days after LOSP was 31.1% (Fig. 1A). Patients smoking before allo-HSCT (0% vs. 35.4%, $P = 0.002$) and male gender (20.0% vs. 61.9%, $P = 0.026$, Fig. 1B) had lower 100-day survival rate. However, pre-HSCT lung function test did not influence the 100-day OS rate of LOSP patients (FEV1%: ≥median vs. <median: 31.3% vs. 30.7%, $P = 0.761$; DLco%: ≥median vs. <median: 26.0% vs. 39.5%, $P = 0.245$).

Chest CT and BALF

All patients underwent chest CT. For patients who underwent multiple chest CT examinations, only the results from the initial scan were included in this analysis. A patchy appearance was the most common presentation ($n = 35$), followed by ground-glass opacity ($n = 30$), pleural effusion ($n = 26$), fibrous stripes ($n = 20$), consolidation opacity ($n = 18$), and nodules ($n = 7$). The chest CT characteristics were comparable between the NI-LOSP and I-LOSP groups (Table 2).

All patients underwent BAL. For patients who underwent multiple BAL procedures, only the initial results

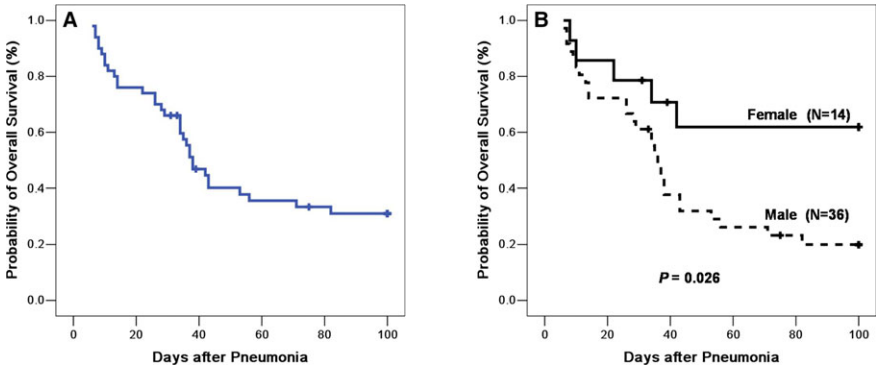

Fig. 1. Probability of overall survival at 100 days after late-onset severe pneumonia (A) in the total study population and (B) according to gender.

Characteristics of late-onset severe pneumonia after transplantation

Characteristics	NI-LOSP group (n = 22)	I-LOSP group (n = 28)	P-value
Median temperature at pneumonia diagnosis, °C (range)	38.0 (36.5–40.0)	38.2 (36.4–40.0)	0.914
Median breaths per minute at pneumonia diagnosis, n (range)	20 (18–33)	28 (14–45)	0.008
Median value of blood test at pneumonia diagnosis			
WBC, $\times 10^9$ cells/L (range)	5.2 (1.4–14.0)	4.0 (0.3–16.6)	0.452
ANC, $\times 10^9$ cells/L (range)	2.6 (0.0–12.0)	3.0 (0.0–15.2)	0.777
LYC, $\times 10^9$ cells/L (range)	0.7 (0.0–3.8)	0.9 (0.0–6.8)	0.710
CRP, mg/L (range)	60.0 (1.2–127.0)	71.1 (2.7–430.0)	0.066
ESR, mm/h (range)	54.0 (9.0–102.0)	66.0 (12.0–143.0)	0.143
Arterial partial oxygen pressure, mmHg (range)	76.5 (55.7–130.0)	74.1 (33.0–120.0)	0.282
Oxygenation index (range)	166.0 (55.0–328.6)	117.8 (64.5–329.8)	0.401
Median BALF cell subset values at pneumonia diagnosis, $\times 10^6$ cells/L (range)			
Absolute total cell count	2.25 (0.70–69.00)	2.40 (0.04–57.2)	0.915
Absolute lymphocyte count	0.61 (0.03–28.98)	0.36 (0.02–8.01)	0.765
Absolute macrophage count	0.94 (0.05–31.05)	0.65 (0.08–18.88)	0.348
Absolute neutrophil count	0.26 (0.00–38.48)	0.52 (0.00–30.32)	0.237
Chest CT, n (%)			
Consolidation opacity	5 (22.7)	13 (46.4)	0.083
Patch	15 (68.2)	20 (71.4)	0.804
Nodule	1 (4.5)	6 (21.4)	0.117
Ground-glass opacity	12 (54.5)	18 (64.3)	0.485
Fibrous stripes	7 (31.8)	13 (46.4)	0.295
Pleural effusion	9 (40.9)	17 (60.7)	0.164
ICU administration, n (%)	13 (59.1)	23 (82.1)	0.072
Median duration of ICU administration, days (range)	9.0 (3.0–31.0)	13.0 (3.0–63.0)	0.253
Need for mechanical ventilation, n (%)	10 (45.5)	21 (75.0)	0.033
Median duration of mechanical ventilation, days (range)	4.0 (1.0–20.0)	8.0 (1.0–60.0)	0.053

