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MAJOR ARTICLE

Detrimental Effect of Broad-spectrum Antibiotics on Intestinal Microbiome Diversity in Patients After Allogeneic Stem Cell Transplantation: Lack of Commensal Sparing Antibiotics

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Background. Maintaining gastrointestinal (GI) microbiome diversity plays a key role during allogeneic stem cell transplantation (ASCT), and loss of diversity correlates with acute GI graft versus host disease (GvHD) and poor outcomes.

Methods. In this retrospective analysis of 161 ASCT patients, we used serial analyses of urinary 3-indoxyl sulfate (3-IS) levels and GI microbiome parameters within the first 10 days after ASCT to identify potential commensal microbiota–sparing antibiotics. Based on antibiotic activity, we formed 3 subgroups (Rifaximin without systemic antibiotics, Rifaximin with systemic antibiotics, and Ciprofloxacin/Metronidazole with/without systemic antibiotics).

Results. Mono-antibiosis with Rifaximin revealed higher 3-IS levels ($P < .001$), higher *Clostridium* cluster XIVa (CCXIVa) abundance ($P = .004$), and higher Shannon indices ($P = .01$) compared to Ciprofloxacin/Metronidazole with/without systemic antibiotics. Rifaximin followed by systemic antibiotics maintained microbiome diversity compared to Ciprofloxacin/Metronidazole with/without systemic antibiotics, as these patients showed still higher 3-IS levels ($P = .04$), higher CCXIVa copy numbers ($P = .01$), and higher Shannon indexes ($P = .01$). Even for this larger cohort of patients, the outcome was superior with regard to GI GvHD ($P = .05$) and lower transplant-related mortality ($P < .001$) for patients receiving Rifaximin plus systemic antibiotics compared to other types of systemic antibiotic treatment. Antibiosis with Ciprofloxacin/Metronidazole (n = 12, $P = .01$), Piperacillin/Tazobactam (n = 52, $P = .01$), Meropenem/Vancomycin (n = 16, $P = .003$), Ceftazidime (n = 10, $P = .03$), or multiple systemic antibiotics (n = 53, $P = .001$) showed significantly lower 3-IS levels compared to mono-antibiosis with Rifaximin (n = 14) or intravenous Vancomycin (n = 4, not statistically significant).

Conclusions. Different types of antibiotic treatments show different impacts on markers of microbiome diversity. The identification of antibiotics sparing commensal bacteria remains an ongoing challenge. However, Rifaximin allowed a higher intestinal microbiome diversity, even in the presence of systemic broad-spectrum antibiotics.

Keywords. broad-spectrum antibiotics; gut microbiome; allogeneic stem cell transplantation; acute intestinal graft versus host disease.

The human gastrointestinal (GI) tract harbors a complex and diverse community of commensal microbiota, providing a variety of beneficial effects to the host. They contribute to the maintenance of intestinal homeostasis and epithelial integrity and exert anti-inflammatory effects by interacting with the mucosal immune system [1, 2]. Therefore, it is hardly surprising that intestinal dysbiosis is associated with inflammatory processes [3] contributing to the pathophysiology of different diseases, ranging from inflammatory bowel disease [4] via gastrointestinal carcinogenesis [5, 6] to metabolic syndromes [7] to neurological disorders [8].

A major risk factor causing disruptions of the intestinal microbiome is the use of broad-spectrum antibiotics. Patients undergoing allogeneic stem cell transplantation (ASCT) are at high risk for intestinal microbiota disruptions [9]. To prevent neutropenic infections, they usually receive prophylactic antibiotics, but still the majority of patients develop fevers and require additional therapeutic antibiotics [10, 11]. Furthermore, conditioning-related epithelial damage, changes of alimentary habits, and parenteral nutrition enhance intestinal dysbiosis during transplantation [12]. Alterations of the intestinal microbiota diversity seem to be linked with the outcome after ASCT, as low intestinal microbiota diversity was found to be associated with increased graft versus host disease (GvHD)-related mortality and worse overall survival (OS) [12, 13]. Even

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correlations between the timing of antibiotic treatment, the kind of antibiotics used for the prophylaxis and treatment of neutropenic infections, and outcomes could be observed, resulting in the need to reevaluate the benefits of current antibiotic standards in ASCT [9, 14]. As a first step, we addressed the question of whether specific antibiotics have differential impacts on microbiota diversity and, thus, can lead providers to the selection of antibiotics with microbiota-sparing effects.

PATIENTS AND METHODS

Patients

A total of 161 adult patients undergoing ASCT at the University Hospital Regensburg were included in our retrospective analysis. Inclusion criteria were hemato-oncologic disease requiring ASCT, an age above 18 years, and receiving non-T cell depleted grafts. The Ethics Committee of the University Hospital Regensburg approved the study (02/220) and, after written informed consent, the patients' stool and urinary specimens were collected on a weekly basis from admission until day 10 after ASCT: prior to admission, specimens were collected at least once between days -2 to +2 and +2 to +10. All specimens were stored at -80°C until analysis. Urinary samples were available in 161 patients and additional stool samples were available in a subgroup of 62 patients. Pretransplant urinary and stool samples were not available for 2 patients.

