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Infection with a respiratory virus before hematopoietic cell transplantation is associated with alloimmune-mediated lung syndromes

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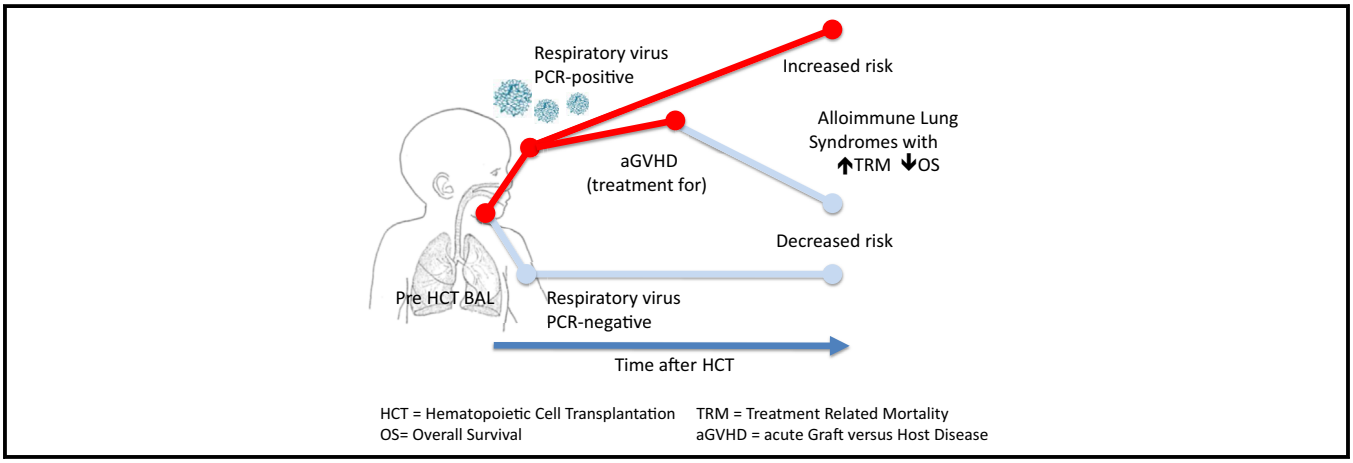
GRAPHICAL ABSTRACT



Background: Alloimmune-mediated lung syndromes (allo-LSs) are life-threatening complications after hematopoietic cell transplantation (HCT). Respiratory virus (RV) has been suggested to play a role in the pathogenesis.

Objective: We studied the relation between RV DNA/RNA detection in the upper/lower airways before HCT and the occurrence of allo-LSs.

Methods: We retrospectively analyzed all HCT recipients between 2004 and 2014, in whom real-time PCR for RV was performed in nasopharyngeal aspirates (NPAs) and bronchoalveolar lavage (BAL) fluid before HCT. The main outcome of interest was the presence of an allo-LS, which was defined as idiopathic pneumonia syndrome or bronchiolitis obliterans syndrome. Other outcomes were overall survival and treatment-related mortality. We used Cox proportional hazard models, logistic regression models, and Fine-Gray competing risk regression for analyses.

Results: One hundred seventy-nine children (median age, 6.8 years) were included. RVs were found in 61% (41% in BAL fluid/NPAs and 20% in NPAs only). Rhinovirus was the most frequently detected RV (42%). Allo-LSs occurred in 13%. RV positivity in BAL fluid was a predictor for allo-LSs (hazard ratio, 3.8; 95% CI, 1.4-10.7; $P = .01$), whereas RV positivity in NPAs only was not. No other predictors were found. Grade II to IV acute graft-versus-host disease related to steroid treatment shows a trend toward a protective effect (odds ratio, 0.16; 95% CI, 0.0-1.3; $P = .08$). Allo-LSs significantly increased treatment-related mortality (52% ± 10% in allo-LSs and 20% ± 4% in non–allo-LSs, $P = .007$).

Conclusions: These results show that pre-HCT BAL fluid RV positivity was a predictor for allo-LSs. Screening for RVs before HCT might identify patients at risk for allo-LSs. This could have implications for prevention and treatment and might subsequently influence the outcomes of HCT. (J Allergy Clin Immunol 2018;141:697-703.)

Key words: *Hematopoietic cell transplantation, respiratory virus, bronchoalveolar lavage, alloimmune lung syndromes, graft-versus-host disease, bronchiolitis obliterans syndrome, idiopathic pneumonia syndrome*

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Abbreviations used
aGVHD: Acute graft-versus-host disease
Allo-LS: Alloimmune-mediated lung syndrome
BAL: Bronchoalveolar lavage
BOS: Bronchiolitis obliterans syndrome
CB: Cord blood
Ct: Cycle threshold
GVHD: Graft-versus-host disease
HCT: Hematopoietic cell transplantation
HR: Hazard ratio
HRCT: High-resolution computed tomography
IPS: Idiopathic pneumonia syndrome
NPA: Nasopharyngeal aspirate
PFT: Pulmonary function test
RV: Respiratory virus
TRM: Treatment-related mortality
URTI: Upper respiratory tract infection

Allogeneic hematopoietic cell transplantation (HCT) is a curative treatment for several malignant and nonmalignant childhood diseases. Its success is limited by toxic events. Pulmonary complications contribute to posttransplantation morbidity and mortality. Noninfectious causes, such as alloimmune-mediated lung syndromes (allo-LSs), are responsible for a significant proportion of posttransplantation lung injury in pediatric HCT recipients.[1]

There is growing interest in the role of the microbiome in patients with graft-versus-host disease (GVHD). Pretransplantation conditioning regimens disrupt the intestinal barrier, and the gut flora shows major changes during HCT, causing dysregulation of intestinal immune homeostasis, which can eventually lead to acute graft-versus-host disease (aGVHD).[2,3] Little is known about the respiratory microbiome and its relation to health and disease.[4]

Studies in patients undergoing lung transplantation or allogeneic HCT[5,6] suggest that the presence of common cold viruses early after transplantation is associated with either graft rejection (in lung transplantation) or development of alloimmune lung disease (in HCT). On this basis, we hypothesize that early presence of viruses in the respiratory tract can cause tissue damage, resulting in activation of the alloimmune system. However, distinguishing allo-LSs from progressive viral infection remains a point of controversy.