The level of statistical significance was set at $P < 0.05$.
NI-LOSP, non-infectious late-onset severe pneumonia; I-LOSP, infectious late-onset severe pneumonia; WBC, white blood cell; ANC, absolute neutrophil count; LYC, lymphocyte; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; BALF, bronchoalveolar lavage fluid; CT, computed tomography; ICU, intensive care unit.

Table 2

were included in this analysis. The median BALF cell subset values were comparable between the NI-LOSP and I-LOSP groups (Table 2). Patients who died after LOSP had significantly higher BALF neutrophil counts than did survivors with LOSP (0.58×10^6 cells/L vs. 0.20×10^6 cells/L, $P = 0.030$), and patients with a higher BALF neutrophil percentage (using the median as the cutoff point) had a significantly lower survival rate than did the other patients (16.7% vs. 45.5%, $P = 0.012$; Figure S1).

Pathogens

Pathogens were identified in blood and/or BALF samples from 28 patients (I-LOSP); 16 had positive blood samples, 26 had positive BALF samples, and 14 had simultaneous positive blood and BALF samples. Fifteen and 13 patients had infections caused by a single and multiple organism, respectively (Tables 3 and 4). CMV was the most common established virus, as it was identified simultaneously in plasma and BALF samples from 5 patients, with median plasma and BALF viral loads of 1.71 (1.09–2.70) $\times 10^3$/copies and 2.41 (0.43–94.9) $\times 10^3$/copies, respectively. The second most common virus was EBV, which was identified simultaneously in plasma and BALF samples from 2 patients, with median plasma and BALF viral loads of 1.90 (1.30–2.49) $\times 10^3$/copies and 32.08 (0.85–63.30) $\times 10^3$/copies, respectively. The third most common virus was RSV, which was identified simultaneously in plasma and BALF samples from 1 patient. The most common bacterium was *Acinetobacter baumannii*, followed by *P. aeruginosa*, *Staphylococcus epidermidis*, and *Enterococcus faecium*. Several multiresistant bacteria were recovered (*A. baumannii*, $n = 4$; *P. aeruginosa*: $n = 1$; *E. faecium*: $n = 1$). *Candida* was identified in the BALF of 2 patients, which might suggest a bystander organism (Table 4). In the NI-LOSP group, 15, 5, and 2 patients could be classified as having idiopathic pneumonia syndrome (IPS), organizing pneumonia, and bronchiolitis obliterans syndrome, respectively. The mechanical ventilation rate was higher and the median mechanical ventilation duration was longer in the I-LOSP group (Table 2). The 100-day

Type of pneumonia

Type of pneumonia	Number
Infectious	28
Only viral	6
Only bacterial	7
Only fungal	2
Multiple organism	13
Non-infectious	22
Idiopathic pneumonia syndrome	15
Organizing pneumonia	5
Bronchiolitis obliterans syndrome	2

