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Monocenter study on epidemiology, outcomes and risk factors of infections in recipients of 166 allogeneic stem cell transplantations during one year

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Monocenter study on epidemiology, outcomes, and risk factors of infections in recipients of 166 allogeneic stem cell transplantations during 1 year

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Abstract

Objectives: During allogeneic hematopoietic stem cell transplantation (allo-SCT), infections significantly contribute to morbidity and mortality. A monocentric prospective analysis was performed to assess epidemiology, risk factors, and outcomes of infections during the peri-transplant period.

Methods: Data were recorded prospectively using a predefined questionnaire.

Results: In 2015, 163 consecutive patients, 37.4% female, median age 59 (range 18-79) years received 166 allo-SCT. Median duration of leukopenia $<10^9$/L was 14.5 days (range 4-43 days). Fever of unknown origin (FUO) occurred in 118/166 patients (71.1%). Severe sepsis developed in 95, and septic shock developed in 26 patients. Intensive diagnostic workup helped to identify causative microorganisms only in a small number of infectious courses. All but 13 patients needed antibiotic therapy, each according to the standard operating procedures of the department. Cumulative incidence of death by infection after 1 year was 16.6% (95% CI: 11.3-22.7). The only risk factor for FUO in neutropenia was duration of neutropenia </≥14 days (55.4% vs 85.5%, $P < .001$).

Conclusion: Results of an elaborate diagnostic workup of infections in the peri-transplant period are scarce. Attention to risk factors might help to identify patients at risk for severe infections.

KEYWORDS

allogeneic stem cell transplantation, FUO, infection, neutropenia

1 | INTRODUCTION

Regarded the only curative approach for many hematologic neoplasms and some non-malignant hematopoietic disorders, allogeneic stem cell transplantation (allo-SCT) still is associated with considerable non-relapse mortality (NRM). During the acute phase of allo-SCT, infections, particularly bacterial ones, significantly contribute to morbidity and NRM.[1–4] Factors that have been associated with an increased risk of infections in the pre-engraftment period are older age, poor functional status, high-risk disease, duration of neutropenia, iron overload, disruption of physiological and anatomical barriers, and type of conditioning (myeloablative, irradiation). Next to

the situation of fever of unknown origin, several bacterial infection syndromes can occur. These include bloodstream infections (BSI), to some extent central-venous catheter (CVC)-associated, pneumonia, neutropenic colitis, and more rarely, central nervous system infections. Professional bodies such as the Infectious Diseases Society of America (IDSA) [5] or the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Medical Oncology (DGHO) [6–11] regularly review and publish guidelines on evidence-based diagnostics and treatment of infections of patients with neoplastic disorders.

Data on epidemiology and risk factor analyses frequently originate from multicenter studies. Those reports all come with an inherent heterogeneity and methodological flaw due to different standard operating procedures that are followed at respective centers. Additionally, those data usually are collected over a long period of time. Despite the benefit of high patient numbers, these studies are potentially biased by the considerable variability of their population at risk, added to anti-infective policies that differ between centers and change over time. Also are those data mostly historic. Newer analyses will incorporate more state-of-the-art anti-infective measures. Furthermore, the patient population proceeding to transplant has become older and carries a higher degree of comorbidities than during earlier years of allo-SCT.

In this study, we analyzed the epidemiology, outcome, and risk factors for infections during the pre-engraftment phase of 163 patients that were transplanted with a graft from an allogeneic donor in 1 year (2015) at one institution with uniform local standard operating procedures.

Novelty Statement

1. New aspect of this work: Cardinal risk factor for infections in neutropenia after allogeneic SCT is duration of neutropenia.
2. Central finding of this work: Antibiotic therapy after allogeneic SCT will remain empiric as results of diagnostic workup are scarce.
3. Specific clinical relevance of this work: Even maximal antibiotic therapy after allogeneic SCT will result in a considerable infection-related mortality.

2 | METHODS AND ANALYSIS

The study was performed on adult patients who received an allo-SCT between January 1, and December 31, 2015, at the Department of Stem Cell Transplantation at the University Medical Center Eppendorf, Hamburg, Germany. Data were recorded during the patients' hospitalization from the digital medical record software Soarian (Siemens) in a prospective fashion using a predefined questionnaire and entered into a Microsoft Excel database. Follow-up data were collected 1 year after transplantation.

The performance status of all patients prior to allo-SCT was evaluated using the ECOG score.[12] The conditioning regimen (myeloablative, reduced intensity, and non-myeloablative) was classified according to Bacigalupo et al.[13] Neutropenia was defined as an absolute neutrophil count $<10^9$/L.[14]

All patients received anti-infectious prevention; acyclovir (3× 400 mg/d p.o. or 3× 500 mg/d iv), trimethoprim/sulfamethoxazole (2× 800 mg/160 mg/d Tuesdays and Fridays), and ciprofloxacin (2× 500 mg/d p.o. or 2× 400 mg/d iv) starting with conditioning; micafungin (1× 100 mg/d) starting from day of transplantation; or leukocytes $<10^9$ cells/L and metronidazole (3× 500 mg/d p.o. or 3× 400 mg iv) from day 1 to 30 after allo-SCT.

CVCs used were rifampicin- and miconazole-impregnated (30 cm length, number 6155.307, Multistar 3+, Vygon).

Fever was defined as body temperature above 38.3°C once or body temperature of 38.0-38.2°C twice within 12 hours. Severe sepsis was defined, according to the definition valid at the time of study, as sepsis with organ dysfunction.[15] The definition of septic shock required severe sepsis with the need of systemic vasopression (epinephrine). An infection was assumed if one or more of the following occurred: fever, elevated CRP level, severe sepsis, septic shock, and documented infection (positive microbiological culture, infiltration upon thoracic CT [tCT]). tCT scans were ordered for patients with 48 hours of continuing episodes of fever, and follow-up scans were ordered when the initial scan showed lung infiltrates. The evaluation of an invasive fungal infection was conducted in accordance with the definition of EORTC/MSG.[16] Empiric first-line therapy in emerging fever consisted of ceftazidime (3 × 2 g) and vancomycin (2 × 1 g initially, then TDM-guided). Second line, after 48 hours of ongoing fever, contained meropenem (3 × 1 g) plus vancomycin (2 × 1 g). Liposomal amphotericin B (3 mg/kg body weight) was added to the previous drugs in third-line therapy, replacing micafungin after 72 hours of fever. The fourth-line therapy included gentamicin (5 mg/kg body weight initially, then TDM-guided) in addition to the drugs in third-line therapy. In all lines of antibiotic therapy, vancomycin was replaced by daptomycin if renal function was poor, an allergy against vancomycin was present, or a vancomycin-resistant enterococcus was present. Linezolid was chosen instead of vancomycin/daptomycin if vancomycin was not possible due to the aforementioned reasons and a pulmonary focus was suspected or present. In general, the department's standard operating procedures follow recommendations that had been laid out in several guidelines.[6,7,10,11,17]

To identify patients at increased risk for treatment-related infection, specific patients' characteristics and treatment variables were analyzed with regard to overall infection rate, days to onset of infection, days with febrile neutropenia, days of neutropenia, and duration of antibiotic treatment as well as documented infection such as positive microbiological culture and/or pulmonary infiltrate upon tCT scan.

For statistical analysis, SPSS (Statistical package for the Social Science, IBM) version 22.0, and R 3.2.3 5 (R Core Team [18] https://

www.R-project.org/) were used. In the epidemiological part of the study, frequencies for nominal and ordinal variables and descriptive analyses for metric variables were reported. For the comparison of patient groups, chi-square and Fisher's exact test in nominal variables were calculated. *T* test and ANOVA were used for evenly distributed metric variables. As non-parametric tests, Mann-Whitney *U* and Kruskal-Wallis were performed. Metric variables were analyzed by Pearson correlation. The cumulative incidence of a fatal infection was calculated as described,[19] with death through other cause as competing risk, and vice versa. All *P*-values reported are two sided, and significance is given with $P < .05$. Confidence intervals are set at 95%.