In our cohort, 55 patients received Ciprofloxacin at 500 mg 2 times a day and Metronidazole at 400 mg 3 times a day for antibiotic prophylaxis, typically starting 8 days prior to ASCT and going until 28 days post-transplant. In April 2012, we switched our standard gut decontamination regimen to Rifaximin at 200mg twice a day (n = 106), due to an increasing number of patients with Vancomycin-resistant enterococci. Rifaximin is a Rifamycin-derivative with broad-spectrum activity and negligible intestinal resorption [15]. In cases of neutropenic infections, Piperacillin/Tazobactam was standardly used according to the guidelines of the European Leukemia network for empiric first-line therapy and was followed by Meropenem/Vancomycin as a second-line therapy [16]. Patients with known colonization with gram-negative bacteria, penicillin-resistant bacteria, or clinical signs of sepsis were initially treated with Meropenem/Vancomycin. In case of a known intolerance against penicillin or carbapenem, Ceftazidime was used as an antibiotic first- or second-line therapy instead. Patients with local signs of an infection of the central venous catheter were treated with intravenous Vancomycin. The clinical standard of therapeutic administration of systemic antibiotics did not differ between the 2 decontamination groups. For each patient, the time point of the beginning and the duration of systemic antibiotic therapy were exactly documented. In this retrospective analysis, we focused on patients who received monotherapy with different kinds of antibiotics within the first 10 days after ASCT and classified patients, according to their antibiotic exposure, into the following groups: patients with gut decontamination only (Rifaximin vs. Ciprofloxacin/Metronidazole) and patients with gut decontamination and a single kind of systemic broad-spectrum antibiotic (Piperacillin/Tazobactam vs. Meropenem/Vancomycin vs. Ceftazidime vs. Vancomycin). In case of administration of several broad-spectrum antibiotics within the first 10 days, patients were classified into the group with multiple systemic antibiotics.

The interval until day 10 was chosen because many patients with antibiotic monotherapy switch to second-line antibiosis in this timeframe, therefore not allowing for the differentiation of effects at later times post-transplant. Additionally, we have previously shown that the first 10 days after ASCT are the most critical with regard to microbiota effects on patients' outcomes post-transplant [17]. Patients were attributed to the different antibiotic groups according to the antibiotic with the broadest spectrum of efficacy that was given more than 2 days. We subsequently divided patients into 3 different subgroups: (1) Rifaximin treatment without further broad-spectrum antibiotics (n = 14), (2) Rifaximin followed by additional systemic broad-spectrum antibiotics (n = 92), and (3) Ciprofloxacin/Metronidazole administration with/without systemic broad-spectrum antibiotics (n = 55). None of the patients (0/14) in the Rifaximin without systemic antibiotics group, 8.7% (8/92) of patients in the Rifaximin group followed by systemic antibiotic treatment, and 25.5% (14/55) of patients in the Ciprofloxacin/Metronidazole group with/without systemic broad-spectrum antibiotics received the standard systemic conditioning regimen. A separate analysis of Ciprofloxacin/Metronidazole alone and Ciprofloxacin/Metronidazole followed by systemic antibiotics revealed a comparable time course of 3-indoxyl sulfate (3-IS) levels. As the number of patients receiving Ciprofloxacin/Metronidazole alone was very small with regard to microbiota analyses, we therefore combined both groups.

Within the first 10 days after ASCT, 74.5% (n = 41) of patients with Ciprofloxacin/Metronidazole and 86.8% (n = 92) of patients with Rifaximin required additional systemic broad-spectrum antibiotic treatment. Altogether, 89.1% (49/55) of Ciprofloxacin/Metronidazole patients and 93.4% (99/106) of Rifaximin patients received systemic antibiotics during the course of ASCT (data are not statistically significant [ns]). Patients' characteristics are shown in Table 1.

Analysis of Urinary 3-Indoxyl Sulfate Levels

In 161 patients, urinary 3-IS levels were analyzed by reversed-phase liquid chromatography–electrospray ionization tandem mass spectrometry, as previously described, and corrected in relation to the creatine value [17].

