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Strong Association between Respiratory Viral Infection Early after Hematopoietic Stem Cell Transplantation and the Development of Life-Threatening Acute and Chronic Alloimmune Lung Syndromes

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Alloimmune lung syndromes (allo-LS), including idiopathic pneumonia syndrome, bronchiolitis obliterans syndrome, and bronchiolitis obliterans organizing pneumonia, are severe complications after hematopoietic stem cell transplantation (HSCT). In our cohort of 110 pediatric patients, 30 had allo-LS (27.3%), 18 with idiopathic pneumonia syndrome and 12 with bronchiolitis obliterans syndrome. Multivariate analysis showed that respiratory viral infection early after HSCT is an important predictor for the development of allo-LS ($P < .0001$). This was true for all viruses tested. In multivariate analysis, allo-LS was the only predictor for higher mortality ($P = .04$). Paradoxically, prolonged administration of immunosuppressive agents because of acute graft-versus-host disease had a protective effect on the development of allo-LS ($P = .004$). We hypothesize that early infection of the respiratory tract with a common cold virus makes the lungs a target for alloimmunity.

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INTRODUCTION

Pulmonary complications are common after allogeneic hematopoietic stem cell transplantation (HSCT). Between 30% and 60% of adult HSCT recipients reportedly experience pulmonary complications, representing a major cause of mortality [1-4]. In children undergoing HSCT, the incidence of pulmonary complications varies

from 10% to 25%, and onset is a poor prognostic event carrying a significantly increased risk of mortality [5,6]. At one time, most pulmonary complications were directly related to infection; today, however, noninfectious pulmonary complications, such as idiopathic pneumonia syndrome (IPS) and bronchiolitis obliterans syndrome (BOS), are seen more frequently [2,5,6].

Respiratory virus (RV) infections occur in 1%-56% of HSCT recipients. Most previous studies have examined the progression from upper respiratory tract infection (URTI) to lower respiratory tract infection (LRTI) and described the risk factors for this progression [7-16]. Some have reported an association of early RV infection with late, obstructive lung injury [17]. An association between RV infection and alloimmunity in lung transplant recipients was recently reported [18]. Lung transplant recipients develop more acute and chronic graft rejection after common RV infection early (<100 days) after transplantation [18].

Isolated alloimmune lung disease (ie, BOS or IPS) after HSCT suggests a specific trigger making the lung a target organ for alloreactivity. This is in line with the 3-step process reflecting the current view of the development of alloreactivity: (1) tissue damage, resulting in (2) release of inflammatory cytokines, resulting in (3) activation and influx of T lymphocytes [19]. We speculated that the presence of a common RV might

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trigger alloimmune lung syndrome (allo-LS) in HSCT. We prospectively studied the influence of these RVs on the development of allo-LS and overall survival (OS) in a cohort of pediatric HSCT recipients.

PATIENTS AND METHODS

Study Design and Study Populations

All patients who underwent allogeneic HSCT between January 2004 and May 2008 at the pediatric Hematology and Immunology Department of the Wilhelmina Children's Hospital/University Medical Center were included in this prospective study. Patients were enrolled in the HSCT protocol after providing written informed consent for the HSCT and the research protocol.

Supportive Care and Graft-versus-Host Disease Prophylaxis

All patients received antiemetic drugs. Prophylactic anticonvulsive therapy (clonazepam) was given to those patients receiving busulfan. Antibiotic prophylaxis involved daily ciprofloxacin and fluconazole from the start of conditioning until the resolution of neutropenia (3 days of $>500,000$ neutrophils/ μL). Additional prophylaxis against *Streptococcus viridans* in the mucositis phase was given with cefazolin. Starting 1 month after transplantation, cotrimoxazole 3 times a week was given as *Pneumocystis carinii* pneumonia prophylaxis. Only in cases of positive serology for herpes simplex virus was prophylaxis (with acyclovir) administered. No prophylaxis for other viruses was given. IgG levels were checked every 2 weeks; intravenous immunoglobulin was given only to those patients with an IgG level <4 g/L.

Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine (aiming for a trough level of 100-250 $\mu\text{g/L}$, based on national protocol guidelines), supplemented with methylprednisolone (MP; 1 mg/kg/day for 28 days) in patients receiving a cord blood (CB) transplant, or methotrexate (short course, 10 mg/ m^2 on days 1, 3, and 6) in patients receiving an unrelated bone marrow (BM) or peripheral blood stem cell (PBSC) transplant. In patients receiving an unrelated donor graft (CB, BM, or PBSC), antithymocyte globulin (ATG) serotherapy was administered until day -1, with ATG-fresenius for patients with acute lymphoblastic leukemia and thymoglobulin for all other indications.

Infection Monitoring

Bacterial/Fungal

To monitor bacterial colonization, nose/throat swabs and stools were cultured weekly and processed in accordance with standard microbiological procedures. Up to June 2006, we tested for galactomannan (Platelia *Aspergillus* enzyme immunoassay; Bio-Rad,

Hercules, CA) in cases of suspected *Aspergillus* infection, based on such clinical symptoms as prolonged fever during systemic broad antibiotic therapy and radiologic findings. After June 2006, we routinely monitored galactomannan twice weekly.