To assess the effect of common cold viruses on the development of allo-LSs, we performed a retrospective analysis to relate the presence of viral DNA/RNA in either nasopharyngeal aspirates (NPAs), bronchoalveolar lavage (BAL) fluid, or both to various outcome parameters, such as allo-LSs and survival.

METHODS

Study design and patients

We included all consecutive pediatric patients receiving their first allogeneic HCT from January 2004 to October 2013 who underwent routine BAL and nasal aspiration according to our previously described pre-HCT screening protocol, which consisted of chest high-resolution computed tomography (HRCT), pulmonary function tests (PFTs; in children >5 years of age), nasal aspiration for viral tests, and BAL for viral, bacterial, and fungal diagnostics.[7] Clinical data were collected prospectively, starting before conditioning, and registered in the clinical database. Minimum follow-up for surviving patients was 6 months. Patients were included and data were collected after written informed consent was obtained in accordance with the Declaration of Helsinki. Institutional ethics committee approval for sample and data collection was obtained through trial numbers 05/143 and 11/063-k.

Procedures

BAL was performed after achievement of general anesthesia (only if patients had a procedure requiring anesthesia planned [ie, central line placement or HRCT in younger children]) by instilling 10 mL of normal saline aliquots through an endotracheal catheter wedged in the distal bronchi. From 2007, all patients underwent BAL (except for patients who did not have general anesthesia); before 2007, all patients underwent nasal aspiration, but not all had a paired BAL sample taken. Nasal aspiration was done with a disposable catheter connected to a mucous trap. Dry nasopharyngeal suction was performed, followed by instillation and immediate suction of 2 to 3 mL of sterile normal saline through the catheter.

Real-time PCR for RVs was performed, as described previously.[8] For detection of RNA viruses, cDNA was synthesized with MultiScribe Reverse Transcriptase and random hexamers (Applied Biosystems, Foster City, Calif). Detection of viral pathogens was performed in parallel by using real-time PCR assays specific for the following viruses: bocavirus; human herpesvirus 6; respiratory syncytial virus; influenza virus A and B; parainfluenza virus 1 to 4; rhinoviruses; adenoviruses; human coronaviruses OC43, NL63, and 229E; human metapneumovirus, and *Mycoplasma pneumoniae*. Semiquantitative viral load was expressed in cycle threshold (Ct) values.

In case of a positive RV result, we postponed the HCT procedure with 2 weeks in elective HCT (non–primary immunodeficiency benign disorders) and/or we prolonged immunosuppressive therapy after HCT as allo-LS prophylaxis. This fits our hypothesis that RV positivity is a predictor for allo-LSs and that steroid treatment for aGVHD has a protective effect on the occurrence of allo-LSs.[6] Apart from the pre-HCT screening, no routine monitoring for RV was performed. Only in the case of onset/progression of respiratory symptoms was nasal aspiration, BAL, or both repeated.

Conditioning regimens were performed according to international protocols. In patients with nonmalignant disease, thus consisted of targeted busulfan (area under the curve, 90 mg h/L in 4 days) and fludarabine (160 mg/m^2 in 4 days). In patients with malignant disease, either fractioned total-body irradiation–based conditioning (3 × 2 × 2 Gy; etoposide, 60 mg/kg) or targeted busulfan (area under the curve, 90 mg h/L) plus fludarabine (160 mg/m^2) or fludarabine plus clofarabine (40 and 120 mg/m^2) was given, depending on the patient's age, myeloid or lymphoid origin of disease, central nervous system involvement, and high-risk disease characteristics. In patients receiving an unrelated donor transplant, serotherapy was performed with antithymocyte globulin (thymoglobulin). In patients with very high-risk malignancies (relapsed myeloid leukemia and early relapsed lymphoid leukemia) receiving a cord blood (CB) donor, we omitted antithymocyte globulin from December 2012 onward.

Stool samples and nose/throat swabs were cultured weekly to monitor for bacterial colonization. Plasma was tested weekly for the presence of EBV, cytomegalovirus, human herpesvirus 6, and adenovirus DNA by using real-time PCR. Weekly galactomannan testing (Platelia *Aspergillus* enzyme immunoassay; Bio-Rad Laboratories, Hercules, Calif) was performed to screen for *Aspergillus* species infection.

Antimicrobial prophylaxis involved daily ciprofloxacin and fluconazole during neutropenia, with additional prophylaxis against *Streptococcus viridans* with cefazolin in the mucositis phase. *Pneumocystis jirovecii* pneumonia prophylaxis was administered as co-trimoxazole 3 times a week. In case of positive serologic results for herpes simplex virus (in all patients) and varicella zoster virus (in CB recipients), prophylaxis with acyclovir was given. No other antiviral prophylaxis was given. In high-risk patients for invasive fungal infection, *Aspergillus* species prophylaxis was done with daily voriconazole or twice-weekly amphotericin B.

