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Microbiologic Diagnostic Workup of Acute Respiratory Failure with Pulmonary Infiltrates after Allogeneic Hematopoietic Stem Cell Transplantation: Findings in the Era of Molecular- and Biomarker-Based Assays

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A B S T R A C T

Allogeneic hematopoietic stem cell transplantation (HSCT) recipients frequently develop acute respiratory failure (ARF) with pulmonary infiltrates. Molecular- and biomarker-based assays enhance pathogen detection, but data on their yield in this population are scarce. This was a retrospective single-center study of 156 consecutive HSCT recipients admitted to the intensive care unit (ICU) between May 2013 and July 2017. Findings from a microbiologic diagnostic workup using currently available methods on bronchoalveolar lavage (BAL) and blood samples from 66 patients (age, 58 years [range, 45 to 64]; HSCT to ICU, 176 days [range, 85 to 407]) with ARF and pulmonary infiltrates were analyzed. In 47 patients (71%) a causative pathogen was identified (fungal, n = 28; viral, n = 26; bacterial, n = 18). Polymicrobial findings involving several pathogen groups occurred in 20 patients (30%). Culture (12/16, 75%), galactomannan (13/15, 87%), and *Aspergillus*-PCR (8/9, 89%) from BAL but not serum galactomannan (6/14, 43%) helped to diagnose invasive aspergillosis (n = 16, 24%). *Aspergillus*-PCR detected azole resistance in 2 cases. Mucorales was found in 7 patients (11%; BAL culture, n = 6; Mucorales-PCR, n = 1). Patients with identified pathogens had higher Simplified Acute Physiology Score II scores ($P = .049$) and inferior ICU survival (6% versus 37%, $P < .01$), which largely related to the presence of an invasive fungal infection. Eight patients (12%) had 1 or more viruses with uncertain lung pathogenicity as the sole microbiologic finding. A diagnostic microbiologic workup incorporating molecular- and biomarker-based assays identified pathogens in most HSCT recipients with ARF and pulmonary infiltrates admitted to the ICU. Implications of polymicrobial infection and pathogen patterns in these patients warrant further investigation.

INTRODUCTION

Pulmonary complications are a significant contributor to morbidity and nonrelapse mortality after allogeneic hematopoietic stem cell transplantation (HSCT). Acute respiratory failure (ARF) occurs in up to 16% of patients within the first year after HSCT [1] and is the main reason for intensive care unit (ICU) admission in this population [2]. Because of the broad range of possible causative pathogens or noninfectious processes, dedicated algorithms have been proposed to determine the cause of pulmonary infiltrates in immunocompromised hosts [3,4].

In the course of recent decades diagnostic testing has become more sophisticated, and modern approaches now include molecular (eg, PCR) and other nonculture tests (eg, antigen assays) to detect fungal, bacterial, and/or viral pathogens in various types of specimens [5]. In addition to these technical advances, the list of respiratory and opportunistic viruses linked to documented cases of lower respiratory tract disease in immunocompromised hosts has been extended over the last years [6-8]. These developments have led to an increase in the number of diagnosed infections in HSCT recipients

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with ARF that would have previously been diagnosed with a noninfectious post-HSCT lung injury syndrome. In fact, a study was able to identify pathogens in more than half of the cases previously diagnosed with idiopathic pneumonia syndrome using molecular and nonculture tests for a wide array of pathogens on bronchoalveolar lavage (BAL) samples [9]. Still, situations remain where noninfectious post-HSCT lung injury syndromes become the diagnosis of exclusion [10].

In the absence of comparable data in the literature, our study had 2 aims. The first was to analyze the findings of a modern infection detection panel performed on BAL and blood samples in a real-world cohort of critically ill HSCT recipients with ARF and pulmonary infiltrates. Second, we wanted to infer possible clinical and scientific implications.

METHODS

This retrospective study included consecutive adult (ie, ≥18 years old) HSCT recipients treated at the Department of Bone Marrow Transplantation at the University Hospital Essen, Germany. The study was approved by the ethics committee of the University of Duisburg-Essen (EC 15-6446-BO) and conducted in accordance with Good Clinical Practice guidelines and the amended Declaration of Helsinki. Our center performs around 200 adult HSCTs per year in 2 inpatient units with a total capacity of 20 beds. Critically ill patients are treated in a third affiliated unit (ICU) providing 17 closed beds run by trained intensivists and hemato-oncologists.

Consecutive adult HSCT recipients admitted to the ICU between May 2013 (introduction of a PCR-based viral panel for respiratory specimen testing) and July 2017 were screened and included in the study cohort (1) if they had a BAL with available results performed during their ICU stay and (2) if the BAL was conducted as part of a workup of ARF (new requirement of supplemental oxygen or ventilatory support during hospitalization) with pulmonary infiltrates documented on x-ray or computed tomography (CT) of the lungs ±7 days from the procedure.