Table 3

Characteristics of pathogens

	Type of organism	No.
Multiple organism		
Multiple virus	CMV + RSV	2
	CMV + EBV	1
	EBV + RSV	1
	Coxsackie virus + RSV	1
	CMV + EBV + Influenza A virus+ Parainfluenza virus	1
Multiple bacteria	*Staphylococcus epidermidis + Enterococcus faecium*	1
	Pseudomonas aeruginosa + Klebsiella pneumonia + Stenotrophomonas maltophilia	1
Virus + fungal	EBV + CMV + *Aspergillus fumigatus*	1
	CMV + *Pneumocystis jirovecii*	1
Virus + bacterium	Influenza A virus + Coxsackie virus + *Acinetobacter baumannii*	1
Bacterium + fungal	*Enterococcus faecium + Candida albicans*	1
Virus + bacterium + fungal	EBV + Parvovirus B19 + *Enterobacter cloacae + Aspergillus flavus + Aspergillus niger*	1
Single organism		
Virus	CMV	4
	Adenoviridae	1
	Coxsackie virus	1
Bacterium	*Acinetobacter baumannii*	5
	Pseudomonas aeruginosa	1
	Staphylococcus epidermidis	1
Fungal	*Candida tropicalis*	1
	Pneumocystis jirovecii	1

CMV, cytomegalovirus; RSV, respiratory syncytial virus; EBV, Epstein–Barr virus.

Table 4

survival rate of the I-LOSP group was significantly lower than that of the NI-LOSP group (19.1% vs. 46.9%, $P = 0.043$, Fig. 2).

LOSP therapy

All patients with LOSP received oxygen therapy and empirical broad-spectrum antimicrobial therapies. Forty-two patients received trimethoprim–sulfamethoxazole. The initial antifungal drugs used for treatment in 47 cases were as follows: caspofungin for 28 cases, itraconazole for 13 cases, voriconazole for 10 cases, amphotericin B for 6 cases, and micafungin for 3 cases. Three patients received targeted antifungal therapy and the others received empirical antifungal therapy. Thirteen patients received combination antifungal therapy. Forty-four patients received antivirus therapy: IV ganciclovir or foscarnet sodium, 43 patients; IV ribavirin, 5 patients (RSV: $n = 4$; parainfluenza virus: $n = 1$); and oral oseltamivir, 4 patients. Ten patients received combination antiviral therapy. Patients who received ribavirin therapy tended to have better OS (75.0% vs. 26.8%, $P = 0.088$; Figure S2A), whereas no associations were observed between the other anti-infection therapies and outcomes (data not shown).

All patients received corticosteroid therapy. The initial methylprednisolone (MP) dose was 1.3 (0.2–15.38) mg/kg/day, and the median time from LOSP diagnosis to MP therapy was 7 (0–43) days. Patients initially given low-dose MP (≤2 mg/kg/day) had a better 100-day OS rate after LOSP than did patients initially given high-dose MP (>2 mg/kg/day). In addition, the 100-day OS rate after LOSP for patients given late MP therapy (≥1 week after LOSP diagnosis) was better than that of patients given early MP therapy (<1 week after LOSP diagnosis). Patients who received late, low-dose MP therapy had the best outcomes (Fig. 3A–C).

Eight patients received DLI, and the median time from LOSP diagnosis to DLI was 12 (4–32) days. The median $CD3^+$ cell dose was 0.5 (0.3–0.6) $\times 10^8$ cells/kg. DLI did not improve survival among patients with LOSP (Figure S2B).

