Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Infection with a respiratory virus before hematopoietic cell transplantation is associated with alloimmune-mediated lung syndromes

Birgitta Versluys, MD, a Marc Bierings, MD, a Jean Luc Murk, MD, b Tom Wolfs, MD, c Caroline Lindemans, MD, a Kors vd Ent, MD, d and Jaap Jan Boelens, MD a,e Utrecht, The Netherlands

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% vs 10% in allo-LSs and 20% \( \pm \) 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
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 HCT5,6 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.1 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.5 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) and fludarabine (160 mg/m² 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/m²) or fludarabine plus clofarabine (40 and 120 mg/m²) 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 (Patella 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 jiroveci 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/m² on days 1, 3, and 6) was given.
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 source 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).11 BOS is defined according to the National Institutes of Health Consensus Criteria on Chronic GVHD 2014 as an FEV1/vital capacity ratio of less than 0.7, FEV1 of less than 75%, and evidence of air-trapping (on PFTs or HRCT scans) in the absence of respiratory tract infection.11 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).11

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 (URT) 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

| Virus Type      | NPA only (n = 36) | BAL fluid (n = 74) |
|-----------------|-------------------|-------------------|
| Rhinovirus      | 14 (39%)          | 38 (51%)          |
| Multiple viruses| 10 (28%)          | 20 (27%)*         |
| Parainfluenza   | 4 (10%)           | 6 (8%)            |
| Adenovirus      | 3 (8%)            | 2 (3%)            |
| Coronavirus      | 2 (6%)            | 6 (9%)            |
| RSV             | 1 (3%)            | 1 (1%)            |
| Bocavirus       | 2 (6%)            | 1 (1%)            |
| Influenza       | —                 | 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, 1.4-10.7; P = .01), as shown in Table III (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 NPA 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)

| Predictors                | Univariate P value | Multivariate 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
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, 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. The effect of RVs in an HCT population is conflicting. Some groups describe progression to viral pneumonia, some describe spontaneous recovery, and others describe an association with poor outcome. Furthermore, there are emerging reports on rhinovirus being more than just a common cold virus.

The respiratory microbiome, which consists of viruses, bacteria, and fungi, closely interacts with local and systemic immunity. Viral immunomodulation is complex and multidimensional. 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). 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 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.
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. Therefore further studies on the influence of the microbiome/viriome on the development of alloimmune 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. 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.

In a recent study on RVs and IPS after HCT, Seo et al 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.

REFERENCES
1. Cooke KR. Acute lung injury after allogeneic stem cell transplantation: from the clinic, to the bench and back again. Pediatr Transplant 2005;9:24-36.
2. Shono Y, Docampo MD, Peled JU, Perobelli SM, Jenq RR. Intestinal microbiota-related effects on graft-versus-host disease. Int J Hematol 2015;101:428-37.
3. van Montfrans J, Schulz L, Versluys B, de Wildt A, Wolfs T, Bierings M, et al. Viral PCR positivity in stool before allogeneic hematopoietic cell transplantation is strongly associated with acute intestinal graft-versus-host disease. Biol Blood Marrow Transplant 2015;21:772-4.

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.
1. Yanik GA, Grupp SA, Pulsipher MA, Levine JE, Schultz KR, Wall DA, et al. Clinical impact of community-acquired respiratory viruses on bronchiolitis obliterans after hematopoietic stem cell transplantation in children. Biol Blood Marrow Transplant 2015;21:1622-6.

2. van de Pol AC, Wolff TF, Jansen NJ, van Loo AM, Rossen JW. Diagnostic value of real-time polymerase chain reaction to detect viruses in young children admitted to the paediatric intensive care unit with lower respiratory tract infection. Crit Care 2006;10:R61.

3. Panoskaltsis-Mortari A, Griese M, Madtes DK, Belperio JA, Haddad IY, Folz RJ, 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;183:1262-79.

4. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant 2015;21:389-401.e1.

5. Glucksberg H, Storb R, Buckner CD, Neiman PE, Clift RA, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HLA-matched sibling donors. Transplantation 1974;18:295-304.

6. Yanik GA, Grupp SA, Pulsipher MA, Levine JE, Schultz KR, Wall DA, et al. Tumour necrosis factor receptor inhibitor therapy for the treatment of children with idiopathic pneumonia syndrome after hematopoietic stem cell transplantation and the development of life-threatening acute and chronic allograft dysfunction and allograft survival after solid organ transplantation. Am J Transplant 2011;11:1071-8.