Data shown in Tables 1 and 2 were obtained by reviewing the patients' medical charts, radiographic images, and discharge letters. Disease risk was categorized according to Seo et al. [11]. The Simplified Acute Physiology Score II (SAPSII) was used to assess the severity of illness at ICU admission [12]. Absolute neutrophil counts and absolute lymphocyte counts were included if results were available ±2 days from BAL.

Standard diagnostic tests performed on BAL included conventional culture for bacteria and fungi; direct microscopy; galactomannan (Bio-Rad, Munich, Germany); PCR testing for the detection of 2 fungal, 3 bacterial, and 20 viral pathogens (*Pneumocystis jirovecii* [Sacace, Como, Italy], *Aspergillus* spp. [AsperGenius PN001, Maastricht, The Netherlands; available from March 2015], atypical bacteria [*Legionella pneumophila*, *Mycoplasma pneumoniae*,

Table 1
Cohort Characteristics

	All Patients (n = 66)	Established Pathogen (n = 47)	Without Established Pathogen (n = 19)	*P*
Sex				.29
Male	38 (58)	25 (53)	13 (68)	
Female	28 (42)	22 (47)	6 (32)	
Age at ICU admission, yr	58 (45-64)	58 (47-64)	49 (32-63)	.20
Disease risk at transplantation				.78
Standard	43 (65)	30 (64)	13 (68)	
High	23 (35)	17 (36)	6 (32)	
Cell source				.57
Bone marrow	4 (6)	2 (4)	2 (11)	
Peripheral blood stem cells	62 (93)	45 (96)	17 (90)	
Donor type				.16
Matched related	16 (24)	11 (23)	5 (26)	
Mismatched related	2 (3)	0	2 (11)	
Matched unrelated	36 (55)	26 (55)	10 (53)	
Mismatched unrelated	12 (18)	10 (21)	2 (11)	
Conditioning regimen				.85
MA including TBI ≥ 8 Gy	29 (44)	20 (43)	9 (47)	
MA without TBI	7 (11)	6 (13)	1 (5)	
Reduced intensity	30 (45)	21 (45)	9 (47)	
GVHD prophylaxis				.22
CNI + MTX	52 (79)	36 (77)	16 (84)	
CNI + MMF	10 (15)	9 (19)	1 (5)	
Others	4 (6)	2 (4)	2 (11)	
ATG during conditioning	47 (71)	34 (72)	13 (68)	.77
HSCT to ICU admission, days	176 (85-407)	173 (76-288)	201 (87-415)	.46
ICU during hospitalization for HSCT	14 (21)	11 (23)	3 (16)	.74
SAPSII at ICU admission	38 (27-51)	45 (28-53)	32 (26-39)	**.05***
GVHD during ICU or at admission				
Acute GVHD	16 (24)	10 (21)	6 (32)	.53
Chronic GVHD	18 (27)	14 (30)	4 (21)	.55
Etiology of ARF according to discharge diagnosis				**<.01**
Fungal pneumonia	20 (30)	20 (43)	0	
Viral pneumonia	15 (23)	11 (23)	4 (21)	
Clinically documented pneumonia	15 (23)	3 (6)	12 (63)	
Polymicrobial pneumonia	7 (11)	7 (15)	0	
Bacterial pneumonia	6 (9)	6 (13)	0	
Noninfectious etiology	3 (5)	0	3 (16)	
Life-supporting interventions				
Invasive mechanical ventilation	63 (96)	45 (96)	18 (95)	1.0
P/F ratio at initiation of IMV	167 (129-250)	162 (127-249)	177 (140-253)	.32
Vasopressors	61 (92)	45 (96)	16 (84)	.14
Renal replacement therapy	38 (58)	31 (66)	7 (37)	.05
Extracorporeal life support	12 (18)	8 (17)	4 (21)	.73
ICU length of stay, days	16 (6-27)	14 (5-24)	22 (11-38)	.09
ICU survivors	10 (15)	3 (6)	7 (37)	**<.01**

Values are absolute number (%) or median (IQR). $P < .05$ are shown in bold type. MA indicates myeloablative; TBI, total body irradiation; GVHD, graft-versus-host disease; CNI, calcineurin inhibitor; MMF, mycophenolate mofetil; MTX, methotrexate; ATG, antithymocyte globulin; P/F, PaO2/FiO2 ratio.

* Exact $P = .049$.