3 | RESULTS

3.1 | Patient characteristics, donor characteristics, and transplantation

In 2015 (January 1-December 31), 163 adult patients received 166 allo-SCT. The clinical features are shown in Table 1. The median age of the 61 females (37.4%) and 102 males (62.6%) was 59 years (range 18-79 years). Graft source was bone marrow in 10.2% (n = 17) and mobilized peripheral blood stem cells in 89.8% (n = 149) of the patients with 51 female (30%) and 115 male (69.3%) donors. Transplantations were from matched related (18.7%, n = 31), matched unrelated (55.4%, n = 92), mismatched unrelated (21.1%, n = 35), and haploidentical related (4.8%, n = 8) donors. CMV IgG matching was negative/negative in 31.3% (n = 52), positive/positive in 50.6% (n = 84), positive patient and negative donor in 7.8% (n = 13), and negative patient and positive donor in 10.2% (n = 17). The conditioning regimen prior to transplantation was myeloablative in 94 patients (56.6%), with reduced intensity in 71 patients (42.8%) and non-myeloablative in one patient (0.6%). Median duration of neutropenia was 14.5 days (range 4-43 days). Underlying diseases were chronic myeloproliferative neoplasm, lymphatic neoplasms, acute myeloid leukemias, myelodysplastic syndromes, myeloma, and aplastic anemia.

3.2 | Fever and results of diagnostic evaluation

Regardless of the white blood cell count (WBC), 138 patients (83.1% of n = 166) became febrile during their allo-SCT inpatient stay at least once, with a median duration of 3 febrile days (range 1-23), and with a median time to onset of infection of 9 days (range 0-25 days) since admission. Fever of unknown origin presented in 118 patients (71.1%, n = 166). In 20.5% (n = 34) of the cases, fever occurred before an increase in CRP. The CRP was increased before the patient became febrile in 36.1% (n = 60), and an increase in CRP and fever co-occurred in 26.5% (n = 44). During neutropenia, 117 patients (70.5%) were febrile, with a median duration of 2 days (range 1-11). Severe sepsis developed in 95 (57.2%), and septic shock developed in 26 patients (15.7%).

Blood cultures were drawn from 142 patients (85.5%) on a median of 4 days (range 1-38). Of those, 131 were febrile, 11 showed signs of an infection that justified BC analysis but were not febrile, and 7 patients who were febrile did not receive a BC analysis. In 34 patients (23.9%), at least one blood culture was positive. 23/34 patients (67.6%) had one positive culture, 7/34 patients (20.6%) had two, and each 2/34 patients (5.9%) had three and four positive blood cultures. In 7 of the 34 patients with positive blood cultures, the same species grew in more than one blood culture: three times *Staphylococcus epidermidis*, two times *Candida parapsilosis*, and one time each *Cryptococcus neoformans* and *Pseudomonas aeruginosa*. *Escherichia coli* was the most frequent pathogen detected that was not susceptible to be a contamination. *S. epidermidis* and *staphylococcus hominis* were detected more frequently but with long times to positivity and susceptible to be a contamination, thus their appearance is likely to be overestimated. Results of positive blood cultures are shown in Table 2.

During their hospital stay, 94 patients (56.6%) received at least one tCT scan. Out of these, 34 patients had one scan, 35 patients had two scans, and 15 patients had three scans. Four, five, six, and seven scans were ordered for 5, 1, 1, and 3 patients. In 64/94 patients (68.1%), at least one tCT scan showed pulmonary infiltrates. Signs of invasive fungal pulmonary infection were appreciated as "possible" in 38 patients (22.9%) and "probable" in 11 patients (6.6%). A bronchial alveolar lavage followed the pathologic tCT scans in 51 patients (79.7%), and of those, 50 patients had at least one bronchoalveolar lavage (BAL) result positive for a pathogen, regardless of an attributable causal connection to pneumonia or pneumonitis. In total, 89 BALs were performed for 51 patients. The top five species discovered were coagulase-negative staphylococci (n = 31, 62%), *Candida spp.* (n = 27, 54%), *Enterococcus species* (n = 26, 52%), *Aspergillus* (n = 14, 28%), and *Streptococcus viridans* (n = 10, 20%). Cytomegalovirus was seen in 5 (10%) BALs. Pathogens with a plausible connection to pneumonia or pneumonitis were each seen in 1 (2%) lavage. Those were Mucor, *Klebsiella pneumonia*, *Enterococcus faecalis*, *P. aeruginosa*, and *Respiratory syncytial virus*. Results of the BAL are shown in Table 3.

All patients received one or more impregnated central venous catheters (CVCs) during hospitalization (one CVC: 121 pts [72.9%]; two CVCs: 27 pts [16.3%]; three CVCs: 10 pts [6%]; four CVCs: 4 pts [2.4%]; six CVCs: 3 pts [1.8%]; nine CVCs: 1 pt [0.6%]). Mean duration of intravenous persistence of the first CVC was 25.6 ± 8.5 days, and mean duration of intravenous persistence of the second CVC was 13.3 ± 6.2 days. In 86 patients (51.8%), a minimum of one CVC was analyzed for microbial growth. A positive result was returned in 10 patients (11.6%) of which 5 (50%) results were positive for *S. epidermidis*, 2 (20%) positive for *C. parapsilosis*, and 1 (10%) was positive each for *Candida glabrata*, *E. faecalis*, and *Enterococcus faecium*.

Physicians of the department evaluated mucositis of the patients according to the Bearman scale. With two missing (n = 164), 21.3%

TABLE 1 Patient characteristics and clinical features

Characteristics	All patients (n = 163)
Age in years at admission	
Mean ± SD	56.0 (13.19)
Median	58.6
Range	18-79
Sex, n (%)	
Female	61 (37.4)
Male	102 (62.6)
Patient median age in years (range) by sex	
Female	60.1 (24-75)
Male	58.0 (18-79)
Characteristics	**All cases (n = 166)**
Disease, n (%)	
Myeloma	16 (9.6)
Chronic myeloproliferative neoplasm	38 (22.9)
Lymphatic neoplasm	25 (15.1)
Acute myeloid leukemia	47 (28.3)
Secondary acute myeloid leukemia	15 (9)
Myelodysplastic syndrome	22 (13.3)
Aplastic anemia	2 (1.2)
Acute biphenotypic leukemia	1 (0.6)
ECOG score at admission, n (%)	
0	58 (34.9)
1	93 (56)
2	14 (8.4)
3	1 (0.6)
Cardiovascular disease at admission, n (%)	15 (9)
COPD at admission, n (%)	7 (4.2)
Conditioning regimen, n (%)	
Myeloablative	94 (56.6)
Reduced intensity	71 (42.8)
Non-myeloablative	1 (0.6)
Graft source, n (%)	
Bone marrow	17 (10.2)
Peripheral blood stem cells	149 (89.8)
Median CD34 + cell number transplanted in 10^6 per kg body weight (range)	6.53 (0.5-13.6)
Donor sex, n (%)	
Female	51 (30.7)
Male	115 (69.3)
Patient/donor HLA matching, n (%)	
Match-related	31 (18.7)
Match-unrelated	92 (55.4)
Mismatch-unrelated	35 (21.1)
Haploidentical-related	8 (4.8)
Patient/donor CMV matching, n (%)	
Negative/negative	52 (31.3)
Positive/positive	84 (50.6)
Positive/negative	13 (7.8)
Negative/positive	17 (10.2)
Median duration of neutropenia in days (range)	14.5 (4-43)
Median neutrophil engraftment, days (range) (n = 164)[a]	12 (6-38)
Primary outcome, n (%)	
Discharge	145 (87.3)
Death	21 (12.7)
Reason of death (n = 21), n (%)	
Infection/Sepsis	19 (90.5)
Other	2 (9.5)

Abbreviations: CMV, cytomegalovirus; ECOG, Eastern Cooperative Oncology Group; HLA, human leukocyte antigen; SD, standard deviation.

[a]Two death before neutrophil engraftment.

TABLE 2 Species found in blood cultures

Species	Number of patients (n = 34) (%)	Mean time to positivity, hours (SD)
Streptococcus pneumoniae	1 (2.9)	13.8
Streptococcus mitis	3 (8.8)	6.9 (0.14)
Staphylococcus epidermidis	13 (38.2)[a]	25.5 (8.24)
Staphylococcus hominis	3 (8.8)	21.6 (5.44)
Staphylococcus haemolyticus	3 (8.8)	14.3 (1.81)
Escheria coli	6 (17.6)	8.6 (3.43)
Enterococcus faecium	1 (2.9)	9.5
Micrococcus luteus	1 (2.9)	34.3
Cryptococcus neoformans	1 (2.9)	79.3
Pseudomonas aeruginosa	2 (5.9)	11.7 (0.07)
Candida albicans	1 (2.9)	47.7
Candida parapsilosis	2 (5.9)	27.7 (2.90)
Rothia mucilanginosa	2 (5.9)	17.7 (0.71)

Note: Two "time to positivity" missing (1 *Escheria coli* and 1 *Streptococcus mitis*).