GVHD prophylaxis consisted of cyclosporine (through a level of 150-250 μg/L) in all patients. In CB recipients we added prednisolone (1 mg/kg/d for 28 days); in patients receiving an unrelated volunteer donor transplant, methotrexate (short course, 10 mg/m^2 on days 1, 3, and 6) was

TABLE I. Demographics and baseline characteristics (n = 179)

Age at HCT (y [range])	6.8 (0.6-22.7)
Sex	
Male	106 (59%)
Female	73 (41%)
HCT indication*	
Malignancy	90 (50%)
Bone marrow failure syndrome	16 (9%)
Inborn error of metabolism	34 (19%)
Primary immune deficiency	39 (22%)
Conditioning	
TBI based	27 (15%)
Chemotherapy based	152 (85%)
Donor	
MSD	47 (26%)
MUD	35 (20%)
uCB	97 (54%)
HLA matching	
Matched	120 (67%)
Mismatched	59 (33%)
CMV serology recipient	
CMV positive	103 (58%)
CMV negative	70 (39%)
CMV unknown	6 (3%)
BAL	
RV positive	74 (41%)
RV negative	105 (59%)
NPA	
RV positive	105 (59%)
RV negative	74 (41%)

CMV, Cytomegalovirus; *MSD*, matched sibling donor; *MUD*, matched unrelated donor; *TBI*, total-body irradiation; *uCB*, unrelated cord blood.
*HCT indications: *Malignancy*, acute lymphoblastic leukemia, acute myeloblastic leukemia, myelodysplastic syndrome, juvenile myelomonoblastic leukemia, and lymphoma; *bone marrow failure syndromes*, Fanconi anemia, congenital agranulocytosis, and severe aplastic anemia; *inborn errors of metabolism*, Hurlers syndrome, hemoglobinopathies, and other; *primary immune deficiencies*, severe combined immune deficiency and combined immunodeficiency.

added to cyclosporine. From 2013, we also administered a short course of methotrexate to patients receiving bone marrow from an HLA-matched sibling.

Treatment of allo-LSs consisted of 10 mg/kg/d intravenous methylprednisolone for 3 days and 2 mg/kg/d thereafter, tapering by 25% per week to 0.5 mg/kg/d. Methylprednisolone pulses were repeated monthly until recovery up to a maximum of 6 pulses. Recovery was defined as normalization of PFTs, resolved symptoms, or both. In between subsequent pulses, 0.5 mg/kg/d prednisone was administered. Other immunosuppressive agents (usually cyclosporine) were continued. In addition, azithromycin was given because of its suggested immunomodulatory effect. Along with immunosuppressive therapy, supportive care was provided with extra oxygen and mechanical ventilation, when necessary.

Outcomes

The main outcome of interest was the occurrence of allo-LSs, which were defined as idiopathic pneumonia syndrome (IPS) or bronchiolitis obliterans syndrome (BOS). IPS is defined by the American Thoracic Society as evidence of widespread lung injury by clinical symptoms and radiologic abnormalities in the absence of active lower respiratory tract infection and other factors explaining pulmonary dysfunction (cardiac dysfunction, fluid overload, or renal failure).[9] BOS is defined according to the National Institutes of Health Consensus Criteria on Chronic GVHD 2014 as an FEV_1/vital capacity ratio of less than 0.7, FEV_1 of less than 75%, and evidence of air-trapping (on PFTs or HRCT scans) in the absence of respiratory tract infection.[10] We adjusted these definitions by not excluding patients only for a longer existing positive PCR result for RVs when they fulfilled all other criteria for allo-LSs (as described previously[6]).

Additionally, we investigated the association between allo-LSs and overall survival, treatment-related mortality (TRM), and aGVHD and chronic GVHD in other organs (classification according to the Glucksberg criteria[11]).

Statistical analysis

The duration of follow-up was the time to development of allo-LSs or death or the last assessment for survivors. We assessed the association between outcome and patient-related variables (age at transplantation, sex, RV status, RV viral load expressed as Ct value, and cytomegalovirus status), disease (malignancy, bone marrow failure syndromes, and inborn errors of metabolism and primary immune deficiency), conditioning regimen (chemotherapy or total-body irradiation based), and donor factors (stem cell source and HLA disparity). Because of sample size, the median Ct value (as semiquantitative viral load) was taken to dichotomize the group in high or low RV viral load.

Variables associated with a P value of less than .1 by using univariate analysis were selected for multivariate analysis. Probabilities of event-free and overall survival were calculated by using the Kaplan-Meier estimate; we used the 2-sided log-rank test for univariate comparisons. Time-dependent outcomes were analyzed by using Cox proportional hazard models. For the end points of allo-LSs, overall survival, and TRM, we used Fine-Gray competing risk regressions. For dichotomous variables, univariate and multivariate logistic regression analyses were done. All statistical analyses were performed with either SPSS 22 (SPSS, Chicago, Ill) or R, version 3.0.1, software.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. BV and JJB had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Patients' characteristics

A total of 273 patients underwent transplantation during the study period. One hundred seventy-nine patients had paired NPA and BAL samples before HCT available for analysis; they were evaluated in the study. The other 94 patients did not have NPAs and BAL fluid taken. The median age at transplantation was 6.8 years (range, 0.6-22.7 years); half of the patients underwent transplantation for a malignant disease, and CB (54%) was the main source of stem cells, followed by bone marrow (44%). Baseline characteristics are shown in Table I. Median follow-up of surviving patients was 4.3 years (range, 0.7-9.7 years).

Overall, RVs were detected in 110 (61%) of the patients. In BAL fluid RVs were detected in 74 (41%) samples; 36 (20%) children had RVs detected in NPAs only. Only 5 (6%) patients with positive BAL fluid RV results had negative NPA RV results. Rhinovirus was the most frequently detected RV (43%), followed by multiple RVs (38%), with a similar distribution in BAL fluid and NPAs, as shown in Table II. No patients with negative RV results had signs of upper respiratory tract infection (URTI) during hospitalization.

Most patients had no or very mild signs of a URTI and no signs of lower respiratory tract infection at the time of sampling. The median PCR Ct value for RV in BAL fluid was 32 (range, 17-43), which is comparable with the Ct values found in NPAs (29; range, 14-43).

TABLE II. Distribution of RVs in pre-HCT samples

	NPAs only (n = 36)	BAL fluid (n = 74)
Rhinovirus	14 (39%)	38 (51%)
Multiple viruses	10 (28%)	20 (27%)*
Parainfluenza virus	4 (10%)	4 (6%)
Adenovirus	3 (8%)	2 (3%)
Coronavirus	2 (6%)	6 (9%)
RSV	1 (3%)	1 (1%)
Bocavirus	2 (6%)	1 (1%)
Influenza virus	—	1 (1%)
hMPV	—	1 (1%)

hMPV, Human metapneumovirus; *RSV*, respiratory syncytial virus.
*Multiple = 2 to 4 different RVs, 14 with rhinovirus.