Table 2
Characteristics at the Time of BAL

	All Patients (n = 66)	Established Pathogen (n = 47)	Without Established Pathogen (n = 19)	*P*
ICU admission to BAL, days	2 (1-6)	2 (0-8)	3 (1-6)	.76
Infiltrates on chest x-ray*				.91
Focal	11 (17)	7 (15)	4 (22)	
Multifocal/diffuse	45 (70)	33 (72)	12 (67)	
None	8 (13)	6 (13)	2 (11)	
Pleural effusion	15 (23)	10 (22)	5 (28)	.74
Number of quadrants involved on x-ray	3 (1-4)	4 (1-4)	3 (1-4)	.76
Absolute neutrophil count, g/L†	1.89 (.3-5.1)	1.89 (.21-5.99)	2.04 (.39-4.80)	.88
Absolute lymphocyte count, g/L†	.17 (.06-0.54)	.14 (.02-0.51)	.40 (.13-0.56)	.05
Immunosuppressive therapies‡				
Prednisone > 1 mg/kg	21 (34)	17 (39)	4 (24)	.37
Calcineurin inhibitor	13 (21)	9 (21)	4 (24)	1.0
Mycophenolate mofetil	17 (28)	13 (30)	4 (24)	.76
No. of immunosuppressants	1 (1-2)	2 (1-2)	1 (1-2)	.79
Antimicrobial therapy‡				
Intravenous broad-spectrum antibiotic	57 (93)	41 (93)	16 (94)	1.0
Trimethoprim-sulfamethoxazole	15 (25)	8 (19)	7 (41)	.10
Anti-mold coverage	54 (89)	39 (89)	15 (88)	1.0
Antiviral therapy	26 (43)	18 (41)	8 (47)	.78

Values are number (%) or median (interquartile range).
* Data available for 64 patients; the remaining 2 patients had a chest CT around BAL showing infiltrates.
† Data available for 59 patients.
‡ Data available for 61 patients.

and *Chlamydia pneumoniae*; all sacace], cytomegalovirus [CMV], herpes simplex virus types 1 and 2, varicella zoster virus [all Qiagen, Hilden, Germany], human herpesvirus 6 [HHV-6; Altona, Hamburg, Germany]; and the respiratory panel (all from Qiagen) including influenza A (including H_3N_2 and H_1N_1) and B viruses, parainfluenza virus types 1 to 3, human metapneumovirus, human coronavirus (types OC43, NL63, and 229E), respiratory syncytial virus, adenoviruses (AdV), enteroviruses, human bocavirus, and human rhinoviruses (HRV). Because of limited sample material or technical reasons, not all tests were performed or provided results in all patients. Positive reads of CMV were only included if the viral load was >500 copies/mL as measured by quantitative PCR [13]. Molecular testing for azole-resistant strains of *Aspergillus* spp. (AsperGenius PN002 [14], Maastricht) and for the detection of Mucorales [15] were available from January 2017. Other BAL tests were performed as clinically indicated (Table 3).

Results from blood cultures, serum CMV, and serum galactomannan testing were analyzed if they were obtained ±7 days from BAL. Results of galactomannan testing from BAL or blood are reported as positive using an optical density index ≥ .5 [16]. Viral pathogens were graded as "established" or "uncertain" lung pathogenicity in the setting of HSCT according to Seo et al. [9]. The recovery of *Candida* spp. or *Enterococcus* spp. from BAL was not reported in the absence of positive blood cultures. Documented diagnoses of probable invasive aspergillosis were in line with De Pauw et al. [17]. Standard antimicrobial prophylaxis consisted of ciprofloxacin and metronidazole from conditioning start until engraftment [18]. Patients with prolonged neutropenia or on systemic corticosteroids at a dose of >.5 mg/kg body weight prednisone or equivalent received anti-mold prophylaxis with voriconazole or posaconazole. Prophylaxis against *P. jirovecii* using trimethoprim-sulfamethoxazole or pentamidine as well as antiviral prophylaxis using acyclovir or valaciclovir followed national guideline recommendations [19].

Continuous data are presented as median and interquartile range (IQR; 25% to 75%) and dichotomous data as number and percentage. Group comparisons were made using Fisher's exact test and the Mann-Whitney U test as appropriate. Results were considered statistically significant when $P < .05$. Because of the exploratory nature of the study, no adjustments for multiple testing were made. Statistical tests were performed using the SPSS 23 software package (IBM, Armonk, NY).

RESULTS

Patient Demographics and Characteristics

Of 156 HSCT recipients who were admitted to the ICU between May 2013 and July 2017, 66 patients (42%; 38 men and 28 women; age, 58 years [IQR, 45 to 64]) fulfilled the inclusion criteria for this study (Figure 1). Table 1 shows the patients' characteristics regarding HSCT, severity of illness and organ support measures, and ICU outcome.