Viral

Plasma was tested weekly for Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpes 6 virus (HHV6), and adenovirus DNA positivity by real-time polymerase chain reaction (PCR) (see next section). In patients deemed positive (viral load >400 cp/mL), this test was done twice a week. Adenovirus (viral load >1000 cp/mL) was treated preemptively with cidofovir. CMV (viral load >1000 cp/mL) was treated preemptively with foscavir or ganciclovir. Depending on the viral load, the immunosuppressive regimen, and signs of posttransplantation lymphoproliferative disease, EBV was treated preemptively with anti-CD20 (rituximab).

Respiratory Viral

Before August 2005, nasal pharyngeal aspirate (NPA) samples were obtained for PCR only in the presence of symptoms of a URTI or LRTI, and then only up to day +100 posttransplantation. From August 2005 onward, we performed surveillance studies on NPA samples of all patients admitted to our HSCT unit. Reverse-transcriptase (RT)-PCR was done for all common RVs (see later). We repeated the NPA weekly in patients negative for RV and twice weekly in patients positive for RV.

Real-Time PCR for Respiratory Viruses

Nucleic acids were extracted using the total nucleic acid protocol with the MagNA Pure LC nucleic acid isolation system (Roche Diagnostics, Basel, Switzerland). For detection of RNA viruses, cDNA was synthesized using MultiScribe RT and random hexamers (Applied Biosystems, Foster City, CA). Detection of viral and atypical pathogens was performed in parallel, using real-time PCR assays specific for the following viruses: CMV; EBV; HHV-6; respiratory syncytial virus A and B; influenza virus A and B; parainfluenza virus 1-4; rhinoviruses; adenoviruses; human coronaviruses OC43, NL63, and 229E; human metapneumovirus; *Mycoplasma pneumoniae*; and *Chlamydia pneumoniae*. Real-time PCR procedures were performed as described previously [20]. In brief, samples were assayed in duplicate in a 25- μL reaction mixture containing 10 μL of cDNA, 12.5 μL of TaqMan Universal PCR Master Mix (Applied Biosystems), 300-900 nmol/L of the forward and reverse primers, and 75-200 nmol/L of each probe. All samples had been spiked before extraction with an internal control virus (murine encephalomyocarditis virus [RNA virus] and porcine herpesvirus [DNA virus]) to monitor for efficient extraction and amplification, essentially as described

previously [21]. The cycle of threshold (Ct) gives an impression of the quantity of the viral load (ie, a semi-quantitative value).

Recording Pulmonary Complications

All patients were observed for signs of respiratory disease early and late after transplantation. All clinical symptoms were recorded. In patients with URTI symptoms, NPA samples were obtained and tested for RV infection by PCR (see earlier). In patients with signs of LRTI, chest X-rays were obtained. Other tests, performed as indicated, included bronchoalveolar lavage (BAL) for broad infectious screening with bacterial/fungal cultures, viral PCR, and galactomannan, as well as high-resolution computed tomography (HRCT) scans. In cases of suspected allo-LS, HRCT and BAL were always performed (see definitions of disease shortly).

Pulmonary Function Tests

Because performing pulmonary function tests (PFTs) is difficult in young children, in our cohort routine PFTs before transplantation were performed only in children aged ≥ 6 years. PFTs also were performed in all children with such respiratory symptoms as shortness of breath, dry cough, and tachypnea after discharge. In patients with a diagnosis of allo-LS, PFTs were repeated at least monthly until the disorder resolved. Patients aged ≥ 6 years underwent spirometry testing and, when technically possible, body plethysmography and CO diffusion testing according to European Respiratory Society guidelines [22]. Total lung capacity (TLC) and forced expiratory volume in 1 second (FEV₁) were expressed as percentage of the predicted normal value, using published equations for children and adults [23], giving TLC % predicted and FEV₁ % predicted. A TLC % predicted of $<80\%$ was designated a "restrictive" pattern; an FEV₁ % predicted of $<80\%$ and FEV₁/forced vital capacity (FVC) of $<70\%$, an "obstructive" pattern; a TLC % predicted of $<80\%$ and FEV₁/FVC of $<70\%$, a "mixed" pattern; and CO diffusion of $<80\%$, "impaired diffusion."

Definitions of Disease

URTI was defined as rhinorrhea and/or dry cough only. LRTI/pneumonia was defined as cough and/or fever and pulmonary infiltrates on chest x-ray, with elevated C-reactive protein and/or positive microbiological cultures from sputum, BAL fluid, or blood. IPS was defined as the presence of acute bilateral pulmonary infiltrates with cough, dyspnea, and hypoxemia in the absence of infection (excluding an RV) or heart failure. By this definition, IPS included such entities as diffuse alveolar bleeding and periengraftment syndrome [2]. BOS was defined as typical HRCT changes, such as bronchial wall thickening,

air trapping, and mosaic parenchymal attenuation [1], in the absence of signs of infection and, whenever pulmonary function testing could be done, abnormal pulmonary function test results (ie, decrease in FEV₁ of $>20\%$ or in FEV₁/FVC of $<70\%$). bronchiolitis obliterans organizing pneumonia (BOOP) was defined as restrictive PFT (if PFT were done) and consolidation on chest x-ray [1]. Allo-LS was defined as IPS, BOS, and BOOP, subdivided into acute (IPS) and chronic (BOS/BOOP) forms.