Abbreviation: SD, standard deviation.

[a]Only three blood cultures were deemed pathogenic.

(n = 35) had no mucositis, 17.1% (n = 28) had Grade 1, 59.1% (n = 97) had Grade 2, 1.8% (n = 3) had Grade 3, and 0.6% (n = 1) had Grade 4 mucositis. Excluding mucositis, 84 patients (50.6%) were found to have a documented infection by diagnostic investigation, see Table 4.

TABLE 3 Results of BAL

BALs in patients with infiltrate in tCT	Number of patients (n = 64) (%)
No BAL	13 (20.3)
1 BAL	26 (40.6)
2 BALs	16 (25)
3 BALs	6 (9.4)
4 BALs	2 (3.1)
5 BALs	1 (1.6)
Results of BAL	**Number of patients (n = 50) (%)**
Coagulase-negative staphylococci	31 (62)
Enterococcus species	26 (52)
Adenovirus	1 (2)
Candida spp.	27 (54)
Cytomegalovirus	5 (10)
Stenotrophomonas maltophilia	1 (2)
Human metapneumovirus	1 (2)
Aspergillus	14 (28)
Rhinovirus	1 (2)
Enterovirus	1 (2)
Parainfluenza virus	3 (6)
Streptococcus viridans	10 (20)
Mucor	1 (2)
Coronavirus	2 (4)
Rothia mucilaginosa	1 (2)
Coryneforme	2 (4)
Lactobacillus	3 (6)
Haemophilus parainfluenzae	1 (2)
Klebsiella pneumoniae	1 (2)
Enterococcus faecalis	1 (2)
Pseudomonas aeruginosa	1 (2)
Respiratory syncytial virus	1 (2)
Pseudomonas oryzihabitans	1 (2)
Herpes simplex virus	1 (2)

Abbreviations: BAL, bronchoalveolar lavage; tCT, thoracic computer tomography.

3.3 | Antibiotic treatment

The reason to start antibiotic treatment was fever in 44% (n = 73), an increase in CRP in 25.9% (n = 43), fever and a simultaneous increase in CRP in 9% (n = 15), and other reasons in 21.1% (n = 35) of the patients. In 7.8% (n = 13) of all patients, no antibiotic therapy was initiated. In accordance with the standard operating procedures of the department, 129 patients (77.7%) received first-line antibiotic therapy, 75 patients (45.2%) proceeded to second-line therapy, 42 patients (25.3%) needed third-line therapy afterward, and 20 patients (12%) received fourth-line therapy. Out of 54 patients not receiving second-line therapy after first-line therapy, the anti-infectious therapy was discontinued in 36 patients (21.7%), an antifungal drug was added in six patients (3.6%), an antiviral therapy was started in two patients (1.2%), therapy was changed to second-line plus liposomal amphotericin B in seven patients (4.2%), and in one patient each (0.6%), therapy was changed to vancomycin and meropenem at a higher dose (3 × 2 g/d), meropenem was added to first line, or therapy was changed to ampicillin/sulbactam, respectively. A complete list of administered anti-infectious drugs is shown in Table 5.

TABLE 4 Documented infections

Diagnostic investigation	Number of patients (n = 84) (%)
Infiltrate on tCT scan	64 (76.2)
Positive culture of BAL fluid	50 (59.5)
Positive blood culture	36 (42.9)
Positive culture of CVC	5 (5.9)

Abbreviations: BAL, bronchial alveolar lavage; CVC, central venous catheter; tCT, thoracic computer tomography.

TABLE 5 Use of anti-infectious medication

Drug	Number of patients (n = 166) (%)	Median days of treatment (range)
Ceftazidim	139 (83.7)	10 (1-28)
Vancomycin	126 (75.9)	15 (1-43)
Meropenem 1g	107 (64.5)	12 (2-64)
Meropenem 2g	32 (19.3)	9 (2-42)
Gentamicin	34 (20.5)	6 (2-21)
Colistin	15 (9)	7 (3-21)
Linezolid	53 (31.9)	12 (1-69)
Daptomycin	42 (25.3)	9 (1-34)
Liposomal amphotericin B	72 (43.4)	13 (2-74)
Voriconazole	35 (21.1)	8 (1-69)
Caspofungin	11 (6.6)	13 (2-41)
Tigecycline	3 (1.8)	5 (3-6)
Fosfomycin	3 (1.8)	5 (3-6)
Piperacillin/Tazobactam	1 (0.6)	8
Duration of overall anti-infectious medication		19 (3-94)

3.4 | Outcome and follow-up

Discharge was possible for 145 patients (87.3%). Twenty-one patients (12.7%) died during hospitalization of their allo-SCT. Reasons for death were infection in 19 patients (90.5%) and other causes (relapse, heart failure) in 2 patients (9.5%). After 1 year, an additional 8 patients had died due to infection-related causes. Thus, after 1-year follow-up, 27 patients died from infection without relapse (cumulative incidence 16.6%, 95% CI: 11.3-22.7), and 22 patients died from non-infectious causes (relapse, other, cumulative incidence 13.5%, 95% CI: 8.8-19.2).

TABLE 6 Univariate analysis of infection

	Infection			Documented infection		
	Yes	No	*P* value	Yes	No	*P* value
Median age (years, range)	58 (18-79)	60 (24-71)	.723	59 (18-79)	58 (22-77)	.554
ECOG score, n = 166						
0, n (%)	49 (84.5)	9 (15.5)	.131	26 (44.8)	32 (55.2)	.072
1, n (%)	88 (94.6)	5 (5.4)		46 (49.5)	47 (50.5)	
2, n (%)	14 (100)	0		11 (78.6)	3 (21.4)	
3, n (%)	1 (100)	0		1 (100)	0	
ECOG 0 and 1, n (%)	137 (90.7)	14 (9.3)	.611	72 (47.7)	79 (52.3)	.027
ECOG 2, n (%)	14 (100)	0		11 (78.6)	3 (21.4)	
Mean duration of neutropenia (days)	16.38	11.50	<.001	17	14	<.001
<14 d of neutropenia, n (%)	72 (86.7)	11 (13.3)	.025	32 (38.6)	51 (61.4)	.002
>=14 d of neutropenia, n (%)	80 (96.4)	3 (3.6)		52 (62.7)	31 (37.3)	
Conditioning, n = 166						
Myeloablative, n (%)	87 (92.6)	7 (7.4)	.779	48 (51.1)	46 (48.9)	.892
Non-myeloablative, n (%)	65 (90.3)	7 (9.7)		36 (50)	36 (50)	
CMV status of patient, n = 166						
CMV-IgG positive, n (%)	91 (93.8)	6 (6.2)	.262	56 (57.7)	41 (42.3)	.029
CMV-IgG negative, n (%)	61(88.4)	8 (11.6)		28 (40.6)	41 (59.4)	
CMV status combination						
Patient − ve/ donor + ve, n (%)	17 (100)	0	.365	5 (29.4)	12 (70.6)	.077
All others, n (%)	135 (90.6)	14 (9.4)		79 (53)	70 (47)	
Patient + ve/ donor − ve, n (%)	11 (84.6)	2 (15.4)	.301	4 (30.8)	9 (69.2)	.136
All others, n (%)	141 (92.2)	12 (7.8)		80 (52.3)	73 (47.7)	
Median CD34 + cell number transplanted in 10^6 per kg BW	6.03×10^6	6.60×10^6	.461	6.63×10^6	6.48×10^6	.717
Disease, n = 166						
Myeloma, n (%)	15 (93.8)	1 (6.3)	.842	8 (50)	8 (50)	.441
Chronic myeloproliferative neoplasm, n (%)	33 (86.8)	5 (13.2)		13 (34.2)	25 (65.8)	
Lymphatic neoplasia, n (%)	23 (92)	2 (8)		15 (60)	10 (40)	
Acute myeloid leukemia, n (%)	42 (89.4)	5 (10.6)		26 (55.3)	21 (44.7)	
Secondary acute myeloid leukemia, n (%)	15 (100)	0		8 (53.3)	7 (46.7)	
Myelodysplastic syndrome, n (%)	21 (95.5)	1 (4.5)		12 (54.5)	10 (45.5)	
Aplastic anemia, n (%)	2 (100)	0		1 (50)	1 (50)	
Acute biphenotypic leukemia, n (%)	1 (100)	0		1 (100)	0	

Abbreviations: CMV, cytomegalovirus; ECOG, Eastern Cooperative Oncology Group; IgG, immunoglobulin G.