Table 1. Summary of Patient Characteristics

Age, years	51.0 ± 11.7
Antibiotic treatment	
Ciprofloxacin/Metronidazole	7.4% (n = 12)
Piperacillin/Tazobactam	32.3% (n = 52)
Meropenem/Vancomycin	9.9% (n = 16)
Ceftazidime	6.2% (n = 10)
Multiple systemic antibiotics	32.9% (n = 53)
Vancomycin	2.5% (n = 4)
Rifaximin	8.7% (n = 14)
Diagnosis	
Acute leukemia	54.7% (n = 87)
Lymphatic neoplasia	21.7% (n = 35)
Myelodysplastic syndrome	13.0% (n = 21)
Myeloproliferative syndrome	8.7% (n = 14)
Aplastic anemia	2.5% (n = 4)
Stage of underlying disease	
Early/intermediate	64.6% (n = 104)
Advanced	35.4% (n = 57)
Donor	
Sibling	29.8% (n = 48)
Unrelated donor	70.2% (n = 113)
Conditioning	
Reduced intensity	86.3% (n = 139)
Standard	13.7% (n = 22)

Quantification of *Clostridium* Cluster XIVa 16S-Ribosomal Ribonucleic Acid Gene Copies by Quantitative Polymerase Chain Reaction

Using *Clostridium* cluster XIVa (CCXIVa) group-specific primers [18] and SYBR® Green I Master (Roche) quantitative polymerase chain reaction reagents, 16S-rRNA gene copy numbers of CCXIVa species were determined in fecal deoxyribonucleic acid preparations (n = 62) by real-time quantitative polymerase chain reaction on a LightCycler 480 II instrument (Roche). Full-length 16S-rRNA gene amplicons of *Eubacterium rectale DSM 17629*, cloned into the pGEM® T-Easy vector (Invitrogen), served as quantification standards.

Analysis of Enterococcus spp. 16S-rRNA Gene Copies by Quantitative Polymerase Chain Reaction

Enterococcus species were quantified analogous to CCIVa in the same subgroup (n = 62), except that genus-specific primers [19] and Enterococcus faecalis ATCC 29212 quantification standards were used.

Analysis of Intestinal Microbiome Diversity

In the same 62 patients, 16S-rRNA gene analyses of stool specimens were performed. Extraction of nucleic acids and sequencing of variable V3-V6 16S-rRNA gene regions were performed using a GS FLX+ system, as described before [20]. Reads were demultiplexed and quality filtered with the QIIME 1.9.1 software package, using default parameters [21]. Operational taxonomic units were clustered at 99% pairwise identity using the vsearch 2.4.3 package [22]. Taxonomy was assigned to OTUs using UCLUST v1.2.22q [23] and the SILVA release 128 reference database [24]. The α-diversity was determined at different time points between admission and day 28 after ASCT by calculating the Shannon diversity index [25].

Clinical Outcome

For assessment of clinical outcomes in relation to antibiotic regimen, the incidences of severe GI GvHD (stage II-IV), transplant-related mortality (TRM), and OS were evaluated. In addition, data regarding fevers of unknown origin and bacteremia were analyzed, as well as intestinal infections with *Clostridium difficile*.

Bioinformatics and Data Analysis

Continuous data are presented descriptively as mean ± standard deviation and range. Group comparisons were performed by 2-sided Mann-Whitney U-tests due to non-normal data distributions. Absolute and relative frequencies were given for categorical data and compared between study groups by chi-squared tests. All hypotheses were tested in an exploratory manner on a 2-sided 5% significance level. Statistical analyses were performed using IBM SPSS Statistics 22 (SPSS Inc, Chicago, IL).

RESULTS

Direct Effects of Antibiotic Groups on Commensal Bacteria and Diversity

Based on the systemic activity of Ciprofloxacin/Metronidazole, we classified our cohort in relation to antibiotic treatment into 3 different subgroups, as mentioned above. Again, 3-IS concentrations between day -2 and day 10 were higher in the Rifaximin group without systemic antibiotics (23.4 ± 14.7, 7.2–59.4 µmol/mmol creatinine [crea]) compared to Ciprofloxacin/Metronidazole with/without systemic antibiotics (7.5 ± 9.0, 0–40.2 µmol/mmol crea, $P < .001$) and Rifaximin followed by systemic antibiotics (13.2 ± 16.3, 0–101.9 µmol/mmol crea, $P = .005$). More specifically, we found a higher abundance of CCXIVa in the Rifaximin group without systemic antibiotics compared to the Ciprofloxacin/Metronidazole with/without systemic antibiotics ($P = .004$), whereas there was only a trend compared to the Rifaximin group followed by systemic antibiotic treatment ($P = .06$; Figure 1). Similarly, the Shannon index again was higher in the Rifaximin group without systemic antibiotics than in the Ciprofloxacin/Metronidazole group with/without systemic broad-spectrum antibiotics ($P = .01$), whereas no difference was found between the Rifaximin groups with and without systemic antibiotics (ns; Figure 2). Enterococcal load in the Rifaximin group without systemic antibiotics was lower but, due to the high variability, was not statistically significant compared to the other 2 antibiotic groups (ns; Figure 3). No differences in any microbiome markers were found in the pre-transplant situation between the 3 different antibiotic groups (ns). Detailed information of the subgroups is provided in Supplementary Table 1.