Treatment of Lung Disease

In general, URTI was not treated; only in the 2 patients with influenza A was a neuraminidase inhibitor administered. LRTI/pneumonia was treated with empiric antibiotic therapy (vancomycin and ceftazidime). Whenever a bacterial pathogen was found, therapy was adjusted according to antibiotic resistance. In patients with probable or proven *Aspergillus* spp, voriconazole was administered; if no response to voriconazole was noted (progressive clinical or radiologic findings), granulocyte transfusions were given.

Allo-LS was treated with MP 10 mg/kg/day i.v. for 3 days and 2 mg/kg/day thereafter, tapering by 25% per week to 0.5 mg/kg/day. The MP pulses were repeated every 4 weeks until recovery, up to a maximum of 6 courses. Recovery was defined as normalization of PFTs and/or resolved symptoms, with no extra oxygen requirement. In between the subsequent courses of MP, prednisone 0.5 mg/kg/day was given. Other immunosuppressive agents (usually cyclosporine) were continued. In addition, azithromycin was given, because of its suggested immunomodulatory effect [24]. Along with immunosuppressive therapy, supportive care was provided, with extra oxygen and mechanical ventilation when necessary. IgG level was maintained above 4 g/L.

Endpoints

The primary endpoint of this study was the development of acute and chronic allo-LS. The secondary endpoint was OS.

Statistical Analysis

Differences between the RV-positive and RV-negative groups were tested using Pearson's χ^2 test. Results with a *P* value $<.05$ were considered statistically significant.

The duration of follow-up was the time to the endpoints, the development of an allo-LS and death, or the last assessment for survivors. To analyze risk factors for outcomes, we considered variables associated with the recipient (age at transplantation, sex, CMV serology, RV positivity, single/multiple viruses), the disease (malignant vs nonmalignant), the donor/transplantation technique (cell source, HLA disparity,

donor relationship, conditioning regimen), HSCT complications (allo-LS, acute GVHD [aGVHD], CMV and adenovirus plasma DNA positivity, veno-occlusive disease), and relapse. To examine the influence of the various viruses on the primary endpoint, rhinovirus was compared with the other viruses, and multiple viral infection was compared with single viral infection.

In the analyses, we tested for allo-LS as a group (IPS + BOS/BOOP) based on the hypothesis that early viral infection might be a trigger for both acute and chronic allo-LS. In addition, we tested both syndromes separately (ie, BOS/BOOP excluding IPS from the analyses, and IPS excluding BOS/BOOP).

Associations between variables (including recipient, disease, and HSCT technique) and the primary endpoint were evaluated using Cox proportional hazard models. Dichotomous outcomes (eg, allo-LS: yes/no) were used as dependent variables, and predictors were used as independent variables. Univariate predictors of outcome with a *P* value <.10 were used for multivariate analysis. Results are expressed as hazard ratios (HRs) and corresponding 95% confidence interval (CIs). CIs not including 1 were considered statistically significant.

Analyses for the association between HSCT complications and the primary endpoint (allo-LS) as well as the secondary endpoint (OS) were done using logistic regression. Dichotomous outcomes (eg, allo-LS or survival: yes/no) were used as dependent variables, and predictors were used as independent variables. Univariate predictors of outcome with a *P* value <.10 were used for multivariate logistic regression analysis. Results are expressed as odds ratio (ORs) and corresponding 95% CIs. CIs not including 1 were considered statistically significant.

Probabilities of allo-LS and OS were calculated using the Kaplan-Meier estimate; the 2-sided log-rank test was used for comparisons. All statistical analyses were performed using SPSS 15.1 (SPSS Inc, Chicago, IL).

RESULTS

Patient Characteristics

A total of 110 patients were included in the study, 42 from January 2004 to August 2006 (before routine NPA testing), and 68 after from August 2006 to May 2008. Six patients who underwent transplantation during this period were excluded from the study because they experienced autologous recovery (*n* = 2), early graft rejection (within 1 month after transplantation; *n* = 2), or early death (before engraftment; *n* = 2) and thus were considered not prone to alloreactive disease. The median age at transplantation was 5.0 years (range, 2 months to 21 years), and body weight ranged between 2 and 100 kg. Baseline characteristics of the

RV-positive and RV-negative groups are shown in Table 1. No significant differences between the 2 groups were evident, although there were slightly more matched donor transplants in the RV-negative group and more CB donors in the RV-positive group.