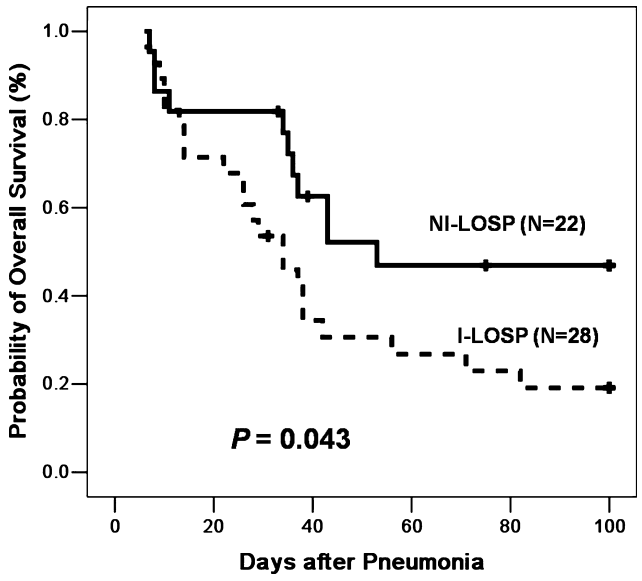

Fig. 2. Probability of overall survival at 100 days after late-onset severe pneumonia (LOSP) according to the detection of pathogens in blood and/or bronchoalveolar lavage fluid. LOSP, late onset severe pneumonia; NI, non-infectious; I, infectious.

Multivariate analysis

Female gender, nonsmoking before allo-HSCT, a lower BALF neutrophil percentage (using the median as the cutoff point), NI-LOSP, and late and low-dose MP therapy were associated with a better OS after LOSP in the univariate analysis. In the multivariate analysis, nonsmoking before allo-HSCT and late and low-dose MP therapy were significantly associated with a better OS after LOSP (Table 5).

Discussion

Although transplant techniques have progressed significantly, LOSP after allo-HSCT continues to seriously influence patient survival. In this study, the 100-day OS rate after LOSP was only 31.1%, despite the use of several therapies. We observed that patients who smoked before allo-HSCT had poorer outcomes and that the BALF neutrophil percentage might predict the outcomes of patients with LOSP. In addition, the correct MP dose and timing might further improve the outcomes of these patients.

In this study, blood and/or BALF samples from 28 of the 50 patients were positive for pathogens, indicating that infection may be an important cause of LOSP. Chen et al. (3) also observed that infection was an important cause of late-onset pneumonia after allo-HSCT. In addition, pathogens were not detected in 22 patients and most of them might thus meet the diagnostic criteria for IPS (18), suggesting that alloimmune reactions play an important role in the development of LOSP (19). However, Seo et al. (20) reported that more than half of the patients previously diagnosed with IPS were found to harbor pathogens according to