Results of univariate analysis are summarized in Table 6. In brief, the only risk factor for FUO in neutropenia and for infections was a duration of neutropenia </≥14 days (55.4% vs 85.5%, *P* < .001).

3.5 | Impact of age on infection and antibiotic treatment

The median age in patients with infection was 58 (range 18-79) and 60 years (range 24-71) in patients with no infection (*P* = .723). Correlation (after Pearson) between the onset of infection and the age of the patients showed no significance (*r* = .021, *P* = .801, n = 152). Patients with and without febrile neutropenia differ in median age but not significantly (febrile neutropenia: 58 years (range 20-79) vs no febrile neutropenia: 59 years (range 18-73); *P* = .141). There is no correlation between age and days with fever in neutropenia (*r* = −.063, *P* = .499, n = 117). The relation between age and the duration of antibiotic treatment during hospital stay is incoherent (*r* = .062, *P* = .449, n = 152). In summary, patient age showed no significant effect on any of the dependent variables.

Febrile neutropenia			Therapeutic option after first-line antibiotics Therapeutic option after first-line antibiotics		
Yes	No	*P* value	End of treatment	Further treatment	*P* value
58 (20-79)	59 (18-73)	.141	59 (22-75)	58 (22-77)	.955
41 (70.7)	17 (29.3)	.930	12 (26.7)	33 (73.3)	.752
66 (71)	27 (29)		21 (28)	54 (72)	
9 (64.3)	5 (35.7)		3 (37.5)	5 (62.5)	
1 (100)	0		0	1 (100)	
107 (70.9)	44 (29.1)	.760	33 (27.5)	87 (72.5)	.686
9 (64.3)	5 (35.7)		3 (37.5)	5 (62.5)	
17.44	12.45	<.001	15	17	.188
46 (55.4)	37 (44.6)	<.001	22 (36.1)	39 (63.9)	.050
71 (85.5)	12 (14.5)		14 (20.6)	54 (79.4)	
64 (66.3)	30 (27.7)	.439	23 (32.9)	47 (67.1)	.172
53 (50.7)	19 (21.3)		13 (22)	46 (78)	
72 (74.2)	25 (25.8)	.210	17 (22.7)	58 (77.3)	.118
45 (65.2)	24 (34.8)		19 (35.2)	35 (64.8)	
11 (64.7)	6 (35.3)	.582	6 (42.9)	8 (57.1)	.212
106 (71.1)	43 (28.9)		30 (26.1)	85 (73.9)	
9 (69.2)	4 (30.8)	1.0	3 (30)	7 (70)	1.00
108 (70.6)	45 (29.4)		33 (27.7)	86 (72.3)	
6.62×10^6	6.40×10^6	.636	7.27×10^6	6.29×10^6	.080
14 (87.5)	2 (12.5)	.089	2 (14.3)	12 (85.7)	.535
26 (68.4)	12 (31.6)		8 (26.7)	22 (73.3)	
13 (52)	12 (48)		5 (29.4)	12 (70.6)	
33 (70.2)	14 (29.8)		14 (41.2)	20 (58.8)	
10 (66.7)	5 (33.3)		3 (25)	9 (75)	
19 (86.4)	3 (13.6)		4 (20)	16 (80)	
2 (100)	0		0	2 (100)	
0	1 (100)		0	1 (100)	

3.6 | Impact of initial ECOG on infection and antibiotic treatment

The initial ECOG scoring had a significant impact on subsequent infection (ECOG 0: n = 49/58 (84.5%); 1: n = 88/93 (94.6%); 2: n = 14/14 (100%); 3: n = 1/1 (100%); *P* = .013). The time to infection was the following (in order ECOG 0, 1, 2, 3): 9.18 days (SD 4.915), 10.23 (SD 4.608), 7.71 (6.318), and 0 days (*P* = .190). As only one patient scored ECOG 3, he was not included in the following comparison of ECOG scores. A significantly higher rate of documented infection was seen in patients with ECOG score 2 compared with ECOG scores 0 and 1 combined (78.6% vs 47.7%, *P* = .027).

No significant effect between ECOG scores was seen in the occurrence of febrile neutropenia or the number of days with febrile neutropenia.

Mean days on antibiotic treatment were 20.6 (SD 10.09) and 20.3 (10.92) for patients with ECOG scores 0 and 1, and 35.5 (SD 25.70) and 43 days in patients with ECOG scores 2 and 3 (*P* = .036). The duration of neutropenia and administration of antibiotics were longer in patients with ECOG 2 as to patients scored ECOG

0 and 1 combined (19.5 days [SD 7.00]/ 35.5 days [SD 25.70] vs 15.5 days [SD 6.25]/ 20.4 days [SD 10.60] $P = .025$/ $P = .013$).

3.7 | Impact of CMV status on infection and antibiotic treatment

Patient groups of positive (n = 97) and negative (n = 69) CMV IgG status did not experience significantly more infections (93.8% vs 88.4%, $P = .217$), febrile neutropenia (74.2% vs 65.2%, $P = .210$), or duration of antibiotic treatment (23 days [SD 15.758] vs 20 days [SD 8.613], $P = .138$) compared with each other. However, patients with negative CMV IgG status (n = 69) showed significantly lower rates of documented infection (40.6% vs 57.7%, $P = .029$). This is also seen in patient-donor matches in which at least one part is CMV IgG negative (n = 82, 39% vs 61.9%, $P = .003$).

3.8 | Impact of disease on infection and antibiotic treatment

The underlying disease did not show significant impact on overall infection, proven infection or the onset of infection, febrile neutropenia, and its duration and the number of days of antibiotic treatment.

The number of patients with infection, clinically apparent and microbiologically proven, was 93.8% and 50% in multiple myeloma (n = 15), 86.8% and 34.2% in chronic myeloproliferative neoplasms (n = 33), 92% and 60% in lymphatic neoplasm (n = 23), 89.4% and 55.3% in AML (n = 42), 100% and 53.3% in secondary AML (n = 15), 95.5% and 54.5% in MDS (n = 21), and 100% and 50% in aplastic anemia (n = 2).

The mean time to infection, counting from the day of admission, for the different diagnoses was 10.5 days (SE 0.93) in patients with multiple myeloma (n = 15, 9.9%), 8.9 days (SE 0.93) in chronic myeloproliferative neoplasm (n = 33, 21.7%), 9.7 days (SE 0.90) in lymphatic neoplasm (n = 23, 15.1%), 10.0 days (SE 0.86) in AML (n = 42, 27.6%), 7.9 days (SE 1.18) in secondary AML (n = 15, 9.9%), 10.3 days (SE 1.01) in MDS (n = 21, 13.8%), 13.5 days (SE 0.50) in aplastic anemia (n = 2, 1.3%), and zero days in acute biphenotypic leukemia (n = 1, 0.6%, $P = .201$).

The mean duration of antibiotic treatment split by the different diagnosis was 18.5 days (SE 1.26) in patients with multiple myeloma (n = 15, 9.9%), 18.9 days (SE 1.28) in chronic myeloproliferative neoplasm (n = 33, 21.7%), 22.7 days (SE 2.62) in lymphatic neoplasm (n = 23, 15.1%), 22.9 days (SE 2.50) in AML (n = 42, 27.6%), 27.9 days (SE 4.79) in secondary AML (n = 15, 9.9%), 22.5 days (SE 3.41) in MDS (n = 21, 13.8%), 16.5 days (SE 3.50) in aplastic anemia (n = 2, 1.3%), and 24 days in acute biphenotypic leukemia (n = 1, 0.6%, $P = .373$).

