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Microbiota Disruption Induced by Early Use of Broad-Spectrum Antibiotics Is an Independent Risk Factor of Outcome after Allogeneic Stem Cell Transplantation

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A B S T R A C T

In allogeneic stem cell transplantation (ASCT), systemic broad-spectrum antibiotics are frequently used for treatment of infectious complications, but their effect on microbiota composition is still poorly understood. This retrospective analysis of 621 patients who underwent ASCT at the University Medical Center of Regensburg and Memorial Sloan Kettering Cancer Center in New York assessed the impact of timing of peritransplant antibiotic treatment on intestinal microbiota composition as well as transplant-related mortality (TRM) and overall survival. Early exposure to antibiotics was associated with lower urinary 3-indoxyl sulfate levels ($P < .001$) and a decrease in fecal abundance of commensal *Clostridiales* ($P = .03$) compared with late antibiotic treatment, which was particularly significant ($P = .005$) for *Clostridium* cluster XIVa in the Regensburg group. Earlier antibiotic treatment before ASCT was further associated with a higher TRM (34%, 79/236) compared with post-ASCT (21%, 62/297, $P = .001$) or no antibiotics (7%, 6/88, $P < .001$). Timing of antibiotic treatment was the dominant independent risk factor for TRM (HR, 2.0; $P \leq .001$) in multivariate analysis besides increase age (HR, 2.15; $P = .004$), reduced Karnofsky performance status (HR, 1.47; $P = .03$), and female donor–male recipient sex combination (HR, 1.56; $P = .02$) A competing risk analysis revealed the independent effect of early initiation of antibiotics on graft-versus-host disease–related TRM ($P = .004$) in contrast to infection-related TRM and relapse (not significant). The poor outcome associated with early administration of antibiotic therapy that is active against commensal organisms, and specifically the possibly protective *Clostridiales*, calls for the use of *Clostridiales*-sparing antibiotics and rapid restoration of microbiota diversity after cessation of antibiotic treatment.

INTRODUCTION

Allogeneic stem cell transplantation (ASCT) is a curative treatment option for a variety of hematopoietic malignancies and other severe hematologic and genetic diseases [1]. Its success, however, is still limited by a significant risk of life-threatening complications, including acute graft-versus-host disease (GVHD) and infection. Because neutropenia and mucosal injury after myeloablative conditioning regimens often lead to neutropenic infections in ASCT recipients [2], most patients require treatment with broad-spectrum antibiotics. However, there is increasing evidence that use of broad-spectrum antibiotics may exert a detrimental impact on intestinal microbiota composition and, subsequently, the outcome of ASCT [3]. Studies have demonstrated that loss of

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intestinal microbiome diversity, in particular commensal *Clostridiales*, appears to contribute not only to the pathogenesis of acute gastrointestinal GVHD but also to increased transplant-related mortality (TRM) [4,5]. Similarly, urinary concentration of 3-indoxyl sulfate (3-IS), a tryptophan metabolite of commensal colonic bacteria, has been identified as an indirect marker of a balanced microbiota and predicts outcome at the time of ASCT [6]. In the present study we investigated the association of the timing of antibiotic treatment with intestinal microbiota composition and clinical outcome in a cohort of 621 patients undergoing ASCT in 2 centers with different practices of antibiotic prophylaxis.

METHODS

Patients

A total of 621 adult patients undergoing ASCT in Regensburg, Germany (n = 380) and New York, New York (n = 241) were included in our retrospective analysis. Inclusion criteria were hemato-oncologic disease requiring ASCT with an age above 18 years and receipt of non–T cell–depleted grafts. The Regensburg cohort consisted of 380 consecutive ASCT recipients enrolled between September 2008 and June 2015, whereas the New York cohort comprised 241 patients who underwent ASCT between October 2009 and May 2015 at the Memorial Sloan Kettering Cancer Center in New York. Further, with approval by the Ethics Committee of the University Medical Center of Regensburg and after receipt of written informed consent, urinary and stool specimens were collected in a total of 130 and 26 patients, respectively, at a minimum of 6 different time points between admission and day 28 after ASCT: before admission, at least once between days −2 to +2, +2 and +10, +11 to +17, +18 to +24, and +25 to +30. In addition, stool samples from 5 healthy stem cell donors served as controls. Similarly, for the New York group stool specimens from 146 ASCT recipients were obtained within 4 days of day 12 after ASCT. All specimens were stored at −80°C until analysis.

All patients received prophylactic antibiotics from the start of conditioning until engraftment, but the type of prophylactic antibiotics differed between the 2 cohorts. In Regensburg, ciprofloxacin 500 mg twice daily and metronidazole 400 mg three times daily were administered orally to 189 patients (49.7%) until March 2012, before prophylaxis was switched to oral rifaximin 200 mg twice daily in the remaining 191 patients (50.3%) to control the emergence of vancomycin-resistant enterococci. In the New York group, all 241 patients received prophylactic intravenous ciprofloxacin 400 mg twice daily, and those receiving myeloablative preparative regimens were additionally given intravenous vancomycin at a starting dose of 1 g twice daily.