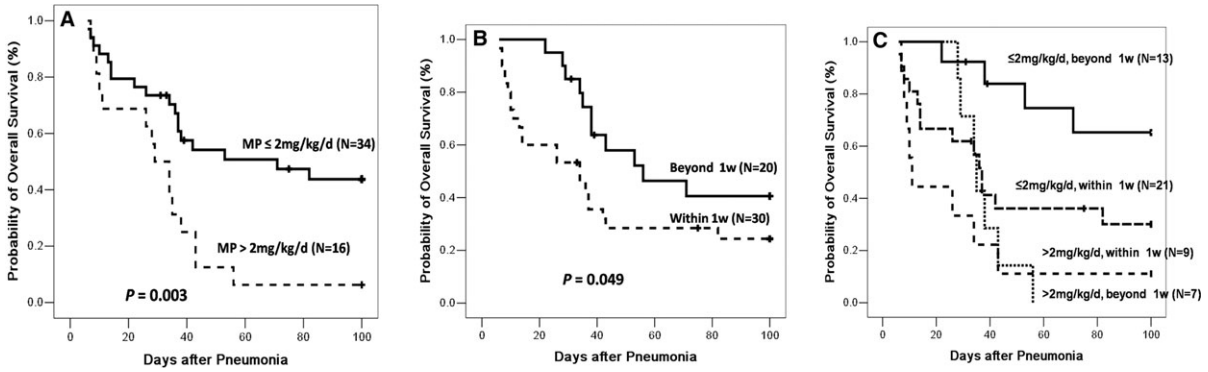

Fig. 3. Probability of overall survival at 100 days after late-onset severe pneumonia (LOSP) according to (A) initial corticosteroid dosage, (B) time from pneumonia diagnosis to corticosteroid therapy, and (C) both corticosteroid dosage and timing. Methylprednisone (MP) ≤2 mg/kg/day beyond 1 week after LOSP vs. MP <2 mg/k/day within 1 week after LOSP, $P = 0.027$; MP ≤2 mg/kg/day beyond 1 week after LOSP vs. MP >2 mg/k/day within 1 week after LOSP, $P = 0.001$; MP ≤2 mg/kg/day beyond 1 week after LOSP vs. MP >2 mg/k/day beyond 1 week after LOSP, $P = 0.001$.

Multivariate analyses of factors prognostic for 100-day overall survival after pneumonia

	Univariate analysis			Multivariate analysis		
Variable	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Gender						
Male	1.00		**0.034**			
Female	0.36	0.14–0.93				
Smoking pre-HSCT						
No	1.00		**0.005**	1.00		**0.033**
Yes	3.85	1.51–9.79		3.40	1.11–10.44	
Type of LOSP						
NI-LOSP	1.00		**0.050**			
I-LOSP	2.07	1.00–4.29				
BALF neutrophil percentage						
≤median	1.00		**0.001**			
>median	8.19	2.47–27.22				
Corticosteroid in LOSP						
MP ≤2 mg/kg/day beyond 1 week after LOSP	1.00		**0.006**	1.00		**0.013**
Others	4.32	1.51–12.37		4.04	1.35–12.08	

Bold indicates statistical significance ($P < 0.05$).
HR, hazard ratio; CI, confidence interval; HSCT, hematopoietic stem cell transplantation; LOSP, late-onset severe pneumonia; NI-LOSP, non-infectious late-onset severe pneumonia; I-LOSP, infectious late-onset severe pneumonia; BALF, bronchoalveolar lavage fluid; MP, methylprednisolone.

Table 5

currently available diagnostic methods, which suggests that infection remains the most important cause of LOSP. In addition, most infectious agents can trigger autoimmunity via different mechanisms (21), and many studies have observed that infections, particularly the viral infections, can trigger a graft-versus-host (GVH) reaction (22–24). Therefore, we suggest that the immune reactions in patients with NI-LOSP might also

have occult infectious etiologies. Although some pathogens detected in BALF likely represent the shedding of pulmonary pathogens rather than invasive disease (25), we observed higher CMV and EBV loads in BALF than in plasma. Patients with LOSP might be too ill to undergo further invasive examinations (e.g., biopsy for histological confirmation). In addition, Seo et al. (20) observed that IPS patients with detectable pathogens had a significantly worse 100-day OS rate, compared to patients without pathogens, which was in accordance with our results. Therefore, the correct method of identifying pathogens from blood and/or BALF might be critical to improving the outcomes of patients with LOSP.