Patients with multiple myeloma (n = 16) did not show a different infection rate compared with all other diagnoses combined (n = 150; infection rate 93.8% vs 91.3%, documented infections 50% vs 50.7%). Although more multiple myeloma patients developed neutropenic fever (87.5% vs 68.7%, $P = .154$), the median days in neutropenic fever differed only by one day (3 days [range 1-9] for multiple myeloma vs 2 days [range 1-11], $P = .445$). The mean duration of antibiotic treatment showed no significant difference in both groups (18 days [range 9-26] in multiple myeloma vs 19 days [range 3-94], $P = .667$), but patients with multiple myeloma more frequently needed antibiotic escalation after first-line treatment than the other patients (85.7% vs 70.4%, $P = .347$).

AML patients (n = 62, including secondary AML) did not show either a significant difference in infection rate (91.9% in AML patients vs 91.3%, $P = .895$) or in proven infections (AML 54.8% vs 48.1%, $P = .399$) when compared to the rest of the study cohort (n = 104). The occurrence of neutropenic fever (AML 69.4% vs 71.2%, $P = .806$) and the median amount of days of neutropenic fever (AML 2 days [range 1-11] vs 2 days [range 1-11], $P = .968$) were similar between the groups. Patients with AML received antibiotic treatment longer (20 days [range 3-94]) than the rest of the cohort (18 days [range 5-82], $P = .146$). However, relatively more AML patients responded to first-line treatment (AML 37% vs 22.9%, $P = .088$) and did not need further escalation in therapy.

3.9 | Impact of conditioning regimen on infection and antibiotic treatment

No significant difference in infection rate was seen between patients who received a myeloablative regimen (n = 94) and those who were treated with a non-myeloablative regimen (RIC + NMA, n = 72; 92.6% vs 90.3%, $P = .779$). There was also no significant difference between these two groups in documented infections (MAC 51.5% vs non-MAC 50%, $P = .892$). In t test analysis, onset of infection, counted from the day of admission, was at a mean of 10.1 days (SD 4.81) in patients with MAC regimen and 8.9 days (SD 5.10) in patients with a non-MAC regimen ($P = .117$). Febrile neutropenia was present in 64 patients (68.1%) in the MAC group compared with 53 patients in the non-MAC group (73.6%, $P = .494$). Duration of febrile neutropenia was significantly longer in patients with non-MAC treatment (4 vs 2.84 days $P = .012$). The mean duration in neutropenia was 15.6 days (SD 5.97) for patients receiving MAC regimen and 16.43 days (SD 7.40) for patients treated with a non-MAC regimen ($P = .434$). No significant difference was seen in the duration of antibiotic treatment (22.1 days in MAC regimen vs 21.8 days in non-MAC regimen, $P = .891$).

3.10 | Impact of the number of transplanted CD34 + cells on infection and antibiotic treatment

When distributed into two groups according to the median amount of CD34+ cells (6.53×10^6 cells per kg body weight), patients above the median showed higher rates of infection (95.2% vs 88.0%, $P = .094$), but no difference was seen in proven infection (50.6% vs 50.6%, $P = 1.000$).

The time to infection showed a significant negative correlation with the number of transplanted CD34+ cells (r = −.220, P = .006, n = 152). This would be regarded as a medium effect.[20] Additionally, a generalized Wilcoxon test was performed to analyze the difference in time to onset of infection between patients in groups of below or above transplanted median amount of CD34 cells. The survival distributions were significantly different from each other and compatible with the negative correlation (χ^2 = 4.489, P = .034).

The mean number of CD34+ cells for patients with febrile neutropenia was 6.62 × 10^6 cells per kg body weight (SD 2.823 × 10^6) and 6.39 × 10^6 cells per kg body weight (SD 2.662 × 10^6) in patients without febrile neutropenia (P = .636). There was no significant correlation between days of febrile neutropenia and the number of CD34+ cells used for the graft (r = −.073, P = .436, n = 117). A significant negative correlation existed between the duration of neutropenia and the number of transplanted CD34+ cells (r = −.208, P = .007, n = 166), although the effect size was medium to small. No correlation was seen in relation to the duration of antibiotic treatment (r = .052, P = .524, n = 152).

3.11 | Impact of neutropenia on infection and antibiotic treatment

An analysis of the appearance of an infection in patients during neutropenia and subsequent antibiotic treatment was performed in 152 patients.

Comparing the mean duration of neutropenia in patients with or without infection, t testing showed significantly longer neutropenia in patients with infection (16.4 vs 11.5 days) ($t(22)$ = −4.37, P < .001). No significant positive correlation can be seen between duration of neutropenia and days to onset of infection (P = .591). Patients with a proven infection spent significantly more days in neutropenia (17 days [SD 7.18] vs 14 days [SD 5.67], P = .004). The occurrence and the duration of febrile neutropenia significantly correlated with the duration of neutropenia. Patients with febrile neutropenia had a mean of 17 days (SD 6.75) of neutropenia, vs 12 days (SD 4.75), P < .001 in those without febrile neutropenia. The duration of neutropenia and febrile neutropenia showed a positive correlation with a strong effect (r = .441, P < .001, n = 166).

Furthermore, durations of antibiotic treatment and neutropenia correlated (r = .418, P < .001, n = 166). The longer a patient experienced neutropenia, the longer she or he received antibiotics in a therapeutic intent, that is, in a non-prophylactic intent.

Patients were grouped according to the median duration of neutropenia (14 days). Patients neutropenia of and exceeding 14 days showed significantly higher rates of infections, generally assumed infections (96.4% vs 86.7%, P = .025) as well as documented infections (62.7% vs 38.6%, P = .002). They also showed a higher occurrence of neutropenic fever (85.5% vs 55.4%, P < .001) and more days with antibiotic therapy (21 [7-94] days, n = 80, vs 16 [3-48] days, n = 72, P = .001).

3.12 | Analysis on successful antibiotic first-line treatment

Patients who did not need further escalation after first-line antibiotic treatment (end of treatment [EoT]) did not differ from patients who did need escalation in terms of age, disease, CMV IgG status, ECOG, conditioning regimen (EoT after first line: 32.9% in MAC vs 22% in non-MAC, P = .172), duration of neutropenia (EoT after first line: 13 [9-30] days, further treatment: 16 [6-43] days, P = .188), or number of CD34 cells transplanted (EoT after first line: 7.26 × 10^6 cells/kg body weight [SD 2.863 × 10^6], further treatment: 6.28 × 10^6 cells/kg body weight [SD 2.814 × 10^6], P = .08). However, the onset of infection had been significantly later in patients whose infection had resolved after first-line treatment compared with patients who needed more than one line of antibiotic therapy (11 days [SD 5.24] vs 9 days [SD 4.43], P = .036).

4 | DISCUSSION

Infections after allo-SCT are a major contribution to non-relapse mortality. Available data mainly stem from multicenter analyses and cover long time periods. Despite high patient numbers as their apparent advantage, these studies are associated with a potential bias regarding the structure of the population at risk and varying center-specific anti-infective policies which additionally change over time.

Here, a monocentric prospective study spanning a defined period of 1 year was performed in order to analyze the epidemiology, outcomes, and risk factors of infections occurring directly around allo-SCT, that is, during the stay that includes the procedure itself. To this end, a doctoral student not involved in treatment decisions prospectively recorded the courses of 163 patients receiving 166 transplantations during 1 year.

FUO occurred in 71.1% of the patients which is well in accordance with published data. Ample, various, and repeated diagnostic measures yielded scarce results. This is most likely a consequence of antibiotic prophylaxis and empiric antibiotic treatment as per guideline recommendations.

Cumulative incidence of death by infection after 1 year was 16.6% (95% CI: 11.3-22.7). In crude numbers, death following an infection during the peri-engraftment phase occurred in 19 patients, and additionally, 8 patients died of infection during the 365 days following their allo-SCT. Thus, risk for infection-related death is higher directly after allo-SCT than during the year after allo-SCT.