ICU referral occurred 5 days (IQR, 0 to 30) after hospital admission, 176 days (IQR, 85 to 407) after HSCT, and during hospitalization for transplantation in 14 patients (21%). BAL was performed 2 days (IQR, 1 to 6) after ICU admission

Table 3
Pathogen Findings in BAL

Findings in BAL	No. of Tests	No. of Positive Tests (% Positive)	Percent of Patients with Positive Test
Fungal			
Culture*	66	18 (27)	27
Aspergillus spp.		13 (20)	20
Mucorales		6 (9)	9
Talaromyces		1 (2)	2
Galactomannan (OD ≥ .5)	64	17 (27)	26
Aspergillus PCR	40	8 (20)	12
Mucorales PCR	3	1 (33)	2
Pneumocystis PCR†	63	5 (8)	8
Viral			
CMV	61	19 (31)	29
Varicella zoster virus‡	57	5 (9)	8
Herpes simplex 1‡	59	5 (9)	8
Herpes simplex 2‡	59	0	0
HHV-6‡	40	10 (25)	15
Influenza viruses	62	3 (5)	5
Parainfluenza viruses	59	1 (2)	2
Human metapneumovirus	59	1 (2)	2
Human coronaviruses‡	59	5 (9)	8
Respiratory syncytial virus	62	2 (3)	3
Adenoviruses‡	61	10 (16)	15
Enteroviruses‡	59	1 (2)	2
Human bocavirus‡	59	5 (9)	8
Human rhinoviruses‡	59	12 (20)	18
Bacterial			
Culture	66	17 (26)	26
Gram-negative		16 (24)	24
Gram-positive		1 (2)	2
Atypical bacteria PCR	63	1 (2)	2
Toxoplasma PCR	10	1 (10)	2
Mycobacteria culture	4	0	0

* Two patients with *Aspergillus* spp. + Mucorales.
† One patient with additional positive direct microscopy.
‡ Classified as virus without established pathogenicity in the lung (see Methods).

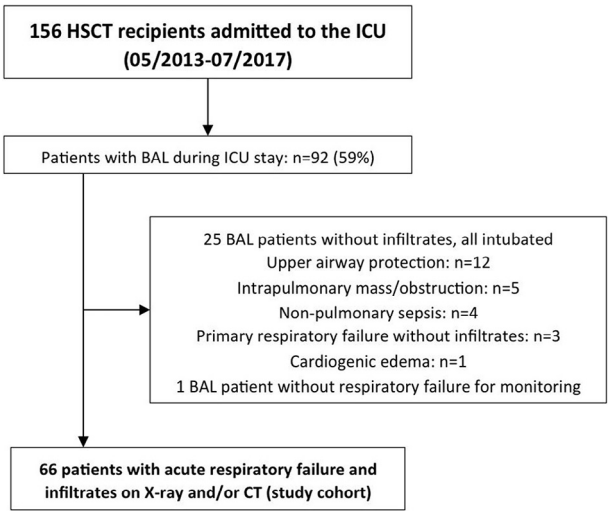

Figure 1. Patient flowchart.

(Table 2), and 1194 tests were performed on the respective samples (Table 3). Most patients presented with multifocal/diffuse infiltrates on chest x-ray (n = 45, 70%), whereas 11 (17%) had a focal infiltrate (3 quadrants involved [IQR, 1 to 4]). A chest CT ±7 days from BAL was available in 41 patients (62%) and was the only method to detect infiltrates in 8 patients (12%) with a negative chest x-ray. At the time of BAL 57 patients (86%) received invasive mechanical ventilation (time from intubation to BAL, 0 days [IQR, 0 to 1]), 4 patients (6%) received noninvasive ventilation via face mask, 3 patients (5%) received high-flow nasal cannula oxygen, and 2 patients (3%) received conventional oxygen therapy. Most patients (n = 55, 90%) were at least on 1 immunosuppressive agent when BAL was performed (medication data available for 61 patients; Table 2). No transbronchial biopsy was performed in any patient.

Bacterial Pathogens

A bacterial pathogen was identified in BAL specimens of 18 patients (27%), mostly in the context of positive culture growth (Table 3). The nonfermenters *Stenotrophomas maltophilia* (n = 5) and *Pseudomonas aeruginosa* (n = 4) were the most abundant bacterial pathogens alongside other gram-negative bacilli. A full list of detected pathogen species is given in Figure 2. There was nearly universal coverage with anti-pseudomonal beta-lactam antibiotics (n = 57, 93%) at the time of BAL. Concomitant blood cultures were available in all patients and yielded noncontaminant bacteria in 5 patients (8%), and only 2 patients with bacteria detected in BAL also had positive blood cultures. Older patient age (62 year [IQR, 59 to 68] versus 52 years [IQR, 42 to 63], $P < .01$) and time from HSCT to ICU admission (234 days [IQR, 155 to 1128] versus 143 days [IQR, 52 to 321], $P = .03$) were associated with the detection of a bacterial pathogen (Supplementary Tables 2 and 3).