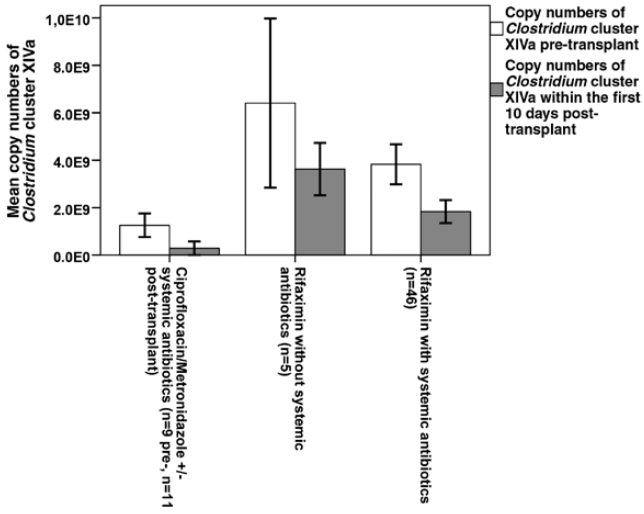

Figure 1. *Clostridium* cluster XIVa (CCXIVa) copy numbers in relation to antibiotic treatment within the first 10 days post-transplant, compared to the pre-transplant situation. CCXIVa copy numbers were significantly higher in the Rifaximin group without systemic antibiotics, compared to the Ciprofloxacin/Metronidazole with/without systemic antibiotics (P = .004), whereas there was only a trend without significance when compared to the Rifaximin group with systemic antibiotic treatment (P = .06). No differences in CCXIVa copy numbers were observed in the pre-transplant situation (P = ns, Mann-Whitney U-test).

Rifaximin Prophylaxis Followed by Systemic Antibiotics Preserves Microbiome Diversity Compared to Prophylaxis With Ciprofloxacin/Metronidazole and Correlates With Superior Outcome

Comparing the Rifaximin group followed by systemic broad-spectrum antibiotics with all other types of systemic antibiotics without Rifaximin, Rifaximin seemed to maintain a microbiome protective effect despite the use of additional broad-spectrum antibiotics. This resulted in higher 3-IS concentrations (13.2 ± 16.3, 0–101.9 µmol/mmol crea to 7.5 ± 9.0, 0–40.2 µmol/mmol crea; P = .04), higher CCXIVa levels ($2.0 \times 10^9 \pm 3.2 \times 10^9$, $0–1.3 \times 10^{10}$ to $1.9 \times 10^8 \pm 5.0 \times 10^8$, $0–1.4 \times 10^9$; P = .01), and higher Shannon indexes (2.2 ± 0.9, 0.2–3.8 to 1.2 ± 1.0, 0.1–3.4, P = .01) for Rifaximin followed by broad-spectrum antibiotics. The enterococcal load was lower in the Rifaximin followed by systemic antibiotics group ($2.8 \times 10^9 \pm 5.2 \times 10^9$, $0–3.2 \times 10^{10}$) compared to all other types of antibiotic treatment without Rifaximin ($7.7 \times 10^9 \pm 9.6 \times 10^9$, $4.4 \times 10^5–2.8 \times 10^{10}$), but the difference did not reach statistical significance (ns). The protective effect of Rifaximin was still observed when a direct comparison of Rifaximin combined with 1 broad-spectrum antibiotic with Ciprofloxacin/Metronodiazole alone was performed (data not shown). The overall duration of additional systemic antibiotic treatments did not differ between the Rifaximin plus systemic antibiotic group (19.8 ± 9.1, 2–40 d) and the Ciprofloxacin/Metronodiazole with/without systemic antibiotic group (16.3 ± 10.4, 0–40 d; ns)

Similarly, Rifaximin followed by additional systemic antibiotics was associated with differences in clinical outcome variables, such as GI GvHD, TRM, and OS, compared to all other types of systemic antibiotic treatment. This resulted in a lower rate of severe GI GvHD (P = .05), lower TRM (P < .001), and higher OS (P = .001) in the Rifaximin plus systemic antibiotic group compared to other types of systemic antibiotic treatments. No differences between the 2 groups were observed for fevers of unknown origin or bacteremia (ns; Table 2). A GI infection with *Clostridium difficile* was observed in only 1 patient of the study