Outcomes of interest

During conditioning and early after HCT, the URTI symptoms did not progress to significant lower respiratory tract infection symptoms. On the contrary, most patients recovered spontaneously. Twenty-four (13%) patients were given a diagnosis of an allo-LS after a median of +59 days (range, 7-201 days). Fifteen patients had IPS, and 9 patients had BOS.

In multivariate analysis detection of RV in BAL fluid was the only predictor associated with allo-LSs (hazard ratio [HR], 3.8; 95% CI, 1.4-10.7; $P = .01$), as shown in Table III (see Table E1 in this article's Online Repository at www.jacionline.org for univariate analysis).

When subdividing into groups with BOS and those with IPS, similar results were found. RV detection in BAL fluid was a predictor for BOS (HR, 5.1; 95% CI, 1.1-24.7; $P = .04$; see Tables E2 and E3 and Fig E1 in this article's Online Repository at www.jacionline.org). For patients with IPS, a positive BAL fluid RV result (HR, 3.6; 95% CI, 1.0-13.8; $P = .06$) was a borderline significant predictor, as was having an inborn error of metabolism as the indication for transplantation (HR, 3.6; 95% CI, 1.0-12.8; $P = .05$; see Tables E4 and E5 and Fig E2 in this article's Online Repository at www.jacionline.org).

The probability of having an allo-LS at 1 year was 26% for patients with positive BAL fluid RV results compared with 6% for those with negative RV results ($P = .005$; Fig 1, *A*). The presence of RVs in NPAs only was not associated with allo-LSs. There was no difference found for rhinovirus and nonrhinovirus in the probability of allo-LS development (Fig 1, *B*). There also was no influence of viral load (defined as high and low based on Ct values) on the occurrence of allo-LSs (see Fig E3 in this article's Online Repository at www.jacionline.org).

In patients with RV in BAL fluid (n = 74), we determined the association between occurrence of grade II to IV aGVHD treated with systemic steroids (occurring after a median of +41 days [range, 6-128 days]) and the development of allo-LSs. aGVHD appears to protect against development of allo-LSs, although results were not statistically significant (odds ratio, 0.16; 95% CI, 0.0-1.3; $P = .08$; Fig 2).

All 24 patients with allo-LSs were treated with immunosuppressive therapy according to the treatment guidelines described in the Methods section. Ten (42%) patients are alive with stabilized lung function, 1 patient died of relapsed disease, and 13 died of TRM (infection or progressive lung disease). Allo-LSs contributed significantly to a higher estimated TRM at 5 years (52% ± 10% in patients with allo-LSs and 20% ± 4% in patients without allo-LSs; $P = .007$; Fig 3, *A*), leading to a trend for lower estimated overall survival at 5 years (48% ± 10% in patients with allo-LSs 66% ± 4% in patients without allo-LSs; $P = .07$; Fig 3, *B*).

TABLE III. Multivariate analyses for predictors for Allo-LSs (BOS plus IPS)

	Univariate	Multivariate	
	P value	HR (95% CI)	*P* value
Sex			
Male		1	
Female	.04*	1.4 (1.0-2.2)	.08
HCT indication*			
Malignancy		1	
Bone marrow failure syndrome	.98	0.0 (0-0)	.98
Inborn error of metabolism	.04*	1.9 (0.7-5.2)	.21
Primary immune deficiency	.07*	2.0 (0.7-5.3)	.19
BAL			
RV negative		1	
RV positive	.001*	3.8 (1.4-10.7)	.01*

*Statistical significance.

DISCUSSION

To our knowledge, this is the largest study analyzing the association between detection of RV DNA/RNA before pediatric HCT and the development of allo-LSs. We noted a very high incidence of RV in pre-HCT samples (61%), predominantly rhinovirus. Despite immunosuppressive treatment of the conditioning and GVHD prophylaxis, no progression to viral pneumonitis occurred. After a median of 8.5 weeks, often coinciding with T-cell immune recovery, 13% of all included patients had respiratory symptoms fitting the diagnostic criteria for allo-LSs. With the limitations of a retrospective cohort study taken into account, our data suggest that detection of RV in BAL fluid (and not from NPAs) before HCT is a strong predictor for the development of allo-LSs in children after HCT. No difference in effect was found between the various viral species detected, nor did we see a relation with the PCR Ct value (as a semiquantitative measure for viral load) of the virus at detection. Grade II to IV aGVHD in another organ occurring earlier in time (median onset after 6 weeks) appears to have a protective effect on the occurrence of allo-LSs, possibly because of earlier initiation of increased immunosuppression. Allo-LSs were treated with high-dose steroids but remained a life-threatening complication; patients with allo-LSs had significantly higher TRM associated with a trend toward lower overall survival. This high TRM warrants novel or additional treatment in prospective trials; etanercept is one of the agents suggested by others, although conflicting data exist.[12,13] Identification of high-risk patients, preventive strategies, and awareness and early detection of allo-LSs could lead to improved survival chances.