7. Campbell AP, Guthrie KA, Englund JA, Furney RM, Minierich EL, Kuyper J, et al. Clinical outcomes associated with respiratory virus detection before allogeneic hematopoietic stem cell transplant. Clin Infect Dis 2015;61:192-202.

8. Strimivasan A, Flynn P, Gu Z, Hartford C, Lovins R, Sunkara A, et al. Detection of respiratory viruses in asymptomatic children undergoing allogeneic hematopoietic stem cell transplantation. Pediatr Blood Cancer 2013;60:149-51.

9. Gerna G, Parilla A, Rovida F, Rognoni V, Marchi A, Locatelli F, et al. Correlation of rhinovirus load in the respiratory tract and clinical symptoms in hospitalized immunocompetent and immunocompromised adults. J Med Virol 2009;81:1498-507.

10. Versluis AB, van der Ent K, Boelens JJ, Wolfs T, de Jong P, Bierings MB. High diagnostic yield of dedicated pulmonary screening before hematopoietic cell transplantation in children. Biol Blood Marrow Transplant 2015;21:1622-6.

11. Hutsupardon S, Essa M, Richardson S, Schechtner T, Ali M, Krueger J, et al. Significant transplantation-related mortality from respiratory virus infections within the first one hundred days in children after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2015;21:1802-7.

12. Hirsch HH, Martino R, Ward KN, Boeckh M, Einsele H, Ljungman P. Fourth European Conference on Infections in Leukaemia (ECIL-4): guidelines for diagnosis and treatment of human respiratory syncytial virus, parainfluenza virus, metapneumovirus, rhinovirus, and coronavirus. Clin Infect Dis 2013;56:258-66.

13. Bredius RG, Templeton KE, Scheltinga SA, Claas EC, Kroes AC, Vossen JM. Prospective study of respiratory viral infections in pediatric hematoepoietic stem cell transplantation patients. Pediatr Infect Dis J 2004;23:518-22.

14. Seo S, Martin E, Xie H, Kuyper J, Campbell AP, Choi SW, et al. Human rhinovirus RNA detection in the lower respiratory tract of hematopoietic cell transplant recipients: association with mortality. Biol Blood Marrow Transplant 2013;19(suppl 1):S167-77.

15. Cadwell K. The virome in host health and disease. Immunology 2015;42:805-13.

16. Tracy M, Cogen J, Hoffman LR. The pediatric microbiome and the lung. Curr Opin Pediatr 2015;27:348-55.

17. Lynch SV. Viruses and microbiome alterations. Ann Am Thorac Soc 2014;11(suppl 1):S57-60.

18. Holtan SG, Pasquin M, Weisdorf DJ. Acute graft-versus-host disease: a bench-to-bedside update. Blood 2014;124:363-73.

19. Brennan TV, Rendell VR, Yang Y. Innate immune activation by tissue injury and cell death in the setting of hematopoietic stem cell transplantation. Front Immunol 2015;6:101.

20. Reddy P, Ferrara JL. Immunobiology of acute graft-versus-host disease. Blood Rev 2007;11:187-94.

21. Kumar D, Husain S, Chen MH, Moussa G, Himsworth D, Manuel O, et al. A prospective molecular surveillance study evaluating the clinical impact of community-acquired respiratory viruses in lung transplant recipients. Transplantation 2010:89:1028-33.

22. Fisher CE, Preiksaitis CM, Lease ED, Edelman J, Kirby KA, Leisenring WM, et al. Symptomatic respiratory virus infection and chronic lung allograft dysfunction. Clin Infect Dis 2016;62:313-9.

23. Vu DL, Breidevaux PO, Aubert JD, Soccal PM, Kaiser L. Respiratory viruses in lung transplant recipients: a critical review and pooled analysis of clinical studies. Am J Transplant 2011;11:1071-8.

24. Martin-Gandul C, Mueller NJ, Pascual M, Manuel O. The impact of infection on chronic allograft dysfunction and allograft survival after solid organ transplantation. Am J Transplant 2013;13:3024-40.
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)

| Predictor                        | 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)

| Predictor                  | 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

| HCT indication* | Univariate | Multivariate |
|-----------------|------------|--------------|
|                 | P value    | HR (95% CI)  | P value |
| Malignancy      | .98        | 0.0 (0.0-0.0)| .98     |
| Bone marrow failure syndrome | .01        | 3.6 (1.0-12.8)| .05*    |
| Inborn error of metabolism | .17        | 2.9 (0.7-11.5)| .14     |

| BAL             | P value    | HR (95% CI)  | P value |
|-----------------|------------|--------------|
| RV negative     | .005       | 3.6 (1.0-13.8)| .06     |
| RV positive     | 1          |              |         |

*Statistical significance.