Several risk factors for FUO and infections found in multicenter analyses cannot be confirmed by our data. These include the type of conditioning. Non-myeloablative and reduced intensity conditioning regimens are used in older patients and patients with preexisting medical conditions. The main goal is to reduce the toxicity that originates from the chemotherapeutic agents and from radiatioin, and consequently to increase the eligibility of these patients for an allogeneic stem cell transplantation.[21] Different

groups report divergent results on whether infection is more likely in either myeloablative or reduced intensity and non-myeloablative regimens.[22–26] Kim et al analyzed 231 allogeneic stem cell transplantations with prior RIC (n = 81) or myeloablative conditioning (n = 150) and found a lower cumulative incidence of infections in patients with RIC (72% vs 87%).[23] In another study with 243 allogeneic stem cell transplantations, RIC was found to be an independent risk factor for bloodstream infections as opposed to MAC as well as autologous stem cell transplantation (HR 4.16).[25] Significantly different numbers of infections, clinically apparent or microbiologically proven, cannot be observed between these two groups in our study cohort. As the boundaries of eligibility for the different types of conditioning have been pushed throughout the years in terms of age and comorbidities, the indifference of infection rates in our study might be a hint of the equalization when it comes to complications in these conditioning regimens.[27] On the other hand, the results point toward an increase in infections in patients with non-myeloablative conditioning due to higher age and more comorbidities as confounders in this group.

Additionally, the underlying disease is not a risk factor for FUO or infection in our study cohort. Although the same is true for age of the patient, we can demonstrate an association between poor ECOG score prior to allo-SCT and infection. In our study, patients with a pretransplantation ECOG score of 2 had a significantly higher rate of infections compared to patients with an ECOG score of 0/ 1. It has been reported that an ECOG score of 2 or higher prior allo-SCT is associated with reduced overall survival.[28] Martino et al reported an increase in infections in allo-SCT-patients who had an ECOG score >1, although no bacterial infections were tracked in this study.[29] We can confirm this association.

As somehow expected and demonstrated several times, duration of neutropenia is a prominent risk factor for both FUO and documented infection.

The assumption of a correlation between patients' CMV serostatus and risk of infection has been discussed before [30–32] and is driven by the idea of a deprived immune system not only by the conditioning prior to stem cell transplantation but also by an improper functioning of immune cells.[33,34] A positive CMV IgG status is a sign for past CMV infection and can also correspond to a latent CMV infection.[35] Opposing Vinuesa et al who did not find a connection between CMV viremia and bacterial infection, we have seen significantly lower rates of bacterial infection in CMV seronegative patients compared to CMV seropositive patients.[36] A consequently lower rate of infection as cause of death in CMV seronegative recipients has been reported by other groups.[31,37] Furthermore, we have seen markedly lower infection rates in any donor recipient combination in which at least one party had a seronegative CMV status. That might support the idea of seropositivity as a sign of latent CMV infection or higher rates of copies of CMV DNA which leads to higher risk of infection in immunocompromised patients. In this study, we did not include the actual CMV load of donors and recipients before transplantation or the CMV load in patients after transplantation. Routine monitoring of CMV viremia and consecutive infections could lead to better screening of high-risk patients before transplantation, a closer monitoring of signs of infection, and quicker start of empirical therapy during post-transplantation hospitalization.

Meanwhile, that is after this analysis was performed, a policy of early discontinuation of antibiotic therapy has been found feasible and safe in trials with patients with hematologic disorders undergoing interventions that included high-risk neutropenic episodes, including allo-SCT.[38–41] The standard operating procedures used in our department advocate a rather extensive use of antibiotics in these critically ill patients. Additionally, strict single room contact precautions were in place as all of our patients were housed in single rooms in 2015. These precautions might not be necessary to avoid hospital-acquisition or patient-to-patient transmission of certain multiresistant bacteria.[42]

Limitations of this study are the heterogeneity in diseases analyzed, HLA matching, donor/recipient relations, and CMV matching. Also, insights from single-center studies are not generalizable as preventive measures and treatment strategies differ. On the other side, during the year (2015) used to address the questions as outlined, the standard operating procedures of the respective department were not changed, and the physicians in charge of the respective wards did not undergo personnel changes. In addition, the person who collected and analyzed that data was not involved in treatment decisions. Thus, the single-center nature of this study together with the fact that the center used strict standard operating procedures counterbalance heterogeneity effects that come with the factors mentioned before as does the large number of patients analyzed and the short time span used for patient analysis.

Taken together, this study provides real-world monocentric data prospectively recorded on the epidemiology, outcomes, and risk factors of infections during the peri-engraftment period of patients undergoing allo-SCT. Future research is necessary to further improve the still substantial mortality of patients during neutropenia after allo-SCT.

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CONFLICT OF INTEREST

MS declares that he has no conflict of interest. KI declares that she has no conflict of interest. CBC declares that she has no conflict of interest. LB declares that she has no conflict of interest. CL declares that she has no conflict of interest. CW declares that she has no conflict of interest. FA declares that he has no conflict of interest. NK declares that he has no conflict of interest. MC declares that he has no conflict of interest.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional

research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

This article does not contain any studies with animals performed by any of the authors.

INFORMED CONSENT

Informed consent was obtained from all individual participants included in the study.

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2. Darstellung der Publikation und Literaturverzeichnis

Transplantation of hematopoietic stem cells was, among others, a result of research in the late 1940´s and early 1950´s after significant findings of radiation injuries in the victims of the atomic bombs in Hiroshima and Nagasaki and further animal-based studies on radiation-sensitivity of bone marrow cells and ways of survival of otherwise lethal radiation (Jacobson et al., 1949a, Jacobson et al., 1949b).

Hematologic diseases were the cause of despair for a long time because treatment options were limited. The concept of stem cell transplantation had the potential of revolutionizing therapy of hematologic diseases, therefore being a curative procedure. The introduction of allogeneic stem cell transplantation in the context of clinical studies began at the end of the 1960's when standardized transplantation programs were first set up, although the first allogeneic bone marrow transplantations in humans were performed on patients in the aftermath of a nuclear accident in Yugoslavia in 1959 (Mathe et al., 1959, Bortin, 1970, Thomas et al., 1977, Thomas and Storb, 1970).

Since then, stem cell transplantation has become a standard treatment while protocols are routinely being checked and refined. Along the way to today's transplantation protocols, this specific treatment modality was associated with severe side effects, relapse, and mortality – and unfortunately still is. The depletion of the immune system during conditioning represents a high risk for the development of infections of bacterial, viral and fungal nature in these patients. Therefore preparation, clinical and laboratory observation, swift recognition of infection and permanent reevaluation of therapy is vital to successful stem cell transplantation with minimal morbidity and mortality.

In the year of our study, 2015, the German Register for Stem Cell Transplantation counted 3351 allogeneic transplantations nationwide. This number increased by 155 to 3506 allogeneic transplantations in 2018 (Beelen and Mytilineos).

A retrospective multicenter data mining study of the European Group for Blood and Marrow Transplantation Acute Leukemia Working Party reported an overall mortality prevalence at day 100 after allogeneic stem cell transplantation of 13.9% for patients with acute leukemia in the period 2000 – 2011 (Shouval et al., 2015).

In our study, we observed the 2015 patient cohort of allogeneic stem cell transplantation recipients at the Department of Stem Cell Transplantation of the University Medical Center Hamburg-Eppendorf. The aim was to document the appearance of infection, the diagnostic work-up and the course of antibiotic treatment. Furthermore, the epidemiology of this cohort and their according donors was crucial to analyze possible risk factors for infection during the peri-transplant period. We designed a predefined questionnaire to document patient data during hospitalization as well as the data of the one-year follow-up. In short, the recorded parameters were: patient characteristics (sex, age, disease, cytomegalovirus (CMV) Ig status), donor characteristics (sex, relation to patient, CMV Ig status), type of transplantation, Eastern Cooperative Oncology Group (ECOG) score at admission, conditioning regimen, neutrophil count (hence days of neutropenia), C-reactive protein (CRP), fever, severe sepsis and septic shock, documented infection, days to onset of infection, days with febrile neutropenia, use of diagnostic tools (microbiology, radiology, endoscopy) and their results, evaluation of fungal infection, anti-infectious treatment according to standard operating procedures (SOP), duration of antibiotic treatment, primary outcome (discharge or death) and one-year follow up data concerning survival and death. Patient data were anonymized, and data protection met the relevant national and European laws. The statistical analysis was performed through the following software: SPSS and R.