A limitation of our sampling method might be the possible contamination of the bronchoscope on its route through the upper airway, influencing the RV DNA/RNA positivity of the BAL samples. However, in all patients BAL was done through a tracheal tube some time after intubation, reducing the risk of direct contamination. Moreover, we have not found any difference in Ct values between NPAs and BAL fluid, and therefore contamination seems unlikely (because one would expect a much higher Ct value and lower viral load when contaminated). If some positive BAL samples were contaminated, the suggested

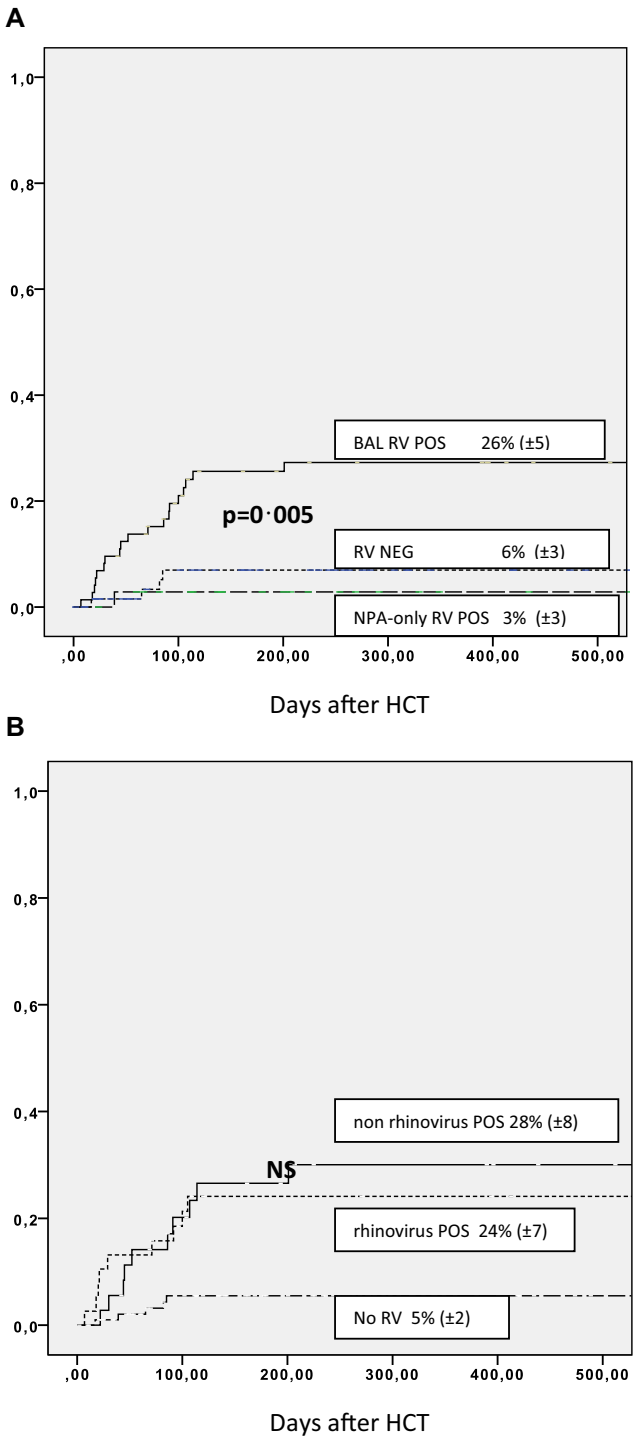

FIG 1. A, Cumulative incidence of allo-LSs for patients with negative RV results, positive BAL fluid RV results, and positive NPA-only RV results. **B,** Cumulative incidence of allo-LSs for patients with negative RV results and both those with and those without rhinovirus (from BAL fluid only, without taking NPA results into account).

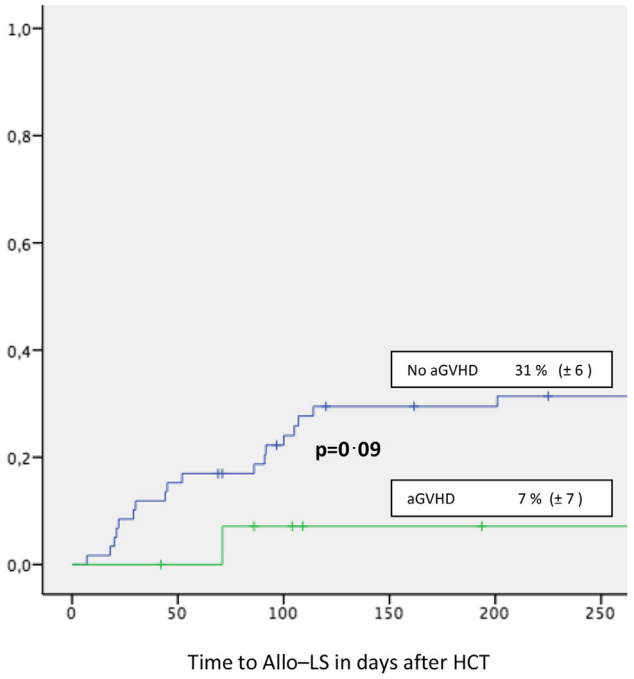

FIG 2. Cumulative incidence of allo-LSs in patients with positive BAL fluid RV results according to the presence of grade II to IV aGVHD in another organ.

association between BAL fluid RV and allo-LSs would be even stronger. An important note is that during the study period, as it became clear there was an association between RV positivity in NPAs and allo-LSs,[6] we started taking preventive measures in the patients with positive NPA RV results by postponing HCT, prolongation of immunosuppressive therapy, or both. This might have influenced the study outcomes and could have led to the observed decrease in the incidence of allo-LSs over time.

A point of discussion could be the fact that the diagnostic criteria for allo-LSs historically insist on the exclusion of infectious causes. Our data suggest that the presence of an RV in the lower airways is rather a warning sign for the development of allo-LSs. The fact that we have pre-HCT, presymptomatic BAL samples gives new insight in this discussion. We show that an RV could be present for weeks (to months) before pulmonary symptoms occur. This is despite the fact that the patients are severely immunocompromised. In an era of more precise detection tools (eg, PCR), it is not surprising that certain disease (exclusion) criteria are subject to changes. Therefore we have allowed RV PCR positivity in the definition of allo-LSs.