We observed that the median duration from LOSP diagnosis to death was 29 days. We also observed that the median value of oxygenation index was <200, and as high as 62% (31/50) of the LOSP patients need mechanical ventilation. It is suggested that LOSP progresses rapidly and acute respiratory distress syndrome occurred in most of the patients. Therefore, our strategy of empirical broad-spectrum antimicrobial therapy administration was reasonable. In addition, we observed that the initial corticosteroid therapy dosage and timing were associated with the outcomes of patients with LOSP; notably, early and high-dose MP therapy did not improve the outcomes. The hypothesized benefit gained from corticosteroid administration for severe pneumonia might be due to reduced proinflammatory cytokine production, downregulated leukocyte adhesion protein expression, and the prevention of excessive alveolar collagen deposition (26). However, infection may be an important cause of LOSP, and high-dose MP did not help to eliminate the pathogens; additionally secondary infections might associate with high-dose MP therapy. Many studies found that low-dose corticosteroid therapy may improve the outcomes of patients with severe pneumonia (27, 28). In a systematic review by Lamontagne et al. (29), treatment with a corticosteroid dosage equivalent to ≤2 mg/kg/day of MP was associated with lower hospital mortality in patients with severe pneumonia, and Tang et al. (30) found that an MP dosage of 0.5–2.5 mg/kg/day or the equivalent was associated with improved mortality and morbidity outcomes without increased adverse reactions in patients with severe pneumonia. We observed better survival rates among patients with LOSP treated with low-dose MP (≤2 mg/kg/day). In addition, as mentioned above, immune reactions, which might also have occult infectious etiologies, could play an important role in the development of LOSP. Although Meduri et al. (28) observed that MP used in early severe acute respiratory distress syndrome was associated with significant improvement in pulmonary and extrapulmonary organ dysfunction and reduction in duration of mechanical ventilation and ICU length of stay, we observed that patients receiving MP therapy within 1 week after LOSP diagnosis had poor outcomes. We speculate that early MP therapy might lead to a loss of infection control and may thus worsen the situation. In cases of LOSP after allo-HSCT, wherein pathogens were cleared using effective anti-infection treatments but infection-related immune reactions remained, corticosteroid therapy could improve patient outcomes. Thus, this is an important finding and underscores that corticosteroid therapy should not be used in all patients with LOSP after allo-HSCT.

We observed that viral infection might be an important cause of LOSP; however, we did not identify effective treatments for most viral infections. As donor leukocytes usually contain cytotoxic T cells presensitized to various viruses, DLI might be a potential treatment for viral infection after allo-HSCT. Liu et al. (4) observed that among 6 patients with LOSP who received DLI, 3 survived until the end of follow-up. However, in our study, DLI did not improve the outcomes of patients with LOSP. We speculated that DLI could trigger GVH effects, which might exacerbate immune-related lung injuries, and could also lead to myelosuppression and secondary infection.

Several factors were also associated with OS in patients with LOSP. For example, we observed that female patients with LOSP had a better OS. Similarly, Schwartz et al. (31) showed that the male gender was associated with diminished survival in idiopathic pulmonary fibrosis. This might be a result of the higher frequency of smokers among male patients (i.e., all smokers in this study were male), as smoking before HSCT was an independent risk factor for worse survival in our multivariate analysis. In addition, patients with a higher BALF neutrophil percentage had a worse OS. Several studies observed that a higher BALF neutrophil percentage was associated with a poor outcome in both infectious and non-infectious lung injury patients (32–34). It has therefore been suggested that overzealous neutrophil activation might result in severe alveolar damage resulting from the release of cytotoxic and immune cell-activating agents (35).

This study had several limitations. First, this was a retrospective study with a relatively small number of patients with LOSP, which might have influenced the accuracy of our findings. Second, we might have underestimated the occurrence of I-LOSP in this study because the number of pathogens that could be tested was relatively small. Third, we could not detect the loads of viruses except for CMV and EBV, and

therefore, we could not further identify the association between the virus load and the outcomes of LOSP. Finally, the large number of different treatments administered to treat patients in both the I-LOSP and NI-LOSP groups introduced heterogeneity to our justification of the generic conclusion regarding the advantage given by late and low-dose MP therapy with respect to the clinical outcomes of patients with LOSP. Future prospective and multicenter studies will provide more information about the prognostic factors and treatments of LOSP after allo-HSCT.

In summary, we observed that LOSP is a severe complication after allo-HSCT. Patients who smoked before allo-HSCT had a worse survival rate. The correct dosage and timing of MP in the context of broad-spectrum antimicrobial therapy could further improve the outcomes of patients with LOSP.

Acknowledgements:

The authors thank Editage for their assistance in editing this manuscript.

Support: This work was supported by the Key Program of the National Natural Science Foundation of China (Grant No. 81230013 and 81530046), National Natural Science Foundation of China (Grant No. 81400145), Project TG-2015-003 supported by the Health Science Promotion Project of Beijing, and Beijing Talents Fund (No. 2015000021223ZK39).