RV Infections and Presenting Symptoms

In this cohort of patients, 55 (50%) had an RV infection. The median day of onset was day +16 post-transplantation (range, day -7 to day +100). Symptoms were usually mild. The majority of patients with RV infection (*n* = 43) had URTI symptoms only. Eleven patients required extra oxygen, and 1 patient needed ventilator support (associated with a bacterial infection). Two patients, both with influenza A infection, were treated with a neuraminidase inhibitor; all other patients experienced spontaneous clinical recovery within 7-14 days. Although symptoms disappeared, virus was detected in NPA samples for weeks to months afterward, with high viral loads (PCR Ct values of 17-24; see Patients and Methods). Thirty-eight patients had a single RV, and 14 patients had multiple viruses. In 3 patients, no RV was detected, but the clinical picture was typical for RV infection. These patients had mild respiratory symptoms (rhinorrhea) with no other cause, and all recovered spontaneously. The distribution of the various viruses is shown in Table 2.

Primary Endpoint: Allo-LS

Thirty patients were diagnosed with allo-LS (27.3%), 12 with BOS (10.9%) and 18 with IPS (16.4%). No patient developed BOOP. One patient presented with pulmonary hypertension with histologically proven vasculopathy, with lymphocyte infiltration that responded to immunosuppressive agents. We considered this patient to have IPS. For the 30 patients with allo-LS, the median time of onset was 8 weeks (range, 2-26 weeks) after transplantation. IPS occurred earlier, with a median time of onset of 7 weeks (range, 2-12 weeks); BOS developed later, after a median of 16 weeks (range, 10-26 weeks).

In univariate analysis, RV positivity, CB stem cell graft, and a chemotherapy-based conditioning regimen were predictors for the development of allo-LS (Table 3). In multivariate analysis, only RV positivity remained a predictor for the development of allo-LS (HR, 8.37; 95% CI, 1.78-39.43; *P* = .007).

Analyzing the separate endpoints acute allo-LS (IPS) and chronic allo-LS (BOS) revealed that RV positivity was the sole predictor for the development of BOS (HR, 107; 95% CI, 0.9-13,347; *P* = .05). For IPS, RV positivity (HR, 11.4; 95% CI, 2.61-49.8; *P* = .001), CB stem cell graft (HR, 4.8; 95% CI, 1.79-12.7; *P* = .002), and nonmalignant indication for transplantation (HR, 3.3; 95% CI, 1.1-10.2; *P* = .034) were found to be predictors in univariate analysis. In multivariate

Table 1. Patient Characteristics

	RV-Negative	RV-Positive	P
Age at HSCT, years, median (range) Years	6.0 (0.5-19)	2.6 (0.2-21)	NS
Follow-up, weeks, median (range) Weeks	70 (4-230)	57 (4-192)	NS
Sex, n (%)			
Male	29 (53)	29 (53)	
Female	26 (47)	26 (47)	NS
Indication, n (%)*			
Malignant	31 (56)	25 (46)	
Nonmalignant	24 (44)	30 (54)	NS
HLA disparity, n (%)†			
Matched	39 (71)	30 (54)	
Mismatched	16 (29)	25 (46)	.076
Number of HSCT, n (%)			
First	52 (94)	50 (91)	
Second	3 (6)	4 (7)	
Third	0 (0)	1 (2)	NS
Conditioning, n (%)‡			
TBI-based	21 (38)	12 (22)	
Chemotherapy-based	34 (67)	43 (78)	NS
Donor relationship, n (%)			
Family	18 (33)	15 (27)	
Unrelated	37 (67)	40 (73)	NS
Graft source, n (%)			
BM/PBSC	43 (78)	34 (62)	
CB§	12 (22)	21 (38)	.061

HSCT indicates hematopoietic stem cell transplantation; BM, bone marrow; PBSC, peripheral blood stem cell; CB, cord blood; TBI, total body irradiation; NS, not significant.

*Malignant indications: Acute lymphoblastic leukemia, 34; myelodysplastic syndrome, 9; acute myelogenous leukemia, 8; juvenile myelomonocytic leukemia, 1; lymphoma, 4. Nonmalignant indications: inborn errors of metabolism, 23; immune deficiency, 13; hemophagocytic lymphohistiocytosis, 5; BM failure, 11; others, 2.

†Matched donor was defined as either 10 of 10 for BM/PBSC grafts molecularly typed or 6 of 6 for CB grafts based on intermediate resolution (HLA-A and HLA-B on serology and HLA-DR on high resolution).

‡Conditioning regimens: TBI-based (n = 33): Fractionated TBI (3 × 2 × 2 Gy) and etoposide 40 mg/kg, 29; thoracoabdominal irradiation/cyclophosphamide 10 mg/kg/fluorouracil (90 mg/m²), 2; TBI (7 Gy)/etoposide (40 mg/kg), 1; TBI/thiotepa/etoposide, 1. Chemotherapy-based (n = 77): busulfan 480 mg/m²/cyclophosphamide 120 or 200 mg/kg, 46; busulfan 480 mg/m²/cyclophosphamide 120 mg/kg/melphalan 140 mg/m², 17; busulfan 480 mg/m²/cyclophosphamide 120 mg/kg/etoposide 40 mg/kg, 3; busulfan 480 mg/m²/fluorouracil 180 mg/m², 3; busulfan 160 mg/m²/cyclophosphamide 40 mg/kg/fluorouracil 90 mg/m², 3; cyclophosphamide 120 mg/kg, 2; treosulfan 42 g/m²/etoposide 40 mg/kg/cyclophosphamide 120 mg/kg, 1; treosulfan 42 g/m², 1; none, 1. Patients receiving an unrelated donor graft received serotherapy (thymoglobulin, 37; ATG-fresenius, 16; campath-1H, 3).