Although there were differences in prophylactic antibiotic regimens between the New York and Regensburg cohorts, treatment of neutropenic fever/infections with additional antibiotics was comparable between the 2 cohorts. In the Regensburg cohort 350 of 380 patients (92%) received additional antibiotic treatment beyond prophylactic regimens. Piperacillin/tazobactam at a thrice-daily dose of 4.0/.5 g was used as empiric first-line therapy in 275 of 350 patients (78%), whereas meropenem 1.0 g thrice daily and vancomycin 1.0 g twice daily served as second-line therapy in 55 of 350 patients (16%). In cases of penicillin intolerance, 20 patients (6%) received alternative first-line treatment with vancomycin and/or ceftazidime (in 13 patients after ciprofloxacin/metronidazole prophylaxis; in 7 patients after rifaximin prophylaxis). In the New York cohort, piperacillin/tazobactam and imipenem/cilastatin were administered 4 times a day at doses of 4.0/.5 g and 500 mg, respectively, as first- and second-line therapy. Overall, 183 of 241 patients (76%) in New York required antibiotics to treat neutropenic fever/infections, among whom 103 (56%) received first-line therapy only and 80 (44%) required second-line therapy. Fifty-eight of 241 ASCT recipients (24%) required no therapeutic antibiotics. The clinical criteria for initiation of first- and second-line antibiotics were comparable with the Regensburg group. At both centers patients received first- and second-line treatment according to international guidelines for treatment of neutropenic fever/infections [7,8].

All patients were classified into 3 groups according to the timing of additional antibiotic initiation: (1) early exposure to antibiotics between days −7 and 0 (early AB group, n = 236 [38%]), (2) exposure to antibiotics on day 0 or thereafter (late AB group, n = 297 [48%]), and (3) no systemic antibiotic treatment during the course of ASCT beyond prophylactic regimens (no AB group, n = 88 [14%]) (Table 1). Because acute leukemia patients usually have a history of several courses of cytotoxic treatment and repeated infections treated with broad-spectrum antibiotics, we additionally defined 2 subgroups and divided patients into an acute leukemia (n = 296) versus nonacute leukemia (n = 325) group. Patient characteristics in the 3 antibiotic subgroups are shown in Table 2.

Table 1
Beginning of Antibiotic Treatment at the 2 Centers

	Total Cohort (N = 621)	Regensburg (n = 380)	New York (n = 241)
No AB group	14% (n = 88)	8% (n = 30)	24% (n = 58)
Early AB group	38% (n = 236)	50% (n = 190)	19% (n = 46)
Late AB group	48% (n = 297)	42% (n = 160)	57% (n = 137)

Analysis of Urinary 3-IS

Urinary 3-IS and creatinine levels were determined by reverse-phase liquid chromatography-electrospray ionization-tandem mass spectrometry as previously described [6].

Quantification of Clostridium *Cluster XIVa 16S rRNA Gene Copies by Reverse Transcriptase PCR*

Using *Clostridium* cluster XIVa group-specific primers and SYBR Green I Master (Roche, Basel, Switzerland) qPCR reagents, 16S rRNA gene copy numbers of *Clostridium* cluster XIVa species were determined in fecal DNA preparations by real-time quantitative PCR on a LightCycler 480 II instrument (Roche). Full-length 16S rDNA amplicons of *Clostridium* cluster XIVa bacteria cloned into the pGEM T-Easy vector served as quantification standards (Invitrogen, Carlsbad, CA).

Clostridial Abundance Measurement

At Memorial Sloan Kettering Cancer Center, stool specimens collected within 4 days of day 12 after ASCT were analyzed for clostridial abundance, as described previously [9]. Briefly, stool specimens were subjected to mechanical disruption (bead-beating), and DNA was extracted with phenol-chloroform [10]. DNA samples were analyzed by the Illumina MiSeq platform (Illumina, San Diego, CA) to sequence the V4-V5 region of the 16S rRNA gene. Sequence data were compiled and processed using mothur version 1.34 [11], screened and filtered for quality [12], and classified to the species level [13] using a modification of the Greengenes reference database [14].

Bioinformatics and Data Analysis

Normally and non-normally distributed continuous data are presented as mean (± standard deviation [STD]) or median (range), respectively. Accordingly, group comparisons were performed by 2-sided *t*, Mann-Whitney U. or Kruskal-Wallis tests. Absolute and relative frequencies were given for categorical data and compared between study groups by chi-square or Fisher's exact tests. All hypotheses were tested in an explorative manner on a 2-sided 5% significance level. Factors associated with early use of systemic antibiotics were assessed using logistic regression analysis. Kaplan-Meier analysis was performed to assess survival and nonrelapse mortality, and Cox regression was used for multivariate assessment of risk factors. Competing-risk analysis [15] for GVHD-related TRM, infectious TRM, and relapse was performed using software package R 3.2.2 (The R Foundation of Statistical Computing, Vienna, Austria). Otherwise, IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL) was used for analysis.

RESULTS

Factors Associated with Early Antibiotic Treatment in ASCT Patients

In the study cohort, multivariate analysis identified several factors associated with higher likelihood of early antibiotic exposure before day 0: advanced stage of underlying disease (hazard ratio [HR], 2.1; 95% confidence interval [CI], 1.7 to 2.7; $P < .001$), matched unrelated donor (HR, 2.2; 95% CI, 1.5 to 3.4; $P < .001$), and interval from first diagnosis to ASCT < 12 months (HR, 2.6; 95% CI, 1.8 to 3.8; $P < .001$). Patient age and donor–recipient sex ratio did not affect timing of antibiotic treatment. The interval from diagnosis to ASCT was shorter in patients suffering from acute leukemia with a median of 5 months (range, 1 to 206) than in nonacute leukemia patients requiring ASCT with a median of 18 months (range, 2 to 156; $P < .001$).

Timing of Antibiotics Affects Intestinal Microbiota Disruption Early after ASCT

Several lines of evidence suggest an impact of early antibiotics on loss of commensal bacteria and therefore raise