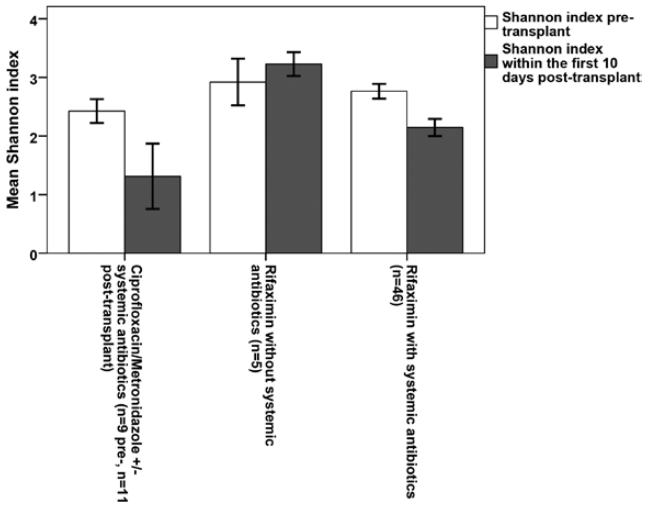

Figure 2. Shannon diversity index in the 3 different antibiotic groups. The post-transplant Shannon index was higher in the Rifaximin group without systemic antibiotics than in the Ciprofloxacin/Metronidazole group with/without systemic broad-spectrum antibiotics (P = .01). No difference was found between the Rifaximin groups with and without systemic antibiotics. No differences in Shannon diversity index were observed in the pre-transplant situation (Mann-Whitney U-test).

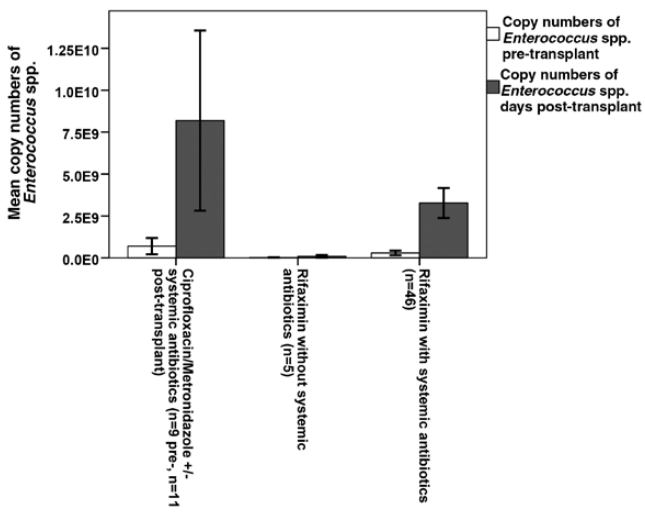

Figure 3. *Enterococcus* species 16S-RNA gene copy numbers in relation to antibiotic treatment within the first 10 days post-transplant compared to the pre-transplant situation. The enterococcal load in the Rifaximin group without systemic antibiotics was lower but, due to the high variability, not statistically significant compared to the other 2 antibiotic groups. No differences in enterococcal load were observed in the pre-transplant situation (Mann-Whitney U-test).

Table 2. Outcome of patients after allogeneic stem cell transplantation is associated with type of prophylactic antibiotics.

N = 147	Neutropenic fever	Bacteremia	GI GvHD (II-IV)	TRM	OS
Rifaximin followed by systemic antibiotics	(32/92) 34.8%	(24/92) 26.1%	(13/92) 14.1%	(12/92) 13.0%	(64/92) 69.6%
Ciprofloxacin/Metronidazole +/- systemic antibiotics	(19/55) 34.5%	(15/55) 27.3%	(15/55) 27.3%	(23/55) 41.8%	(23/55) 41.8%
P-value	.89	.96	.05	<.001	.001

Rifaximin, followed by additional systemic antibiotic treatment, correlated with a lower rate of severe GI GvHD ($P = .05$), lower TRM ($P < .001$) and higher OS ($P = .001$) compared to other types of systemic antibiotic treatment (chi-squared test), whereas no difference was observed for fevers of unknown origin and bacteremia.

Abbreviations: GI, gastrointestinal; GvHD, graft versus host disease; II-IV, severe stages of GvHD; OS, overall survival; TRM, transplant-related mortality.

cohort. This patient received Rifaximin for prophylaxis but also received multiple additional systemic antibiotic treatments.

Impact of Individual Antibiotic Treatment Strategies on Intestinal Microbiome Diversity

Analyzing the type of antibiotic treatment within the first 10 days after ASCT, we observed significantly lower 3-IS levels for different antibiotic groups, including Ciprofloxacin/Metronidazole, Piperacillin/Tazobactam, Meropenem/Vancomycin, and Ceftazidime or multiple systemic antibiotics ($P \leq .03$), except for in those patients receiving intravenous Vancomycin alone (ns) or mono-antibiosis with Rifaximin. In contrast, no statistically significant differences in 3-IS levels were measured between any antibiotic groups in the pre-transplant situation (ns; Figure 4). The extent of suppression of 3-IS levels between day -2 and 10 showed no major differences between the individual groups of antibiotics (Ciprofloxacin/Metronidazole, Piperacillin/Tazobactam, Meropenem/Vancomycin, and Ceftazidime or multiple systemic antibiotics), suggesting a similar suppression of commensal bacteria even for the group receiving Ciprofloxacin/Metronidazole alone. This is also reflected in a comparison of microbiota profiles before transplantation with bacterial distributions from patients receiving Rifaximin only or patients receiving Ciprofloxacin/Metronidazole and/or other broad- spectrum antibiotics (Figure 5). The latter group is distinguished by an overall reduction of bacterial richness, which is related to the relative predominance of various bacterial genera, such as *Bacteroides, Enterococcus, Faecalibacterium*, or *Peptoniphilus*.