§Median cell dose of CB: in nucleated cells, 7.8 (range, 2.7-20.0) × 10⁷ cells/kg; in CD34⁺ cells, 4.5 (range, 1.1-10.0) × 10⁵ cells/kg. All CB grafts were unrelated. One patient received a double CB graft.

analysis, only RV-positivity remained significant (HR, 8.65; 95% CI, 1.9-38.4; *P* = .005).

The median duration from RV positivity and the development of allo-LS was 7 weeks (range, 1.2-20 weeks) (Figure 1A). The timing of development of RV positivity seems to be important as well. Patients who were RV-positive early after transplantation (before the median of day +16) had a slightly greater likelihood of developing allo-LS than those who became RV-positive after day +16 (HR, 2.10; 95% CI, 0.89-5.00 *P* = .089).

Univariate analysis of the influence of HSCT-associated complications on allo-LS showed that adenovirus reactivation (OR, 3.86; 95% CI, 1.56-9.5; *P* = .004) was predictive of allo-LS. aGVHD grade II-IV in other organs appeared to be a negative predictor (OR, 0.22; 95% CI, 0.061-0.78; *P* = .02). In multivariate analysis, only aGVHD remained a strong predictor for preventing allo-LS (OR, 0.1; 95% CI, 0.02-0.47; *P* = .004).

The influence of aGVHD on the development of allo-LS for the whole group and for the RV-positive

patients is shown in Figure 1B and C. In this study, aGVHD clearly developed before the onset of allo-LS. The mean time to onset was 4 weeks for GVHD (range, 2-15 weeks) and 8 weeks for allo-LS (range, 2-26 weeks). In particular, in the RV-positive group, aGVHD grade II-IV in another organ was strongly protective against the development of allo-LS.

Table 2. Viruses Detected by Real-Time RT-PCR

Virus	n
Rhinovirus	28
Parainfluenzavirus-3	4
Influenza-A virus	2
Coronavirus	3
Adenovirus	1
Multiple viruses*	14
"Negative"†	3

*Subdivided: adenovirus/rhinovirus, 4; parainfluenza-3/rhinovirus, 2; human metapneumovirus/rhinovirus, 2; rhinovirus/adenovirus/parainfluenza-3, 2; respiratory syncytial virus/rhinovirus, 1; coronavirus/parainfluenza-3, 1; coronavirus/adenovirus, 1; coronavirus/rhinovirus, 1.

†Typical clinical symptoms of an URTI with no other explanation.

Table 3. Univariate Analysis of Predictors for Allo-LS

	Total, n	Allo-LS				
		n	%	HR	95% CI	P
Overall	110	30	27.3			
Age				0.95	0.88-1.02	.18
Sex						
Male	58	15	25.9	1		
Female	52	15	28.8	1.03	0.5-2.12	.93
Indication for HSCT						
Malignant disorder	56	10	18.0	1		
Nonmalignant disorder	54	20	35.7	2.67	0.87-3.97	.11
HLA disparity						
Matched	69	17	24.6	1		
Mismatched	41	13	31.7	1.14	0.55-2.36	.72
Conditioning						
TBI-based	33	4	12.1	1		
Chemotherapy-based	77	26	34.0	3.05	1.06-8.75	.04
Donor						
Family	33	9	27.3	1		
Unrelated	67	21	31.3	1.07	0.49-2.35	.80
Stem cell source						
BM/PBSC	67	17	25.4	1		
CB	33	13	39.4	2.13	1.03-4.41	.042
RV infection						
No	55	3	6.7	1		
Yes	55	27	52.9	10.3	3.14-34.3	.00
Recipient CMV serology						
Serology-negative	76	19	25	1		
Serology-positive	34	11	32.4	1.41	0.59-4.78	.68

HSCT indicates hematopoietic stem cell transplantation; TBI, total body irradiation; BM, bone marrow; PBSC, peripheral blood stem cell; CB, cord blood; CMV, cytomegalovirus.

We found no influence of the different individual viral species, or of the presence of a single virus or multiple viruses, on the development of allo-LS (data not shown).

All patients who developed allo-LS were treated according to the protocol with MP pulse therapy, as discussed earlier. All patients demonstrated prompt initial improvement of clinical symptoms.