Author contributions: X.-J.H. served as principal investigator, designed and monitored the study, analyzed the data, and wrote the report; X.-D.M. performed the research, analyzed and interpreted the data, performed the statistical analysis, and wrote the report; Y.W., H.C., C.-H.Y., Y.-H.C., and W.H. analyzed and interpreted the data; and L.-P.X., X.-H.Z., F.-R.W., J.-Z.W., and K.-Y.L. performed the research and analyzed the data.

Conflicts: None of the authors have any potential financial conflict of interest related to this report.

References

1. Lucena CM, Torres A, Rovira M, et al. Pulmonary complications in hematopoietic SCT: a prospective study. Bone Marrow Transplant 2014; 49 (10): 1293–1299.
2. Cooke KR, Yanik GA. Lung injury following hematopoietic cell transplantation. In: Appelbaum FR, Forman SJ, Negrin RS, Blume KG, eds. Thomas' Hematopoietic Cell Transplantation Stem Cell Transplantation, Vol. 4. Blackwell Publishing Ltd: Oxford, UK, 2004: 1456–1472.
3. Chen CS, Boeckh M, Seidel K, et al. Incidence, risk factors, and mortality from pneumonia developing late after hematopoietic stem cell transplantation. Bone Marrow Transplant 2003; 32 (5): 515–522.
4. Liu DH, Chen SS, Sun YQ, et al. Clinical characteristics of late-onset severe pneumonia after allogeneic hematopoietic stem cell transplantation. Zhonghua Nei Ke Za Zhi 2013; 52 (10): 819–823.
5. Lu DP, Dong L, Wu T, et al. Conditioning including antithymocyte globulin followed by unmanipulated HLA-mismatched/haploidentical blood and marrow transplantation can achieve comparable outcomes with HLA-identical sibling transplantation. Blood 2006; 107 (8): 3065–3073.
6. Wang Y, Liu DH, Liu KY, et al. Long-term follow-up of haploidentical hematopoietic stem cell transplantation without *in vitro* T cell depletion for the treatment of leukemia: nine years of experience at a single center. Cancer 2013; 119 (5): 978–985.
7. Xiao-Jun H, Lan-Ping X, Kai-Yan L, et al. Partially matched related donor transplantation can achieve outcomes comparable with unrelated donor transplantation for patients with hematologic malignancies. Clin Cancer Res 2009; 15 (14): 4777–4783.
8. Huang XJ, Liu DH, Liu KY, et al. Haploidentical hematopoietic stem cell transplantation without *in vitro* T cell depletion for the treatment of hematological malignancies. Bone Marrow Transplant 2006; 38 (4): 291–297.
9. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood 2005; 106 (8): 2912–2919.
10. Huang XJ, Liu DH, Liu KY, et al. Treatment of acute leukemia with unmanipulated HLA-mismatched/haploidentical blood and bone marrow transplantation. Biol Blood Marrow Transplant 2009; 15 (2): 257–265.
11. Liu J, Kong J, Chang YJ, et al. Patients with refractory CMV infection following allo-HSCT are at high risk for CMV disease and non-relapse mortality. Clin Microbiol Infect 2015; 21 (12): 1121.e9–1121.e15.
12. Xu LP, Zhang CL, Mo XD, et al. Epstein-Barr virus-related post-transplantation lymphoproliferative disorder after unmanipulated human leukocyte antigen haploidentical hematopoietic stem cell transplantation: incidence, risk factors, treatment, and clinical outcomes. Biol Blood Marrow Transplant 2015; 21 (12): 2185–2191.
13. Mo XD, Xu LP, Liu DH, et al. Nonmalignant late effects in survivors of partially matched donor hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2013; 19 (5): 777–783.
14. Mandell LA, Wunderink RG, Anzueto A, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 2007; 44 (Suppl 2): S27–S72.
15. Li PQ, Zhang J, Muller CP, et al. Development of a multiplex real-time polymerase chain reaction for the detection of influenza virus type A including H5 and H9 subtypes. Diagn Microbiol Infect Dis 2008; 61 (2): 192–197.
16. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant 1995; 15 (6): 825–828.
17. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant 2005; 11 (12): 945–956.