From January 1 to December 31 2015 we recorded the courses of 163 recipients of 166 allogeneic stem cell transplantations. Of those 37.4% were female and 62.6% were male, the mean age was 59 years (range 18-79 years). Age was no significant risk factor concerning suspected or documented infection. The graft source for transplantation was 10.2% bone marrow and 89.8% mobilized peripheral blood stem cells. Donors were 30% female and 70% male with 18.7% match related, 55.4% match unrelated, 21.1% mismatched unrelated and 4.8% haploidentical related transplantation.
Underlying diseases in the recipients of transplantation were chronic myeloproliferative neoplasms, lymphatic neoplasms, acute myeloid leukemias, myelodysplastic syndromes, myeloma, aplastic anemia. The underlying disease is not a risk factor for fever of unknown origin (FUO) or infection in our study cohort.

Outcome and follow-up

Of 166 recipients of allogeneic stem cell transplantation 145 patients (87.3%) were discharged, 21 patients (12.7%) died during their hospital stay for transplantation. Of those, 19 patients died of infection, and two of other causes (relapse, heart failure). The one-year follow-up registered 8 more patients dead due to infection-related causes and 20 patients died from causes other than infection (relapse, other).

Fever of unknown origin

Fever of unknown origin and the best empirical treatment is a much-discussed topic in the stem cell transplantation community. Most of the investigations are focused on empirical therapy of either one of the three suspected biological sources, which is bacterial, viral or fungal infection, and the improvement of diagnostic steps to make the fever of unknown origin a fever of known origin.
In our investigation 118 patients (71.1%) developed FUO. Hence, treatment with first line therapy according to SOP (ceftazidime and vancomycin) began. 71 cases (42.7%) remained classified as FUO while 47 cases (28.3%) were classified as documented infection during diagnostic work up. Diagnostic work-up succeeded in 40% of cases of FUO to fathom the cause of fever and classify the case as documented infection. The absolute number of cases of documented infection was 84, with pulmonary infiltration on thoracic computer tomography scan in 64, positive microbiological result in bronchial alveolar lavage fluid in 50, blood culture in 36 and central venous line in 5 cases.

The remaining 42.7% cases of FUO are well within the, admittedly, wide range of FUO observed in other studies which is 23% up to 51% (Hambach et al., 2002, Trenschel et al., 2005, Krüger et al., 1999).

Days of neutropenia

The t-test for mean duration of neutropenia in patients with or without infection showed significantly longer neutropenia in patients with infection (16.4 days *vs* 11.5 days) ($t(22)=-4.37$, $p<.001$). And patients with a proven infection spent

significantly more days in neutropenia (17 days (SD 7.18) *vs* 14 days (SD 5.67), *p*=.004).
The median duration of neutropenia was 14 days. Patients with neutropenia of and exceeding 14 days showed significantly higher rates of infections, generally assumed infections (96.4% *vs* 86.7%, *p*=.025) as well as documented infections (62.7% *vs* 38.6%, *p*=.002). They also showed a higher occurrence of neutropenic fever (85.5% *vs* 55.4%, *p*<.001).

As expected, and demonstrated several times, duration of neutropenia is a prominent risk factor for both FUO and (documented) infection (Hori et al., 2004, Junghanss et al., 2002). In the guidelines of the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Medical Oncology (DGHO) concerning diagnosis and empirical treatment of fever of unknown origin (FUO) in adult neutropenic patients the authors of said guideline agreed upon risk stratification into standard risk for infection (up to 7 days of neutropenia) and high risk of infection (duration of neutropenia of at least 8 days) (Heinz et al., 2017). There was no significant difference in terms of infection between patients with up to seven days of neutropenia (n=9, 88.9%) and patients with 8 or more days of neutropenia (n=157, 91.7%, *p*=.766). The large difference in group size might limit the finding.

Cytomegalovirus status
The supposition of a link between the CMV serostatus of patients and risk of infection has been discussed and shown before several times (Schmidt-Hieber et al., 2013, Broers et al., 2000, Nichols et al., 2002). The reason for that is the idea of a deprived immune system not only by the conditioning prior to allogeneic stem cell transplantation but also by an improper functioning of immune cells (Taylor-Wiedeman et al., 1991, Hokland et al., 1988).

Our study cohort was CMV IgG positive in 97 cases and CMV IgG negative in 69 cases. In our analysis regarding CMV status (positive *vs* negative) we did not experience significant disparities in febrile neutropenia (74.2% *vs* 65.2%, *p*=.210), duration of antibiotic treatment (23 days (SD 15.758) *vs* 20 days (SD 8.613), *p*=.138) or infections (93.8% *vs* 88.4%, *p*=.217). Yet patients with negative CMV

IgG status showed significantly lower rates of documented infection (40.6% *vs* 57.7%, *p*=.029). This is also seen in patient-donor matches in which at least one part is CMV IgG negative (n=82, 39% *vs* 61.9%, *p*=.003).

Vinuesa et al. did not find a connection between CMV viremia and bacterial infection (Vinuesa et al., 2016). It is debatable to compare our results with the before mentioned study, because we did not track the polymerase-chain reaction (PCR) results for CMV, and thereby viral load, in our study. A lower rate of infection as cause of death in CMV seronegative recipients has been reported by other analyses (Kroger et al., 2001, Broers et al., 2000). We have seen significantly lower rates of bacterial infection in CMV seronegative patients compared to CMV seropositive patients and in any donor recipient combination in which at least one party was of seronegative CMV status. This result would be a rationale of CMV seropositivity as a sign of latent CMV infection or higher rates of copies of CMV DNA which leads to higher risk of infection in immunocompromised patients. In this study we did not include the actual CMV load of donors and recipients before transplantation or the CMV load in patients after transplantation.

Conditioning regimens

Non-myeloablative and reduced intensity conditioning regimens were developed to increase the number of eligible patients for the procedure. Those regimens are used in older patients and patients with pre-existing medical conditions because these patients benefit from the reduced toxicity of said regimens (Martino et al., 2001).

The conditioning regimens of ‘reduced-intensity’ were combined with ‘non-myeloablative’ in our study, because there was only one case of reduced-intensity conditioning, and named ‘non-myeloablative’ conditioning. No significance was seen in infection rate among myeloablative conditioning (MAC) (n=94) and non-myeloablative conditioning (non-MAC) ((n=72); 92.6 % vs 90.3 %, p=.779). No significant difference between these two groups was seen in documented infections, too (MAC 51.5% vs non-myeloablative 50%, p=.892). Febrile neutropenia was present in 64 patients (68.1%) in the MAC group compared with 53 patients in the non-myeloablative group (73.6%, p=.494). Thus, we saw a higher percentage, though not significant, of febrile neutropenia in the non-

myeloablative group. Interestingly the duration of febrile neutropenia was also significantly longer in patients with non-myeloablative treatment (4 days vs 2.84 days p=.012).

There are different results from other studies about the incidence of infection in patients after myeloablative versus reduced intensity and non-myeloablative regimens during the early post-transplantation phase (Neofytos et al., 2009, Kim et al., 2013, Larosa et al., 2005, Poutsiaka et al., 2007, Alyea et al., 2006). Kim et al. found a lower cumulative incidence of infections in patients with reduced-intensity regimens compared to myeloablative regimens (72% *vs* 87%) (Kim et al., 2013). Poutsiaka et al. conducted a study were the use of reduced-intensity regimens was found to be an independent risk factor for blood stream infections in comparison to myeloablative conditioning as well as autologous stem cell transplantation (HR 4.16) (Poutsiaka et al., 2007).

No significant differences concerning numbers of infections, clinically apparent or microbiologically proven, were seen in our study. This can be viewed in support of the equivalence of the different types of conditioning when it comes to complications as described in a review by Atilla et al (Atilla et al., 2017). Yet, our results probably stem from higher age and more comorbidities as confounders in the group non-myeloablative conditioning.

Antibiotic treatment

Antibiotic therapy was initiated in 153 patients, 92 % of our cohort. The reason was fever (44%, n=73), increased CRP (25.9%, n=43), both of the before mentioned occurring simultaneously (9%, n=15) or other reasons (21.1%, n=35). 129 patients (77.7%) were treated with first-line therapy initially. In 36 patients (21.7%), this therapy was discontinued successfully, 75 patients (45.2%) required second-line therapy and 18 patients (10.8%) received an adapted antibiotic treatment, deviant of the standard operation procedure.