Secondary Endpoints

OS was 72% (80/110) after a median follow up of 66 weeks (range, 4-230 weeks). Cause of death was relapse in 9 patients (8.2%) and nonrelapse mortality in 21 patients (19.1%). Fourteen of the 30 patients with allo-LS died (47%), all from transplantation-related causes: 3 from refractory aGVHD, 2 from invasive fungal infection, 1 from adenovirus disease, 2 from sudden cardiac death of unknown cause, and 6 from ongoing lung disease. Univariate analysis identified adenovirus reactivation (OR, 0.28; 95% CI, 0.08-0.96; $P = .043$) and the development of allo-LS (OR, 0.25; 95% CI, 0.10-0.61; $P = .003$) as predictors for lower survival. Relapse had no significant influence on OS (HR, 1.89; 95% CI, 0.246-14.59; $P = .54$). In multivariate analysis, only allo-LS remained a predictor in this cohort (OR, 0.29; 95% CI, 0.09-0.94; $P = .04$). The impact of allo-LS on OS is depicted in Figure 1D.

DISCUSSION

Our cohort of 110 patients had a high incidence (50%) of early RV infection, occurring at a median of 16 days after HSCT. Rhinovirus infection was the most common RV detected. The RV infections usually had a mild clinical course, and most patients experienced spontaneous recovery within 2 weeks. RV infection occurring during the first 100 days after HSCT appeared to be the sole predictor for the development of acute and chronic allo-LS, which was found in 27.3% of the patients. All patients had recovered from their initial URTI symptoms before a new episode of respiratory symptoms occurred, leading to the diagnosis of allo-LS. The presence of a single RV or multiple RVs, or the presence of rhinovirus and other (nonrhinovirus) RVs, was not associated with the development of allo-LS. Paradoxically, aGVHD had a protective effect against the development of allo-LS, likely resulting from the prolonged immunosuppressive therapy in the patients with aGVHD. All of the patients with allo-LS initially exhibited good clinical response to MP pulse therapy. The development of allo-LS was associated with high mortality, however.

A possible weakness of our study is that we changed our policy on testing for RV during the study period. In the early phase of the study, we tested NPA samples for RV only in those patients exhibiting symptoms. Later in the study period, once the significance of RV was recognized, weekly surveillance assays were

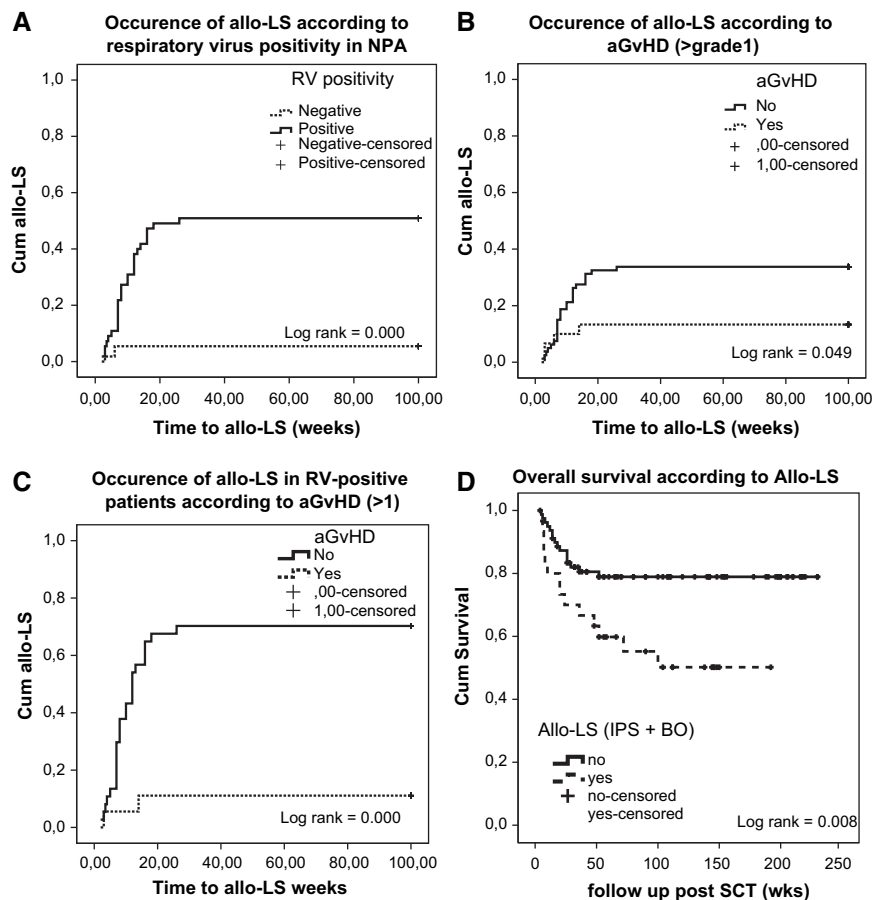


Figure 1. RV= Respiratory Virus, allo-LS = alloimmune lungsyndrome, aGvHD = acute graft versus host disease, IPS = Idiopathic Pulmonary Syndrome, BO = Bronchiolitis Obliterans (Syndrome).

done in all patients. These surveillance assays identified 7 RV-positive patients without symptoms at the time of sampling; however, all of these patients developed URTI symptoms within 14 days after positive sampling, and so these RVs would ultimately have been detected regardless of the testing policy. Thus, we believe that we did not miss any RV-positive patients in the presurveillance period, and that the change in testing policy had no major impact on our results (data not shown), the only difference being that the median time to RV positivity would have been shorter had we monitored the whole group routinely.