DISCUSSION

Intestinal microbiota diversity plays a key role in the pathophysiology of acute GI GvHD and, therefore, significantly influences TRM of patients after ASCT [12, 13]. Commensal bacteria like *Clostridium* cluster XIVa species seem to have beneficial effects

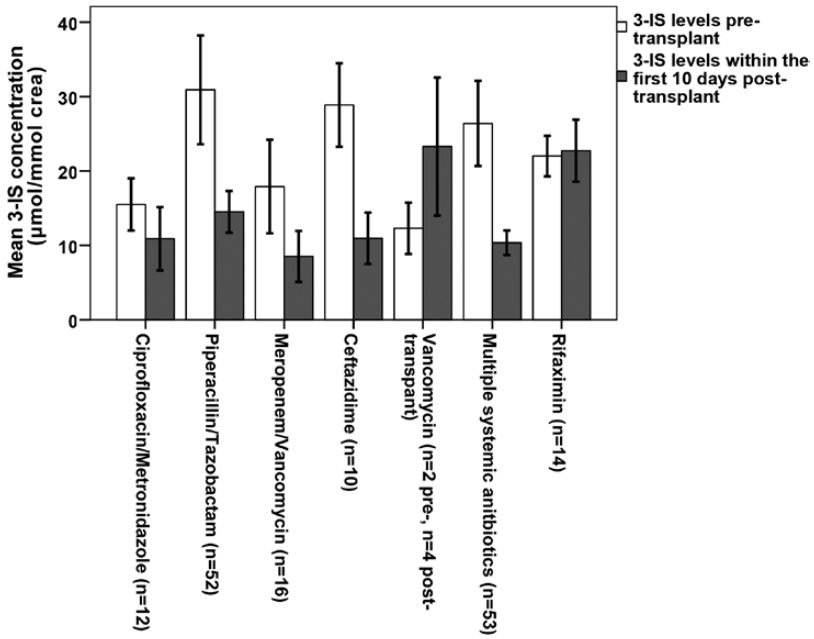

Figure 4. Course of 3-indoxyl sulfate (3-IS) levels in relation to the type of systemic antibiotic treatment. 3-IS levels were significantly lower for all antibiotic groups ($P \leq .03$) within the first 10 days after allogeneic stem cell transplantation, except for the Vancomycin group compared to single administration of Rifaximin. No differences in 3-IS levels were observed in the pre-transplant situation (Mann-Whitney U-test).