The high incidence of RVs in pediatric HCT recipients is in line with other recent studies.[14-16] The effect of RVs in an HCT population is conflicting. Some groups describe progression to viral pneumonia,[17,18] some describe spontaneous recovery,[15,19] and others describe an association with poor outcome.[6,14,20] Furthermore, there are emerging reports on rhinovirus being more than just a common cold virus.[6,14,16,20]

The respiratory microbiome, which consists of viruses, bacteria, and fungi, closely interacts with local and systemic immunity. Viral immunomodulation is complex and multidimensional.[21-23] There is growing interest in understanding how disturbances can lead to lung disease, especially in the field of chronic inflammatory diseases, such as asthma and chronic obstructive pulmonary disease (COPD).[21] Our data suggest that similar processes can occur because of common cold RV persistence in the lungs in immune-deficient patients while reconstituting a donor-derived novel immune system. This concept fits the current understanding of alloimmunity after

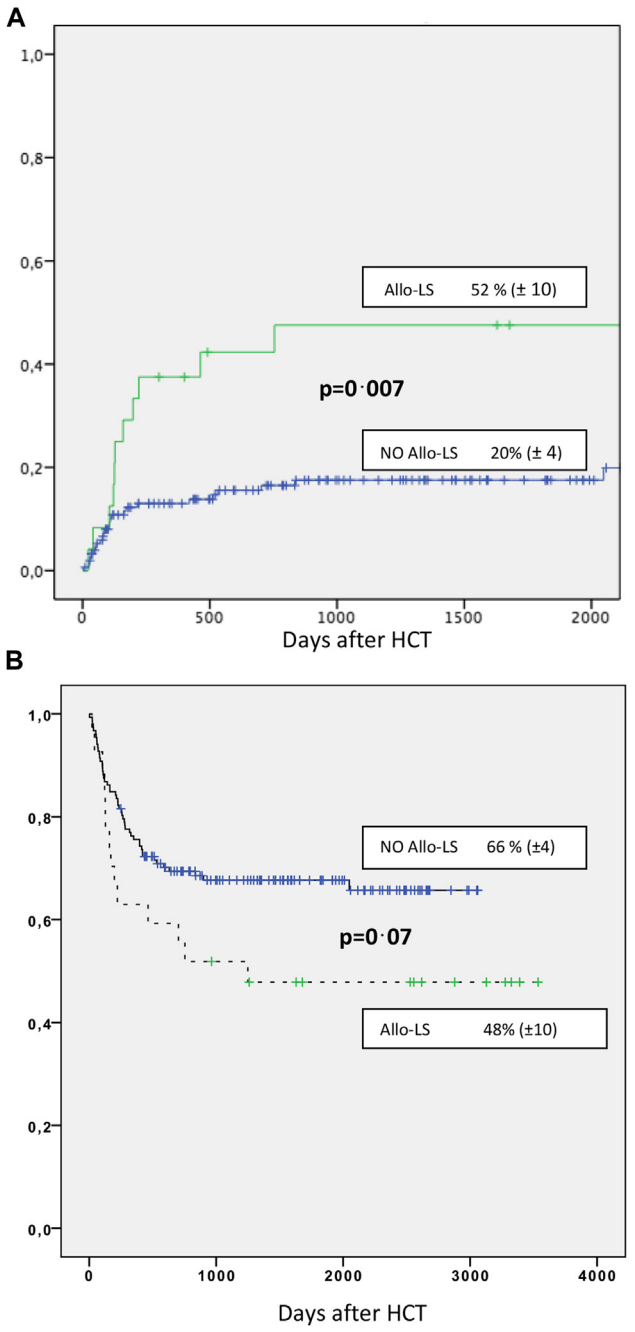

FIG 3. A, Probability of TRM for patients with and without allo-LSs. **B,** Probability of overall survival *(OS)* for patients with and without allo-LSs.

HCT, where host antigen-presenting cells are activated by danger signals expressed on damaged tissues, pathogens, or both, leading to alloactivation and cytokine release and inducing a cycle of inflammation and tissue damage.[24-26] Therefore further studies on the influence of the microbiome/viriome on the development of alloreactive immune phenomena after HCT are of great interest. In line with this, in patients with an inborn error of metabolism, which was found to be a borderline predictor for IPS, storage of glycosaminoglycans in the lungs can lead to low-grade inflammation and activation of antigen-presenting cells as well.

The effects of viral infection and inflammation have been studied in other allograft settings. In lung transplant recipients several groups have found an association between RVs and the development of acute and chronic allograft rejection and BOS, the main limitation to long-term survival.[5,27-29] Also, in patients receiving other solid-organ transplants, there is evidence of infection playing a role in allograft dysfunction both through immunologic factors and nonimmunologic components.[30]

In a recent study on RVs and IPS after HCT, Seo et al[31] described that when using currently available diagnostic methods, in retrospect, they found occult pathogens in the majority of BAL fluid samples taken at diagnosis from patients with IPS. The presence of a pathogen was associated with mortality, even for pathogens with uncertain pulmonary pathogenicity, such as rhinovirus. They reconsidered the diagnosis, suggesting viral pneumonitis instead of IPS, and pointed out the possible harmful effect of high-dose steroids in case of detectable pathogens. This is in contrast with the interpretation of our findings. An important difference with our study is that Seo et al have no information on the RV status of the patients before they experience IPS.

We have shown that in our pediatric population allo-LSs develop at a median of 8 weeks after first detection of RV (during T-cell reconstitution). Therefore we believe that it is not the RV itself that causes the respiratory deterioration but the inflammatory response (similar to immune reconstitution inflammatory syndrome in patients with HIV/AIDS). Timing of symptoms, protection by immunosuppressive therapy (in case of GVHD), and initial response to an increase in immunosuppression support our hypothesis of a primary immune-mediated process. On the basis of these results, we would recommend thorough screening of all pre-HCT patients with chest HRCT and BAL in search of fungal infection but also for RV as a predictor for allo-LSs. In case of RV positivity, we would advise slower tapering of immune suppression after HCT and close monitoring for respiratory symptoms during follow-up with prompt diagnostics and treatment when an allo-LS is suspected.

In conclusion, our findings show that the presence of RV DNA/RNA in BAL fluid before HCT is a strong predictor for the occurrence of allo-LSs weeks to months after HCT. Further studies are needed to unravel the mechanisms underlying alloimmune phenomena in different target organs after HCT and to determine a role for the respiratory microbiome. Recognizing BAL fluid RV positivity before HCT as a predictor for allo-LSs might have clinical implications for prevention (by adapting immune suppressive prophylaxis) and (pre-emptive) therapy. Early recognition might also lead to improved survival chances.