Three patients with symptoms and a clinical course typical of viral URTI were considered RV-positive despite negative RV-PCR results. In these patients, symptoms might have resulted from a virus not detectable by our PCR panel. It would be interesting to test these for more recently identified viruses, such as Boca and Wu/KI. Because of the obvious symptoms and the fact that other study groups have included similar patients in their analyses, we decided to do so as well [10]. Had we considered these patients RV-negative in our analysis, the results would have been the same (only 1 of the 3 patients developed allo-LS). We realize

that we may well have missed some mild URTI symptoms in the period between discharge and day +100 and so do not have full data on RV positivity after discharge from the hospital. No patient who was RV-PCR-negative on discharge developed allo-LS, however. Acquisition of RV very early after HSCT appears to be important for the development of allo-LS; the patients who were RV-positive within 16 days of HSCT tended to be more susceptible to allo-LS.

A remark about the definition of IPS is warranted. In the consensus definition of IPS (established by a 1997 National Institutes of Health workshop), all infectious agents, including RVs, should be excluded. In our patients, we observed prolonged shedding of RV for months, and thus we could not formally diagnose IPS. In all patients, the initial URTI symptoms disappeared spontaneously within 1-2 weeks, however. Subsequently, after a period of at least 14 days without significant respiratory problems, symptoms of hypoxia and/or airway obstruction recurred. We believe that this represents not a direct progression of viral infection, but rather a combination of several factors in which alloreactivity (triggered by tissue damage because of persistent viral infection) plays a pivotal role. Therefore, we chose to define IPS as discussed

earlier, not taking into account the presence of an RV identified by PCR as was done in this study. Moreover, the formal definition of IPS was promulgated in an era when molecular diagnosis of RV was not yet available.

We hypothesize that RVs may contribute to the pathogenesis of any allo-LS as follows. The RV damages the respiratory epithelium, causing an inflammatory response at the time of immune recovery. Normally IPS and BOS/BOOP are viewed as distinct clinical entities, and so we first studied them separately. Because we noted the same strong associations among RV positivity, GVHD, and OS in the 2 groups, we combined both IPS and BOS/BOOP in subsequent analyses.

We also combined BM and PBSC sources in our analysis. It would be interesting to evaluate patients receiving PBSCs as a separate group, because of their apparent higher risk of chronic GVHD (cGVHD). The small number of patients in our cohort who received PBSCs ($n = 7$) precludes meaningful analysis, however.

Numerous studies have explored the incidence and outcome of both nosocomial and community-acquired RV infections after HSCT [7-16]. Reported incidence varies from 1% to 56%. This wide range of incidence can be explained by differences among studies in the definition of RV infection, the period of monitoring for RV infection (eg, inpatients/outpatients, seasonal influence), and sensitivity of the analysis methods used. In our cohort, the high incidence of RV URTI (50%) might be attributed to our close monitoring for respiratory symptoms and performance of RT-PCR surveillance assays. In addition, nosocomial RV infections were frequently observed, and genotyping studies suggested that these had spread throughout the ward during the study period, also contributing to the relatively high incidence (data not shown).

Literature data on the morbidity associated with RV infection after HSCT are conflicting. Some groups found no progression of viral URTI to LRTI in patients after HSCT [10,15], whereas others reported progression in up to 58% of patients [7,8,11,13,16]. In our cohort, we found no direct progression to LRTI. Almost all patients had mild URTI symptoms and recovered spontaneously. The median day of onset of RV infection was only 16 days in our cohort, compared with at least 60 days in previous studies. Progression to LRTI might be expected in patients with RV infection early after transplantation, because of poor immune status, but this was not seen. In our opinion, it is more likely that the moment of immune recovery defines the onset of symptoms. What we define as IPS in this study (ie, symptoms after a period of quiescent RV infection) might have been reported in other studies as progressive viral pneumonia, with symptoms occurring at the onset of RV infection later after HSCT, when some immune recovery has already occurred.

The reported incidence of IPS after HSCT ranges from 2% to 15% of patients [2,4,25,26], and that of BOS ranges from 0% to 26% [1,4,27,28]. Most reported data are from adult studies. The combined incidence of allo-LS in our cohort of 27.3% is comparable to that reported in the literature. Our incidence of IPS is relatively high, most likely because our cohort included a high number of early RV-positive patients.

We found a strong association between RV infection and the development of allo-LS. To the best of our knowledge, this is the first report of such a strong association between RV infection and life-threatening allo-LS. Earlier, Erard et al. [17] described a relationship between RV infection during the first 100 days after HSCT and a decline in airflow leading to increased overall mortality. The decline in airflow was detected immediately after infection and did not return to baseline values, suggesting sustained airway inflammation leading to permanent loss of lung function. Pulmonary disease was much more severe in our cohort compared with the cohort of Erard et al. [17]. This discrepancy can be explained by the early moment of RV infection, when immune recovery has not yet occurred and the persistent RV likely causes more tissue damage, ultimately resulting in a stronger inflammatory response. The absence of immunity at the time of primary RV infection also might explain the absence of an immediate decline in clinical lung function. Pulmonary function deteriorated only after at least 2 weeks after RV infection, when the first signs of immune recovery were evident. It would be interesting to routinely perform PFTs earlier after HSCT, but for reasons of hygiene and patient comfort, we decided not to do this in the present study.