Fungal Pathogens

Fungi represented the most prominent pathogen group and were identified in 28 patients (42%). Most belonged to mold species, which were detected by positive culture growth

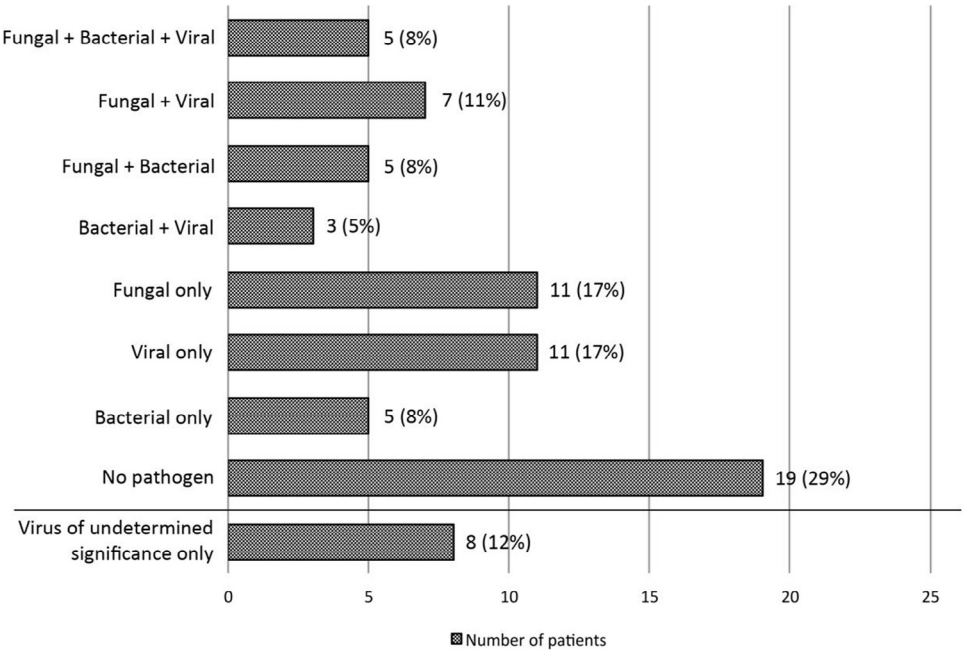

Figure 2. Pathogen distribution among patients. Numbers of patients according to pathogen distribution are plotted against the horizontal axis. The following pathogens were observed in each group: Fungal + Bacterial + Viral: *Talaromyces* (n = 1), *Aspergillus* (n = 4), *Stenotrophomonas* (n = 3), *Pseudomonas* (n = 1), *Escherichia coli* (n = 1), CMV (n = 4), parainfluenza virus (n = 1); Fungal + Viral: *Aspergillus* (n = 4), Mucorales (n = 3), pneumocystis (n = 2), CMV (n = 5), influenza (n = 1), human metapneumovirus (n = 1); Fungal + Bacterial: *Aspergillus* (n = 4), Mucor (n = 1) pneumocystis (n = 1), *Stenotrophomonas* (n = 2), *Klebsiella* (n = 2), *Pseudomonas* (n = 1), *Hemophilus influenzae* (n = 1); Bacterial + Viral: CMV (n = 1), respiratory syncytial virus (n = 1), influenza (n = 1), *Pseudomonas* (n = 1), Enterobacter (n = 1), Legionella (n = 1); Fungal only: *Aspergillus* (n = 8), Mucorales (n = 3), pneumocystis, (n = 2); Viral only: CMV (n = 9), respiratory syncytial virus (n = 1), influenza (n = 1); Bacterial only: *E. coli* (n = 2), *Staphylococcus aureus* (n = 1), *Klebsiella* (n = 1), *Pseudomonas* (n = 1); Virus of uncertain lung pathogenicity: AdV (n = 4), HHV-6 (n = 3), HRV (n = 2), human bocavirus (n = 2), varicella zoster virus (n = 1), herpes simplex virus type 1 (n = 1). Only pathogen findings with established pathogenicity as outlined in Methods section were included in the first 7 groups.