Clinical implications: In children positive RV results in BAL fluid before HCT predisposes to allo-LSs. Screening is important because prevention and treatment of allo-LSs is based on either prolonged or increased immune suppression.

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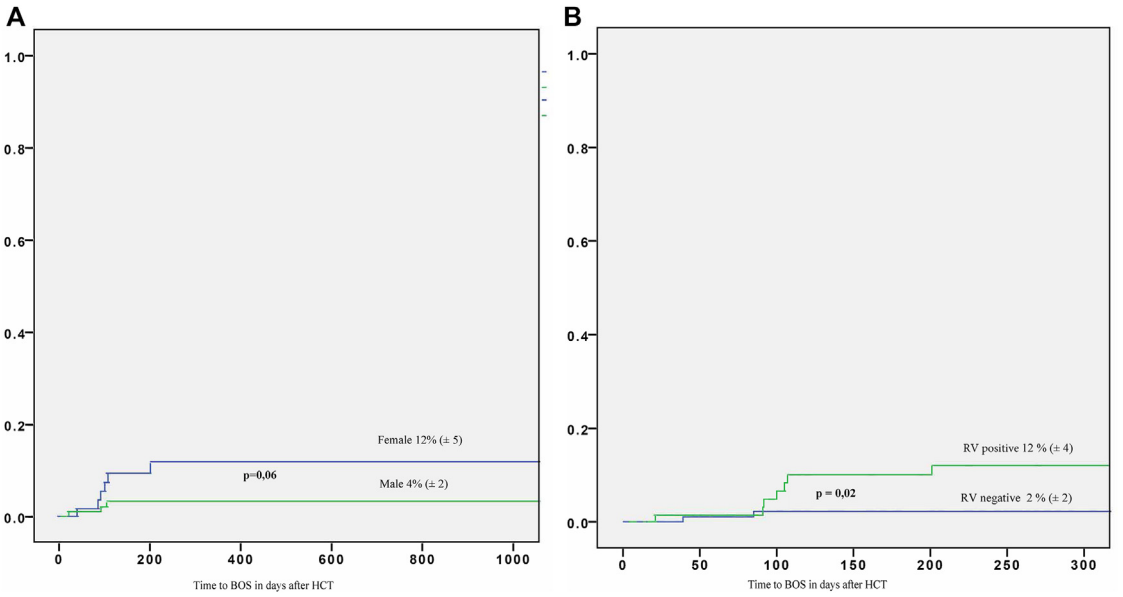

FIG E1. A, Cumulative incidence of BOS according to sex. **B,** Cumulative incidence of BOS according to positive BAL fluid RV results.

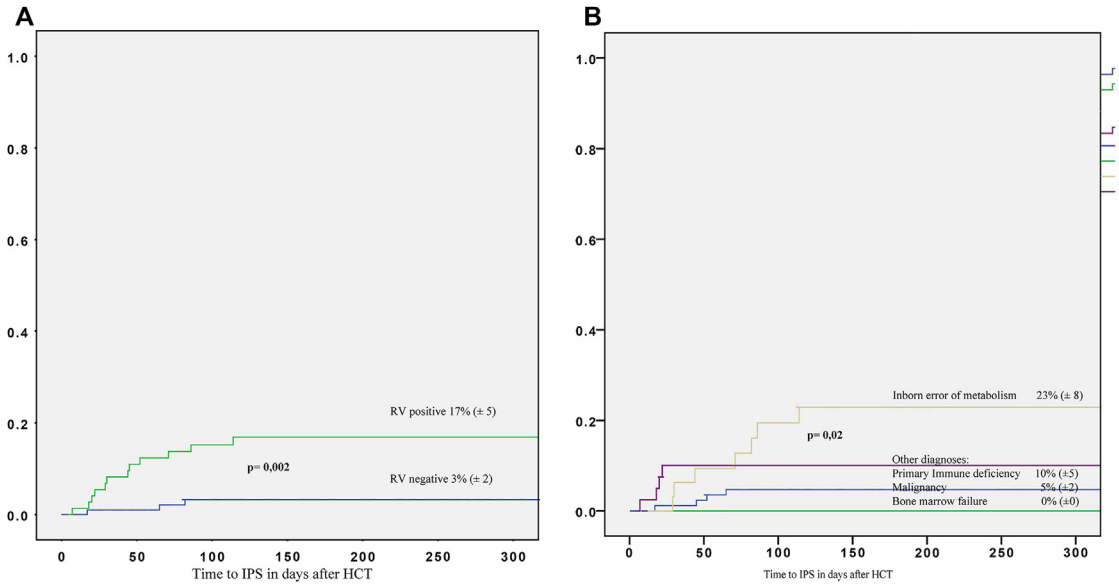

FIG E2. **A,** Cumulative incidence of IPS according to positive BAL fluid RV results. **B,** Cumulative incidence of IPS according to diagnosis.

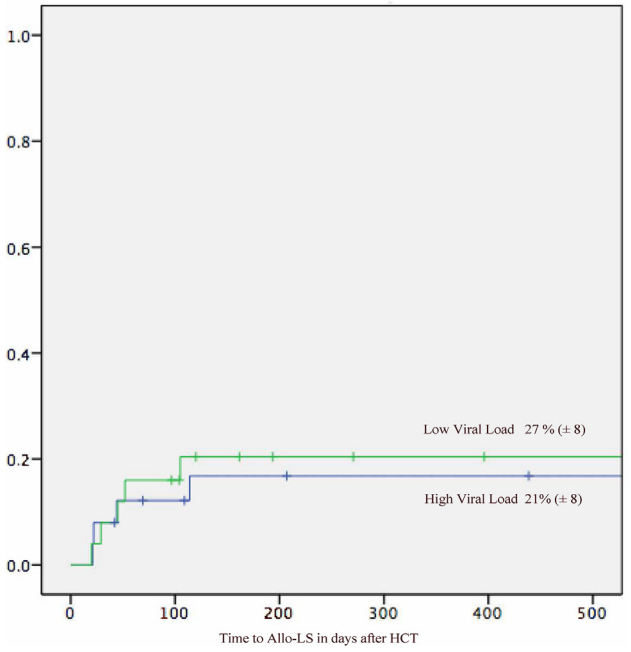

FIG E3. Cumulative incidence of allo-LSs according to RV viral load in BAL fluid (defined as greater or lower than the median Ct value [32] for RV).