To prevent infectious complications during the early post-transplantation phase, the admission of prophylactic antibiotics, as well as anti-viral and -fungal agents, is a well-established procedure, just like the empirical treatment at the advent of

infection. This is a result of a large body of studies carried out over the last decades which showed significantly lower rates of infection and mortality when prophylactic treatment was applied (Bucaneve et al., 2005, Dykewicz, 2001, Lew et al., 1995). These agents against bacteria, virus and fungus are mostly aimed at a broad spectrum of subspecies. In the last decade, research on the negative effects of the microbiome mediated by broad-spectrum antibiotic in patients undergoing allogeneic stem cell transplantation increased and is much discussed. Results show a loss of diversity of the intestinal microbiome as well as an increase in gastrointestinal graft-versus-host disease which therefore results in a significantly higher mortality rate (Holler et al., 2014, Shono et al., 2016, Weber et al., 2017).

A policy of early discontinuation of empirical antibiotic therapy, as a progressive approach to further reduce exposure to antimicrobial agents in patients, was not part of the standard operating procedures at the time of the study but meanwhile different trials showed early discontinuation, i.e. 72 hours after apyrexia and clinical recovery, as a safe procedure in patients with hematologic disorders undergoing interventions that included high-risk neutropenic episodes, thus including allogeneic stem cell transplantation (Aguilar-Guisado et al., 2017, Le Clech et al., 2018, Pasquier et al., 2018, Viscoli, 2017).

This shows one of the difficulties of proper balanced antibiotic therapy in the field of transplantation in general and allogeneic stem cell transplantation in particular.

ECOG score

The assessment of risk prior to the transplantation procedure is very importance for every individual patient. One of many assessment tools is the ECOG score (Eastern Cooperative Oncology Group), which describes and determines the level of a patient’s ability of independent function in their daily routine.

An infection was seen in 84.5% (n=49/58) of patients with initial ECOG score of 0, in 94.6% of patients with an ECOG score of 1, and each 100% in patients classified as ECOG score 2 (n=14/14) and 3 (n=1/1) (p= .027). We also observed a higher rate of documented infection in patients with ECOG score 2 compared to ECOG score 0 and 1 combined (78.6% vs 47.7%, p= .027). Alvarez et al. described a reduced overall survival of patients with a pre-transplant ECOG score

of 2 and higher (Alvarez et al., 2006). Martino et al. published a study which showed an increase of infections in patients who had an ECOG score >1 prior to their allogeneic stem cell transplantation, although no bacterial infections were tracked in this study (Martino et al., 2009). We confirm the usefulness of the ECOG score as a risk assessment tool. Patients with an ECOG score of 2 or higher should receive even more attention during the daily post-transplantation patient assessment.

Strength and limitations of this study

The study had been performed under consistent conditions. The standard operating procedures did not change in 2015 and they had been applied persistently. Also, there was no change of the physicians performing patient treatment on the ward. I, the author of this doctoral thesis and first author of the published article, was not involved in the treatment of the patients or treatment decision and therefore collected and analyzed the data objectively. The follow-up rate was 100 percent and the data we decided to collect beforehand was available for all patients.

A main limitation of this study is the heterogeneity of diseases in the patients undergoing allogeneic stem cell transplantation, thus the small numbers in the different groups. A lot of studies in this field observe only one or few diseases which made it more difficult to compare the respective results. This study also did not include HLA matching and in-depth analysis of donor/recipient relation. Designed as a single center study the results are hardly transferable to generality as transplantation settings and standard operation procedures are various. However, this was not a goal of this study but the description of a specific patient cohort and their progression through the early post transplantation process. Naturally, future research is needed to amplify the knowledge of disease and better the curative approach and peri-transplant management to lessen the still considerable mortality of patients during neutropenia after allogeneic stem cell transplantation.

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3. Zusammenfassung

During allogeneic hematopoietic stem cell transplantation, infections significantly contribute to morbidity and mortality. A monocentric prospective analysis was performed to assess epidemiology, risk factors, and outcomes of infections during the peri-transplant period. The data were recorded prospectively using a predefined questionnaire. In 2015, 163 consecutive patients, of whom 37.4% were female and 62.6% male, with a median age of 59 (range 18-79) years, received 166 allogeneic stem cell transplantation. The median duration of leukopenia (<10^9/L) was 14.5 days (range 4-43 days). Fever of unknown origin (FUO) occurred in 118 of 166 patients (71.1%). Severe sepsis developed in 95, septic shock in 26 patients. Intensive diagnostic workup, i.e. blood culture, thoracic computer tomography scans and bronchoscopy, helped to identify causative microorganisms only in a small number of infectious courses. All but 13 patients needed antibiotic therapy, each according to the standard operating procedures of the department. The cumulative incidence of death by infection after one year was 16.6% (95%CI 11.3-22.7%). The only risk factor for FUO in neutropenia was the duration of neutropenia </≥14 days. FUO occurred in 55.4% of patients with less than 14 days duration of neutropenia and 85.5% of patient with 14 days or more of neutropenia (p<.001). In conclusion, the results of an elaborate diagnostic workup of infections in the peri-transplant period are scarce. Further attention to risk factors might help to identify patients at risk for severe infections.

Während der Durchführung einer allogenen Stammzelltransplantation tragen Infektionen signifikant zu Morbidität und Mortalität bei. Es wurde eine monozentrische Studie zur Erfassung von Epidemiologie, Risikofaktoren und infektiologischem Therapieverlauf und Ausgang vor, während und nach einer Transplantation durchgeführt. Die vor Beginn des Beobachtungszeitraumes festgelegten Parameter wurden in einem standardisierten Datenblatt erfasst. Im Jahr 2015 erhielten 163 Patienten, 37.4% davon weiblich, medianes Alter 59 Jahre (range 18-79), 166 allogene Stammzelltransplantationen. Fieber unbekannter Ursache in der Neutropenie trat bei 118 (71.1%) Patienten auf. Schwere Sepsis entwickelten 95 Patienten, einen septischen Schock 26 Patienten. Die intensive mikrobiologische Diagnostik ergab lediglich eine kleine

Anzahl an pathologischen Befunden während der Infektionsverläufe. Außer 13 Patienten benötigten alle Erkrankten eine Antibiotikatherapie, jeweils nach den Standardarbeitsanweisungen der Klinik für Stammzelltransplantation. Die kumulative Inzidenz der Mortalität durch Infektion nach einem Jahr nach Transplantation betrug 16.6% (95%CI 11.3-22.7%). Der einzige signifikante Risikofaktor für Fieber unbekannter Ursache in der Neutropenie war die Dauer der Neutropenie </≥14 Tage (55.4% vs 85.5%, p<.001). Resultate in der ausgedehnten infektiologischen Diagnostik in der Transplantationsphase sind spärlich, oft ohne pathologischen Befund und nicht wegweisend für die Therapie der Infektion. Eine genauere Betrachtung von möglichen Risikofaktoren kann helfen, Patienten mit einem Risiko für schwere Infektionen zu identifizieren.

4. Erklärung des Eigenanteils an der Publikation

Markus Iversen, né Samek, designed the study, developed the questionnaire and designed the tool for data collection, collected patient data, performed statistical analysis, designed and implemented data tables, analyzed the data, conducted literature review, and wrote the manuscript.

Katharina Iversen performed statistical analysis.

Claudia Langebrake helped with pharmacology and plays an essential role in developing standard operation procedures for the department of stem cell transplantation.

Cristina Belmar-Campos and Laura Berneking performed microbiological diagnostics and interpreted results.

Christine Wolschke and Francis Ayuk supervised patient treatment and the collection of patient data and interpreted results.

Nicolaus Kröger designed the study, supervised patient treatment and the collection of patient data and interpreted results.

Maximillian Christopeit designed the study, performed statistical analysis, analyzed the data, supervised patient treatment and the collection of patient data and wrote the manuscript.

Nurses, technicians and physicians in the departments of stem cell transplantation, radiology, clinical chemistry and microbiology conducted patient treatment, collected patient data and performed various diagnostic testing.

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6. Lebenslauf

Lebenslauf wurde aus datenschutzrechtlichen Gründen entfernt.

7. Eidesstattliche Versicherung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.
Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.

Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Unterschrift: .....