18. Panoskaltsis-Mortari A, Griese M, Madtes DK, et al. An official American Thoracic Society research statement: noninfectious lung injury after hematopoietic stem cell transplantation: idiopathic pneumonia syndrome. Am J Respir Crit Care Med 2011; 183 (9): 1262–1279.
19. Sakaida E, Nakaseko C, Harima A, 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 (12): 4236–4242.
20. Seo S, Renaud C, Kuypers JM, et al. Idiopathic pneumonia syndrome after hematopoietic cell transplantation: evidence of occult infectious etiologies. Blood 2015; 125 (24): 3789–3797.
21. Sener AG, Afsar I. Infection and autoimmune disease. Rheumatol Int 2012; 32 (11): 3331–3338.
22. Gammon B, Cotliar J. Viral infection as a trigger in flares of acute graft-versus-host disease. Br J Dermatol 2013; 168 (4): 906–908.
23. Kawano N, Gondo H, Kamimura T, et al. Chronic graft-versus-host disease following varicella-zoster virus infection in allogeneic stem cell transplant recipients. Int J Hematol 2003; 78 (4): 370–373.
24. Pichereau C, Desseaux K, Janin A, et al. The complex relationship between human herpesvirus 6 and acute graft-versus-host disease. Biol Blood Marrow Transplant 2012; 18 (1): 141–144.
25. Ljungman P, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. Clin Infect Dis 2002; 34 (8): 1094–1097.
26. Deal EN, Hollands JM, Schramm GE, et al. Role of corticosteroids in the management of acute respiratory distress syndrome. Clin Ther 2008; 30 (5): 787–799.
27. Meduri GU, Headley AS, Golden E, et al. Effect of prolonged methylprednisolone therapy in unresolving acute respiratory distress syndrome: a randomized controlled trial. JAMA 1998; 280 (2): 159–165.
28. Meduri GU, Golden E, Freire AX, et al. Methylprednisolone infusion in early severe ARDS: results of a randomized controlled trial. Chest 2007; 131 (4): 954–963.
29. Lamontagne F, Briel M, Guyatt GH, et al. Corticosteroid therapy for acute lung injury, acute respiratory distress syndrome, and severe pneumonia: a meta-analysis of randomized controlled trials. J Crit Care 2010; 25 (3): 420–435.
30. Tang BM, Craig JC, Eslick GD, et al. Use of corticosteroids in acute lung injury and acute respiratory distress syndrome: a systematic review and meta-analysis. Crit Care Med 2009; 37 (5): 1594–1603.
31. Schwartz DA, Helmers RA, Galvin JR, et al. Determinants of survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 1994; 149 (2 Pt 1): 450–454.
32. Tamai K, Tachikawa R, Tomii K, et al. Prognostic value of bronchoalveolar lavage in patients with non-HIV *Pneumocystis* pneumonia. Intern Med 2014; 53 (11): 1113–1117.
33. Mason GR, Hashimoto CH, Dickman PS, et al. Prognostic implications of bronchoalveolar lavage neutrophilia in patients with *Pneumocystis carinii* pneumonia and AIDS. Am Rev Respir Dis 1989; 139 (6): 1336–1342.
34. Kinder BW, Brown KK, Schwarz MI, et al. Baseline BAL neutrophilia predicts early mortality in idiopathic pulmonary fibrosis. Chest 2008; 133 (1): 226–232.
35. Grommes J, Soehnlein O. Contribution of neutrophils to acute lung injury. Mol Med 2011; 17 (3–4): 293–307.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Clinical outcomes of late-onset severe pneumonia according to bronchoalveolar lavage fluid (BALF) neutrophil (A) and lymphocyte (B) percentage.

Figure S2. Clinical outcomes of late-onset severe pneumonia according to patients (A) with and without ribavirin (B) with and without donor lymphocyte infusion (DLI).