TABLE E1. Predictor analyses for outcome of interest allo-LSs (BOS plus IPS): Univariate analyses (n = 24)

	HR (95% CI)	*P* value
Age at HCT	1.0 (0.9-1.0)	.28
Sex		
Male	1	
Female	1.5 (1.0-2.3)	.04*
HCT indication*		
Malignancy	1	
Bone marrow failure syndrome	0.0 (0-0)	.98
Inborn error of metabolism	2.8 (1.1-7.6)	.04*
Primary immune deficiency	2.5 (0.9-6.6)	.07*
Conditioning		
Chemotherapy	1	
TBI based	0.04 (0.0-4.0)	.17
Donor		
MSD	1	
MUD	1 (0.3-3.1)	.99
uCB	0.8 (0.3-2.2)	.73
HLA matching		
MSD 10/10 MUD	1	
9/10 MUD	3.4 (0.7-15.9)	.11
6/6 uCB	0.9 (0.3-2.6)	.82
4-5/6 uCB	1.4 (0.5-3.5)	.52
CMV serology recipient		
CMV negative	1	
CMV positive	1.7 (0.6-4.3)	.30
NPA		
RV negative	1	
RV positive	0.4 (0.0-3.9)	.45
BAL		
RV negative	1	
RV positive	5.5 (2.0-14.7)	.001*

CMV, Cytomegalovirus; *MSD*, matched sibling donor; *MUD*, matched unrelated donor; *TBI*, total-body irradiation; *uCB*, unrelated cord blood.
*Statistical significance.

TABLE E2. Predictor analyses for BOS: Univariate analyses (n = 9)

	HR (95% CI)	*P* value
Age at HCT	1.0 (0.9-1.1)	.74
Sex		
Male	1	
Female	1.9 (0.9-3.8)	.08*
HCT indication*		
Malignancy	1	
Bone marrow failure syndrome	0.0 (0-0)	.99
Inborn error of metabolism	0.8 (0.1-7.1)	.83
Primary immune deficiency	2.5 (0.6-9.8)	.20
Conditioning		
Chemotherapy	1	
TBI based	0.0 (0.0-62.2)	.38
Donor		
MSD	1	
MUD	0.7 (0.1-3.8)	.67
uCB	0.4 (0.1-1.7)	.21
HLA matching		
MSD 10/10 MUD	1	
9/10 MUD	3.5 (0.4-30.4)	.25
6/6 uCB	0.3 (0.0-2.9)	.32
4-5/6 uCB	0.7 (0.1-3.4)	.62
CMV serology recipient		
CMV negative	1	
CMV positive	4.3 (0.5-35.4)	.18
NPA		
RV negative	1	
RV positive	5.7 (0.7-44.5)	.10
BAL		
RV negative	1	
RV positive	5.3 (1.1-25.7)	.04*

CMV, Cytomegalovirus; *MSD*, matched sibling donor; *MUD*, matched unrelated donor; *TBI*, total-body irradiation; *uCB*, unrelated cord blood.
*Statistical significance.

TABLE E3. Predictor analyses for BOS: Multivariate analyses

	Univariate	Multivariate analyses	
	***P* value**	**HR (95% CI)**	***P* value**
BAL			
RV negative	1		
RV positive	.04	5.1 (1.1-24.7)	.04*
Sex			
Male	1		
Female	.08	3.4 (0.8-13.5)	.09

*Statistical significance.

TABLE E4. Predictor analyses for IPS: Univariate analyses (n = 15)

	HR (95% CI)	*P* value
Age at HCT	0.9 (0.8-1.0)	.12
Sex		
Male	1	
Female	1.4 (0.8-2.3)	.21
HCT indication*		
Malignancy	1	
Bone marrow failure syndrome	0.0 (0.0-0.0)	.98
Inborn error of metabolism	4.8 (1.4-16.5)	.01*
Primary immune deficiency	2.6 (0.7-10.6)	.17
Conditioning		
Chemotherapy	1	
TBI based	0.04 (0.0-12.7)	.27
Donor		
MSD	1	
MUD	1.3 (0.3-6.6)	.72
uCB	1.4 (0.4-5.0)	.64
HLA matching		
MSD 10/10 MUD	1	
9/10 MUD	4.3 (0.5-38.5)	.19
6/6 CB	1.5 (0.4-6.0)	.57
4-5/6 CB	2.2 (0.6-7.6)	.24
CMV serology recipient		
CMV negative	1	
CMV positive	1.2 (0.4-3.7)	.75
NPA		
RV negative	1	
RV positive	2.9 (0.8-10.2)	.1
BAL		
RV negative	1	
RV positive	6.2 (1.7-21.8)	.005*

CMV, Cytomegalovirus; *MSD*, matched sibling donor; *MUD*, matched unrelated donor; *TBI*, total-body irradiation; *uCB*, unrelated cord blood.
*Statistical significance.

TABLE E5. Predictor analyses for IPS: Multivariate analyses

	Univariate	Multivariate	
	***P* value**	**HR (95% CI)**	***P* value**
HCT indication*			
Malignancy		1	
Bone marrow failure syndrome	.98	0.0 (0.0-0.0)	.98
Inborn error of metabolism	.01	3.6 (1.0-12.8)	.05*
Primary immune deficiency	.17	2.9 (0.7-11.5)	.14
BAL			
RV negative		1	
RV positive	.005	3.6 (1.0-13.8)	.06

*Statistical significance.