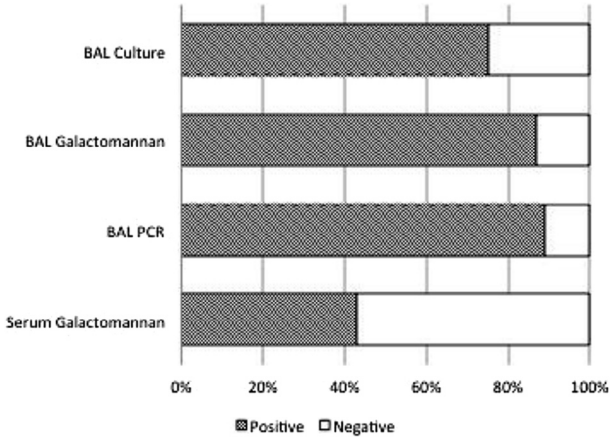

Figure 3. Sensitivity of diagnostic tests in 16 patients with a documented diagnosis of probable invasive aspergillosis. Number of positive tests: BAL culture, 12 of 16 (sensitivity 75%; specificity 98%, PPV 92%, NPV 92%); BAL galactomannan, 13 of 15 (sensitivity 87%, specificity 92%, PPV 76%, NPV 96%); BAL PCR, 8 of 9 (sensitivity 89%, specificity 100%, PPV 100%, NPV 97%); and serum galactomannan, 6 of 14 (sensitivity 43%, specificity 92%, PPV 86%, NPV 86%). NPV indicates negative predictive value; PPV, positive predictive value.

in 18 patients (27%; *Aspergillus* spp., n = 13; Mucorales, n = 6; Talaromyces, n = 1; Table 3). Forty-five patients (89%) received mold-active antifungal agents at the time of BAL. Considering culture results and nonculture tests from BAL (galactomannan and PCR) and blood (galactomannan), the diagnostic workup provided mycologic evidence of *Aspergillus* spp. in 20 patients (30%). Sixteen (80%) of these patients had a documented diagnosis of probable invasive aspergillosis.

Figure 3 shows the sensitivity and gives information about the overall test performance in these patients and in the overall cohort. BAL culture was the only positive test in 2 cases and BAL galactomannan in 1 case, respectively, with the remainder having had at least 2 different positive tests. Serum galactomannan was positive in 43% (6/14 tests) with a documented diagnosis of probable invasive aspergillosis. Of 3 *Aspergillus* DNA-positive samples tested, 2 were found with mutations in the *cyp51A* gene conferring azole resistance. Seven patients (11%) were diagnosed with invasive mucormycosis, 6 patients based on positive culture growth and 1 additional patient by positive PCR. We checked for established risk factors for invasive mucormycosis in our cohort and found an association with relapsed disease (4 [57%] versus 7 [12%], $P = .01$) and neutropenia (absolute neutrophil count, .12 g/L [IQR, 0 to 1.76] versus 2.25 g/L [IQR, .3 to 5.12]; $P = .047$), respectively (Supplementary Table 4). Pneumonia due to *P. jirovecii* was documented as the clinical diagnosis in 4 of 5 patients with a positive PCR, the other patient having been diagnosed with probable invasive aspergillosis. Severity of illness as measured by the SAPSII score (47 [IQR, 35 to 57] versus 34 [IQR, 26 to 46], $P = .02$), high-dose systemic corticosteroids (prednisone > 1 mg/kg; 13 [52%] versus 8 [22%]; $P = .03$), and low absolute lymphocyte counts (.09 g/L [IQR, .01 to .2] versus .35 g/L [IQR, .13 to .61], $P < .01$) were associated with the detection of a fungal pathogen (Supplementary Tables 2 and 3).

Viral Pathogens

A PCR-based panel identified at least 1 viral pathogen in BAL specimens of 26 patients (40%). In relation to the number of tests conducted, CMV (19/61, 31%) was the most abundant virus followed by several viruses with uncertain lung pathogenicity (HHV-6 [10/40, 25%], HRV [12/59, 20%], and AdV [10/61, 16%], etc.) (Table 3). The median CMV DNA load in BAL was 4.08 $\log_{10}$ copies/mL (IQR, 3.39 to 4.96). Fifteen patients (79%) with CMV detected in BAL also were tested positive for viral DNA in plasma at highest median loads of 4.15 $\log_{10}$ copies/mL (IQR, 3.53 to 5.12), whereas 12 of 31 patients (39%) were positive in blood but had CMV not detected or a viral load < 500 copies/mL in BAL.

Viruses with established lung pathogenicity other than CMV were found in 7 cases (influenza type A, n = 3; parainfluenza virus, n = 1; human metapneumovirus, n = 1; and respiratory syncytial virus, n = 2). Eight patients (12%) had 1 or more viruses with uncertain lung pathogenicity identified as the sole pathogens in BAL (AdV, n = 4; HHV-6, n = 3; HRV, n = 2; human bocavirus, n = 2; varicella zoster virus, n = 1; and herpes simplex virus 1, n = 1). Five of these patients had diffuse ground-glass opacities as their main CT finding, 2 patients had patchy alveolar consolidations, and 1 patient had no CT scan but had a focal infiltrate on chest x-ray. Twenty-six patients (43%) in the overall cohort received antiviral therapy at the time of BAL. Regarding patient and baseline characteristics, only the donor type showed an association with the identification of an established viral pathogen ($P = .04$; Supplementary Tables 2 and 3).