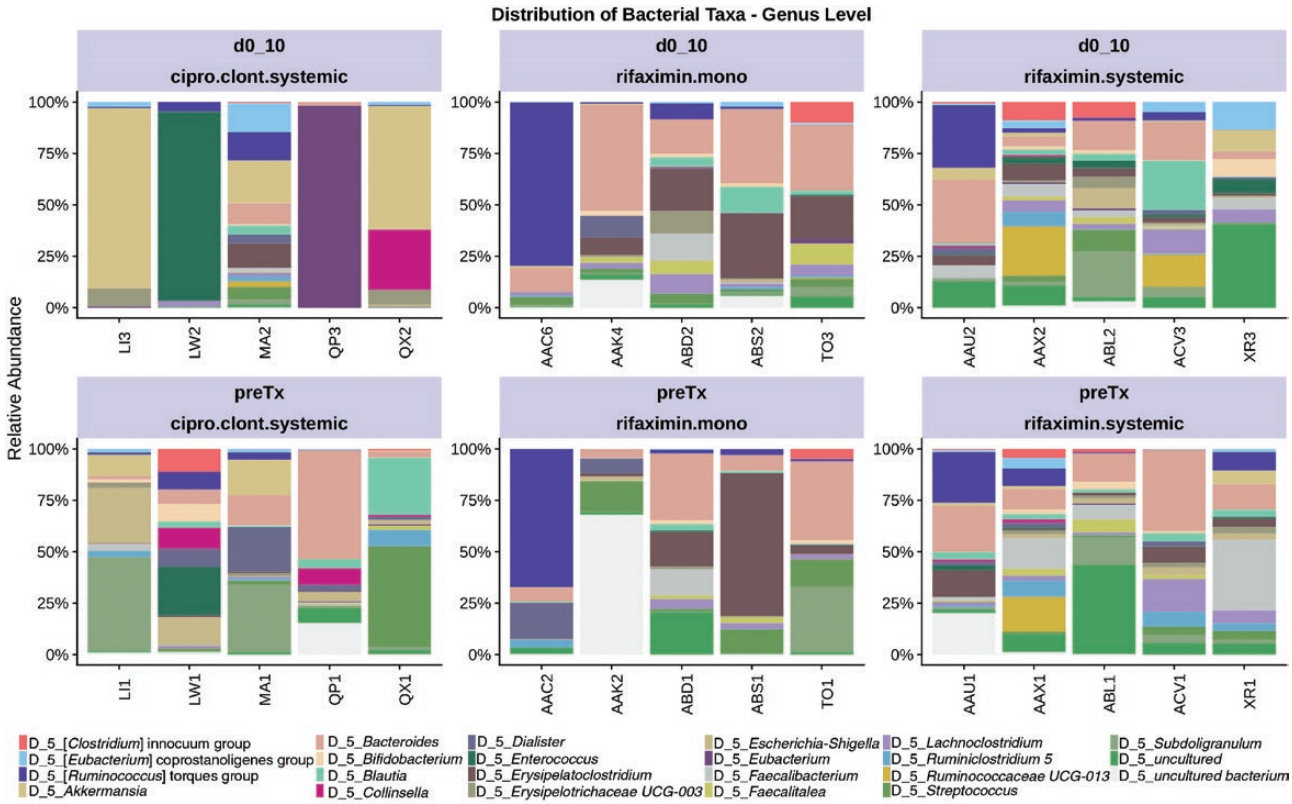

Figure 5. Barplots of bacterial genus distribution in relation to type of antibiotic treatment within the first 10 days post-transplant in representatively selected samples. Rifaximin preserved intestinal microbiota diversity, which was comparable to the pre-transplant situation. On the contrary, treatment with Ciprofloxacin/Metronidazole with/without systemic antibiotics led to a loss of richness. Bacterial profiles were mainly dominated by the genera *Akkermansia* (81%: AAV3 and 51%: QX2), *Eubacterium* (98%: QP3), or *Enterococcus* (90%: LW2). Only genera above 1% abundance in at least 1 sample are depicted.

by maintaining intestinal homeostasis and epithelial integrity and by inducing regulatory T-cells [26]. In contrast, loss of these protective bacteria, as shown for the genus *Blautia*, has been associated with increased GvHD-related mortality [12]. Similar results were found by our group, as we identified low levels of urinary 3-IS, a tryptophan metabolite of colonic commensal bacteria, early after ASCT as a predictor of poor outcomes post-transplant [17]. In the setting of ASCT, the composition of intestinal microbiota is influenced by several factors, like conditioning-related epithelial damage, changes of alimentary habits, and parenteral nutrition [12]. However, a major risk factor causing intestinal dysbiosis is the use of systemic antibiotics for the prophylaxis and therapy of neutropenic infections [27–29]. Even in the non-ASCT setting, it has been shown that antibiotic administration in general reduces gut microbiome diversity: Iizumi et al. reviewed many reports indicating associations between classes, dosages, and periods of exposure of antibiotics, alterations of the gut microbiome composition, and the occurrence of autoimmune diseases like obesity and allergic asthma in children [30].

The increasing understanding of the interactions between antibiotics, bacterial dysbiosis, the pathogenesis of acute GI GvHD, and poor outcomes post-transplant has contributed to providers reconsidering antibiotic standards in the ASCT setting [31]. Since van Bekkum et al. [32] and Beelen et al. [33] demonstrated in mice and in clinical studies that intestinal bacteria play an important role for the development and severity of GI GvHD, strategies of total or selective gut decontamination became the clinical standards for years. However, the development of new, culture-independent techniques like metagenomic sequencing have allowed deeper insights into the complex and dynamic network of intestinal microbiota and have identified the bacteria and metabolites associated with protective effects on gut homeostasis [34]. Shono and colleagues [9] analyzed the impact of different kinds of broad-spectrum antibiotics for the treatment of neutropenic fever on GvHD-related mortality. The use of Imipenem/Cilastatin and Piperacillin/Tazobactam was associated with severe perturbation of gut microbial compositions and an increased GvHD-related mortality rate. In contrast, the administration of Aztreonam or Cefepime, antibiotics with a limited spectrum of activity against anaerobes like *Clostridiales*, was not associated with GvHD-related mortality. This led to their hypothesis that selective antibiotics that offer protection to

commensal bacteria might have beneficial effects on the outcomes of patients after ASCT [9].

In the current retrospective analysis, we aimed to evaluate the different types of antibiotics used for the prophylaxis and therapy of neutropenic infections regarding their impact on microbiome diversity and their protective effects on commensal bacteria within the first 10 days after ASCT. The effects of Ciprofloxacin/Metronidazole, Piperacillin/Tazobactam, and Meropenem/Vancomycin on intestinal microbiota diversity and *Clostridial* abundance were comparable. In addition, treatment with 1 of these antibiotics had a similar impact on intestinal microbiota composition compared with the concurrent administration of several broad-spectrum antibiotics. Even monotherapy with Ceftazidime showed no beneficial effect on intestinal microbiome diversity, although Ceftazidime belongs to the same antibiotic group of cephalosporins as Cefepime, which was reported by Shono et al. to exert protective effects on microbiome composition [9].