Most previous studies have found an association between the presence of GVHD and the development of allo-LS [1,2,25,29]. In those studies, aGVHD was associated with IPS, but other risk factors, including conditioning regimen and infection, might have been involved as well. cGVHD is considered an important risk factor for BOS in adults. Alloreactive T cells, in the context of aGVHD or cGVHD, play an important role in BOS [29]. BOS is also seen in the absence of cGVHD in 7%-18% of patients, however [27]. This percentage may be different in children, who are less susceptible to cGVHD [30]. Our finding that aGVHD grade II-IV in other organs has a protective effect on the development of allo-LS does not necessarily contradict these results. Like aGVHD, allo-LS is a manifestation of alloimmunity. We speculate that the apparent protective effect of aGVHD reflects the influence on alloreactive T cells of immunosuppressive agents used to treat aGVHD grade II-IV. None of our patients had lung involvement at the onset of aGVHD. This may be because the lungs are less susceptible to acute alloreactivity than the classic target organs of gut, liver, and skin. This is also reflected by the fact that the median time to allo-LS was longer

(8 weeks) than the median time to aGVHD (4 weeks). All patients who developed allo-LS did so during tapering of immunosuppressive therapy or after this therapy had been stopped. All of the patients with aGVHD were on prolonged immunosuppressive therapy (including steroids) and thus likely had less chance to develop alloimmunity in the lungs. In other words, the RV-positive patients who did not develop allo-LS had significantly greater immune suppression than the RV-positive patients who did develop allo-LS (data not shown). This effect of immunosuppressive therapy on the development of allo-LS is in line with previous findings [31]. Regarding cGVHD, in contrast to other studies, in our cohort, only 1 of 12 patients (11%) with BOS had signs of cGVHD in other organs. Our finding of no association between cGVHD and BOS/BOOP might be because of the generally low incidence of cGVHD in the pediatric HSCT population. This, together with the fact that viral infections are more frequent in childhood, might make the respiratory epithelium a preferential target for chronic allograft rejection, at least in this pediatric cohort. No protective effect of cGVHD on the development of allo-LS was noted, most likely because cGVHD (mainly the limited form) was not treated with systemic immunosuppressive agents (eg, steroids).

A similar association between RV infection and allo-LS has been reported after lung transplantation. A recent prospective cohort study in 100 lung transplant recipients clearly showed had significantly more acute or chronic rejection episodes in patients with an RV infection occurring within 100 days post-transplantation [18]. BOS occurring after lung transplantation is considered a manifestation of allograft rejection [32,33] because of an alloimmune process. Some animal models of lung transplantation have demonstrated an association between the presence of RV and the development of BOS exclusively in the allogeneic transplantation setting [34]. These results are in line with the association between RV infection and allo-LS seen in our HSCT population, and strongly support the hypothesis that airway damage from an RV infection alone does not lead to severe problems, but triggers alloimmunity. Because of this strong association, the current practice in lung transplantation is to increase immunosuppression by adding steroids in the presence of an RV infection posttransplantation, to avoid rejection (personal communication Lung transplantation program UMC Groningen and UMC Utrecht, 2007). This practice has led to a decreased graft rejection rate.

Increasing immunosuppression solely because of the presence of a RV may sound paradoxical, possibly predisposing the patient to other potentially life-threatening complications, but it is supported by our observation that patients with RV infection early after HSCT were less vulnerable to allo-LS when receiving

immunosuppression for aGVHD. At present, we cannot predict which RV-positive patients are actually at risk for developing allo-LS. Early recognition of the disease by the detection of biomarkers associated with lung damage and the development of allo-LS, or the identification of certain risk groups by studying the genetic polymorphisms of innate immunity in these patients, might be of additional value to fine-tune the initiation or adjustment of immunosuppressive therapy in the HSCT setting.

In conclusion, we have shown a clear relation between early RV infection and the development of allo-LS in pediatric HSCT recipients. Tissue damage because of the persistence of RV in the lung might be a trigger for the development of allo-LS. aGVHD, but more likely greater immunosuppression because of the aGVHD, appears to protect for allo-LS. These findings suggest that prevention of RV infections early after HSCT is of utmost importance. In addition, prolonged immune suppression in transplant recipients with an RV infection might prevent development of allo-LS, in analogy with current practice in lung transplantation.

AUTHORSHIP STATEMENT

A. Birgitta Versluys designed the study, provided clinical data, analyzed data, and wrote the paper. John Rossen developed and performed the viral PCR assays. Rob Schuurman developed and performed the viral PCR assays. Bart van Ewijk performed pulmonary function tests. Marc Bierings designed the study and provided clinical data. Jaap Jan Boelens designed the study, provided clinical data, performed statistical analysis, and wrote the paper.

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