Pathogen Patterns, Polymicrobial Events, and Clinical Outcome

Counting only fungal and bacterial pathogens deemed likely to be causative of pneumonia and viruses with established lung pathogenicity in the setting of HSCT, 47 patients (71%) had at least 1 pathogen identified. In 20 cases (30%) significant pathogens from at least 2 different groups could be found (Figure 2). We checked for associations between pathogen groups and between individual pathogens but found no statistically significant evidence of such (Supplementary Table 1). Patients with an identified pathogen had a higher SAPSII score at ICU admission (45 [IQR, 28 to 53] versus 32 [IQR, 26 to 39], $P = .049$), a trend toward a more frequent need for renal replacement therapy during the ICU stay (31 [66%] versus 7 [37%], $P = .053$), and lower lymphocyte counts at the time of BAL (.14 g/L [IQR, .02 to .51] versus .40 g/L [IQR, .13 to .56], $P = .054$) and showed inferior ICU survival (3 [6%] versus 7 [37%], $P < .01$). In subgroup analysis these findings were largely attributable to the characteristics and outcomes observed in patients with and without a fungal pathogen (see above; ICU survival, 1 [4%] versus 9 [24%]; $P = .04$; Supplementary Table 2). ICU survival was 1 in 20 patients (5%) with polymicrobial infection as compared with 9 in 46 patients (20%) in the remainder ($P = .26$). Of the 8 patients with 1 or more viruses with uncertain lung pathogenicity as the sole microbiologic finding, 2 patients survived to ICU discharge without specific antiviral therapy (1 patient with HRV and 1 with AdV, respectively).

DISCUSSION

Panel-based multiplex pathogen testing designed to achieve rapid turnaround times and to detect a large number of micro-organisms is increasingly used to assist in the diagnosis of respiratory tract infections, bloodstream infections, and others. Immunocompromised patients in particular could benefit from this approach, because a broad range of pathogens may cause their clinical presentation in this population, which often come with atypical signs and symptoms [5]. This

is the first study to report comprehensive findings of a diagnostic workup including currently available panel-based methods in a real-life cohort of critically ill patients with ARF and pulmonary infiltrates after HSCT. We found that around 70% of patients had a pathogen known to cause pneumonia and that polymicrobial findings were frequent. An additional 12% had 1 or more viruses with uncertain lung pathogenicity as the sole microbiologic finding.

Fungi were the most commonly detected pathogens, with microbiologic evidence of such found in 42% of our patients. Their prevalence thus appeared somewhat higher than previously reported in noncritically ill HSCT recipients with pulmonary infiltrates (10% to 30%) [20,21] or in the general population of critically ill immunocompromised patients (30%) [22]. Importantly, probable invasive aspergillosis occurred despite standard prophylaxis and almost universal anti-mold coverage at the time of BAL in several of our patients, and at least 2 of these cases were associated with genotypic azole resistance of the respective strain. Insufficient drug levels during azole therapy may also have led to breakthrough infections [23], but the respective data were not available for analysis. Regarding non-*Aspergillus* mold infections, a significant proportion of patients (11%) suffered from an invasive mucormycosis. Broad anti-mold antifungal is a known risk factor for this condition [24], although other risk factors, such as preceding graft-versus-host disease, older age, disease relapse, or profound neutropenia, have also been taken into account [25]. In our cohort we found an association with mucormycosis only for both of the latter, but statistical power of the analysis was limited. Emerging azole resistance and an increasing number of non-*Aspergillus* mold infections have previously been described in HSCT recipients [26,27], and our findings probably outline this increasing complexity of fungal infection patterns in the HSCT population. Ultimately, their distribution may have clinical implications regarding regimens of antifungal prophylaxis and empirical therapy and the timing of BAL in nonintubated patients, because noninvasive tests (eg, galactomannan) fail to identify cases of mucormycosis or azole-resistant *Aspergillus* strains [3]. However, further studies in that respect are warranted. Importantly, even within this cohort with high baseline immunosuppression, evidence of impaired immune reconstitution as indicated by low absolute lymphocyte counts and high-dose corticosteroid therapy helped to identify individuals at the highest risk for invasive fungal disease.