However, compared to all other types of antibiotics, Rifaximin mono-antibiosis was able to preserve intestinal microbiome heterogeneity, as demonstrated by several intestinal microbiome markers in this analysis. Rifaximin seemed to maintain a protective effect even in the presence of additional broad-spectrum antibiotics. The clinical relevance of these results was demonstrated by lower TRM and higher OS during Rifaximin prophylaxis. Fevers of unknown origin or infectious complications were not increased in the Rifaximin group compared to Ciprofloxacin/Metronidazole. This is in line with our previous study [11] showing that gut decontamination with Rifaximin resulted in lower positivity for *Enterococcus faecalis* and *faecium* than in patients with Ciprofloxacin/Metronidazole. Similarly, higher 3-IS concentrations were measured for patients treated with Rifaximin compared to Ciprofloxacin/Metronidazole. However, only 1 microbiome marker was available in this previous study [11]. Furthermore, 3-IS as a marker of microbiome diversity might not be as robust as traditional fecal parameters. Therefore, in the current study, we expanded the analysis towards a variety of different parameters, which indicate a higher microbiome diversity by the use of Rifaximin.

Rifaximin is a non-absorbable derivative of Rifamycin with so-called eubiotic effects [35]. Rifaximin is able to induce remission in patients with active Crohn's disease and ulcerative colitis, most probably by a modulation of the intestinal microbiome. Whereas Rifaximin doesn't affect the overall gut microbiota composition, it has been demonstrated to enhance the concentration of beneficial bacteria like *Bifidobacterium, Atopobium*, and *Faecalibacterium prausnitzii* [35, 36]. Furthermore, Rifaximin alters bacterial virulence, reduces bacterial adherence to gut mucosa and internalization, and downregulates intestinal inflammatory activity by inducing the pregnane X receptor – nuclear factor 'kappa-light-chain-enhancer' of activated B-cells pathway [35–37]. Whether 1 of these mechanisms contributed to the suggested reduction of microbiota damage in patients receiving Rifaximin and systemic antibiotics in our study is currently unclear and needs further investigation.

Consequently, not only new strategies of gut decontamination, but even no antibiotic prophylaxis is currently considered to be protective toward commensal bacteria. In 2017, Routy and colleagues first analyzed the impact of antibiotic gut decontamination vs no antibiotic prophylaxis on the frequency of severe acute GI GvHD and mortality in 500 patients undergoing ASCT [38]. In the group of patients receiving antibiotics for prophylaxis, the rate of severe, grade II-IV acute GI GvHD was higher (odds ratio = 1.8) and OS was decreased (hazard ratio = 1.6) compared to patients without antibiotics [38].

These new insights into the impact of systemic antibiotics on the pathogenesis of acute GI GvHD and the outcomes of patients after ASCT provide a new aspect to the topic of antibiotic stewardship that is intended to improve the quality of antibiotic therapy for optimizing clinical treatment results and for reducing toxicity and bacterial resistance. A further aspect might result in selecting the most appropriate antibiotics, considering the protection of commensal bacteria and their anti-inflammatory effects, as recommended by Shono et al. [9]. Particularly in the ASCT setting, these associations bring us into conflict: broad-spectrum antibiotics are indispensable to treat neutropenic infections and to save lives, but the use of broad-spectrum antibiotics correlates with the occurrence of severe GI GvHD and affects mortality rates in a detrimental way. Therefore, strategies are required to protect beneficial microbiota: for example, by the use of selective antibiotics with clostridial sparing effects, prebiotics, which stimulate the growth and function of specific gut microbiota, as well as postbiotics. Another possibility is the reconstitution of intestinal microbiota by a fecal microbiota transfer after treatment with systemic antibiotics [39]. The first promising results were reported by Kakihana et al. using fecal microbiota transfers for the treatment of patients with steroid-resistant GI GvHD [40]. The protection of balanced microbiomes becomes more and more important, not only in the setting of ASCT, but also in anti-cancer therapy of other hemato-oncologic diseases, since it has been shown that anti-tumoral immune responses seem to rely on gut microbiota [41].

In summary, the identification of the complex interactions between the intestinal microbiome and acute GI GvHD is of great importance and is required for finding new possibilities to modulate microbiome composition during the course of ASCT. The choice of antibiotics might be a step in this direction.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. D. Weber and E. H. were involved in the conception and design of the study, and D. Wolff and J. H. were responsible for the collection of specimens and of clinical patient data. A. H. and A. G. supported the manuscript by microbiological assessments. K. D. were responsible for the 3-indoxyl sulfate analysis. M. W. contributed to the statistical data analysis. D. Weber and E. H. collected and analyzed the clinical data and wrote the manuscript. W. H., J. H. and D. Wolff were involved in the interpretation and discussion of study results according to the current literature. All authors read, revised, and approved the final draft.

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Potential conflicts of interest. D. Wolff receives personal fees from Novartis, Neovii, and Mallinckrodt outside the submitted work. J. H. reports personal fees from MSD Sharp and Dohme GmbH outside the submitted work. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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