As the second most common pathogen group, 1 or more viruses were detected in BAL samples of 40% of our patients after the implementation of a broad viral PCR panel. CMV was the most abundant virus and was detected at a viral load > 500 copies/mL in every third patient. Although this cut-off was recently established to support the diagnosis of CMV pneumonia in the context of HSCT [13], only very few of these patients had a respective clinical diagnosis documented. However, even in the absence of overt disease, subclinical CMV infection in the lungs has been shown to produce significant biologic effects such as inflammation and fibrosis [28]. Similar considerations apply for the 12% of our patients with the finding of 1 or more viruses of uncertain lung pathogenicity. Although several of these viruses such as HHV-6 [6], human coronavirus [7], or HRV [8] are increasingly recognized to cause lower respiratory tract disease after HSCT, evidence accumulates for a variety of subclinical pathogenic processes to be induced in the lungs upon infection with these viruses [29,30]. Although more research on these host responses is needed, studies including newer antiviral agents and focusing on preventive strategies in patients with asymptomatic shedding or upper respiratory tract disease are already ongoing [31]. Given the inferences from the high rate of polymicrobial findings in our patients (see below), these strategies may be more suited to improve outcomes than interventions in patients who already developed ARF, and we support further such studies.

Bacterial pathogens were detected in BAL samples of 27% of our patients with a clear predominance of gram-negative bacilli. Older patient age and time from HSCT to ICU admission were the only factors associated with the identification of bacterial pathogen, whereas time from hospital to ICU admission—a possible surrogate for the acquisition of hospital-acquired pneumonia—did not correlate with this finding (data not shown). Previous exposure to antibiotics as well as hospitalizations would have been other variables of interest in this context, but we could not obtain the respective data. Interestingly, time from HSCT to ICU admission did not correlate with the detection of the other pathogen groups [32].

The polymicrobial findings in almost a third of cases is another important observation from our study, although polymicrobial pneumonia was the clinical diagnosis in only 11% of patients. This is probably because clinicians attributed lung injury patterns to specific pathogens according to the patients' history, clinical assessment, and radiographic findings [4]. However, both clinical assessment and radiographic findings lack specificity in many instances [33,34]. On the other hand, as outlined above for CMV, microbiologic findings may still be significant even if they do not seem to account for the predominant pattern of lung injury [28,29]. Hence, the mutual interaction of pathogens or their inherent pathogenicity acting as competing conditions could be a significant promoter of lung injury in these patients. In both cases the observation of frequent polymicrobial findings would be important, because it reduced the chance of any single pathogen-directed intervention to be successful, either in clinical practice or in clinical trials.

Detection of a causative pathogen enhances diagnostic certainty and guides antimicrobial therapy. Furthermore, the inability to identify the cause of ARF in hematologic patients is an independent factor associated with mortality [35]. Although the analysis of outcome measures was not the scope of our study, the finding of increased mortality in patients with a detected pathogen was therefore surprising. However, a similar observation was made in the study of Seo et al. [9]. Three factors may have accounted for this observation. First, invasive fungal infections are associated with excess mortality of up to 90% in HSCT recipients [2,22], and our detection rate was high. Indeed, only 1 patient with an invasive fungal infection in our cohort survived to ICU discharge. Second, even with respect to HSCT, patients in our cohort were highly immunosuppressed, most having been on multiagent immunosuppressive therapy including corticosteroids, and having shown very low absolute lymphocyte counts [36]. Detection of a pathogen may therefore have been deleterious in these patients with significantly impaired infection control capabilities, especially if regarded as a possible consequence of high pathogen burden. Third, patients with a detected pathogen were more severely ill than those without. However, this may have been regarded either as a consequence of the respective pathogen(s) or as a factor having influenced detection rates in our patients [37].

A major strength of our study was that it included a reasonable number of patients from a contemporary cohort of

HSCT recipients admitted to the ICU, and findings may thus reflect current patterns observed in this population. On the other hand, microbiologic findings regarding pathogen occurrence and resistance always follow local conditions and have thereby been put into place. Because of the retrospective observational nature of our study, several technical aspects such as standardized conduction of BALs and uniform sample processing cannot be ensured. Other limitations related to the study design concern data completeness, quality, and interpretation. Finally, our study provides a mere description of microbiologic findings and does not allow inferences to relative contributions of single pathogens to ARF and outcome.

In conclusion, by using a microbiologic diagnostic workup incorporating molecular- and biomarker-based assays on BAL and blood samples, pathogens known to cause pneumonia were found in 70% of HSCT recipients with ARF admitted to the ICU. An additional 12% had a virus of undetermined lung pathogenicity as the sole microbiologic finding. BAL may enhance pathogen detection and therapy guidance in settings of high prevalence of invasive mold infections including non-*Aspergillus* strains and of emerging azole resistance. The polymicrobial findings in almost a third of our patients warrant consideration in clinical practice and in the design of future interventional studies.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2018.03.007.

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