Effects of Pre-transplantation Antibiotic Use on Clinical Outcomes in HSCT Patients

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Abstract

Hematopoietic stem cell transplantation (HSCT) recipients receive pre-transplantation treatment regimens varying in conditioning intensity and antibiotic administration. Broad-spectrum, anti-anaerobic antibiotics have the potential to disrupt the normal gut microbiome balance and reduce immune function. We aimed to retrospectively examine the impact of pre-transplantation conditioning and antibiotics on rates of mortality, Clostridium difficile infection (CDI), and graft versus host disease (GvHD) in 59 adults receiving HSCT at Copenhagen University Hospital, Rigshospitalet, between June 18th 2015 and March 9th 2017. Patients had received either non-myeloablative (mini) or myeloablative (MAC) conditioning prior to HSCT. To assess antibiotic use, patients were classified into two groups: those who received high risk antibiotics within 6 months prior to transplantation and those who did not. At transplantation, MAC HSCT patients had a younger median age of 50 compared to a median age of 63 in mini HSCT patients (p-value=0.001). MAC HSCT patients more often received pre-transplantation radiation (p-value<0.0001) and high risk antibiotics (p-value=0.0073) compared to mini HSCT patients. A total of 6 mini HSCT patients (21%) died compared to 2 MAC HSCT patients (7%). However, differences in mortality were not statistically significant (IRR 0.24, p-value=0.097, 95% CI 0.04-1.3). A total of 7 patients (18%) that received high risk antibiotics died compared to 1 patient (5%) in the control group but these differences also were not statistically significant (IRR 4.67, p-value=0.167, 95% CI 0.52-41.8). CDI and GvHD rates did not differ significantly between groups. Overall, there was no evidence that pre-transplantation conditioning and antibiotics could predict clinical outcomes. Treatment with high risk antibiotics may be a risk factor for adverse clinical outcomes, but a larger sample size is necessary to determine whether this trend is significant.

Introduction

The human gut microbiome is populated by a complex ecosystem of primarily anaerobic microbial bacteria that symbiotically influence host immune response, endocrine function, digestion, and protection against pathogen growth.[1,2] The role of the gut microbiome in immune function has been of interest to researchers studying hematopoietic stem cell transplantation

(HSCT), a potential cure for many hematologic disorders.[3] Antibiotics are routinely used in immunocompromised transplant patients but can upset the microbiome balance and lead to the overgrowth of pathogens such as *Clostridium difficile*, causing infection.[1,4] Another complication of HSCT is graft versus host disease (GvHD), which is inflammation and damage to host tissues due to activation of donor T cells.[5] Murine studies have revealed that GvHD is significantly associated with a loss of microbiome diversity and expansion of oxygen-tolerant species at the expense of obligate anaerobes.[5] Since the microflora normally mitigates the severity of intestinal inflammation, broad-spectrum antibiotic exposure may increase risk of GvHD.[5] Further research has demonstrated that increased gut microbiome diversity may be an important predictor of positive clinical outcomes.[6] Therefore, the use of antibiotics with broad-spectrum activity against obligate anaerobes may be detrimental due to the reduction of microbiome diversity, elimination of commensal bacteria associated with gut health and survival, and overgrowth of pathogenic bacteria. In previous studies, the use of broad-spectrum antibiotics with the goal of decontaminating the gut had been thought to prevent GvHD and death.[3] However, as more advanced sequencing methods have developed, several studies have revealed that broad-spectrum antibiotics are associated with a loss of microbiome diversity which was shown to be associated with severe GvHD, infection, and death.[6,7] In this study, we retrospectively analyzed the clinical outcomes of patients that received different pre-transplantation conditioning regimens and antibiotics. We hypothesized that broad-spectrum, anti-anaerobic antibiotic treatment may lead to unfavorable clinical outcomes of mortality, CDI, and GvHD as a consequence of gut microbiome dysbiosis. This research could lead to possibilities of increased specificity of pre-transplantation treatment for HSCT patients and more favorable transplantation outcomes.

Methods

Research Subjects

A retrospective study was conducted on 59 adults who had received allogenic HSCT at Copenhagen University Hospital, Rigshospitalet, between June 18th 2015 and March 9th 2017 and donated a stool sample for microbiome analysis. Subjects were excluded from the study if they had less than 5 months follow up, only had antibiotics recorded post-treatment, or if the treatment date occurred after the study end date. No exclusion was due to an early death date. Patients received either myeloablative (MAC) or non-myeloablative (mini) pre-transplantation

conditioning. MAC HSCT patients received higher intensity pre-transplantation conditioning, consisting of more chemotherapy and anti-thymocyte globulin (ATG), while mini HSCT patients received reduced intensity conditioning.

Antibiotic Administration

Patients received various combinations of 36 different antibiotics, which were recorded within 6 months before and one year after transplantation. Only antibiotics that were administered within 6 months before transplantation were analyzed. When no end date was recorded, the transplantation date was used (5 out of 573 of the antibiotic entries). There was no standard antibiotic regimen administered. Many patients received prophylaxis consisting of sulfamethoxazole and trimethoprim, valaciclovir, fluconazole, or ceftazidime. However, there was considerable variability in antibiotics administered to patients. Rigshospitalet does not administer antibiotics with the purpose to decontaminate the gut.

Antibiotic Groups

The antibiotics were grouped into high risk and low risk groups as depicted in Table 1. The high risk antibiotic group consisted of 10 different antibiotics that have greater broad-spectrum and anti-anaerobic activity, due to evidence that resident anaerobic bacteria promote gut health and limit pathogenic growth. The high risk group consisted of β-lactams with β-lactamase inhibitors, carbapenems, 2nd and 3rd generation cephalosporins, lincosamides, nitroimidazoles, and 4th generation fluoroquinolones. The 59 patients were subdivided into 2 categories based on whether they had received any high risk antibiotics (n=20) or had not received any high risk antibiotics (n=39) within 6 months before HSCT. All patients received low risk antibiotics.

High risk antibiotics	Amoxicillin with clavulanic acid, Ceftazidime, Ceftriaxone, Cefuroxime, Clindamycin, Ertapenem, Meropenem, Metronidazole, Moxifloxacin, Piperacillin with Tazobactam
Low risk antibiotics	Amoxicillin, Ampicillin, Azithromycin, Benzylpenicillin, Ciprofloxacin, Clarithromycin, Colistimethatnatrium, Colistin, Dapsone, Daptomycin, Dicloxacillin, Flucloxacillin, Fucidin, Gentamicin, Linezolid, Lymecycline, Mecillinam, Nitrofurantoin, Phenoxymethylpenicillin, Pivmecillinam, Prevenar 13, Roxithromycin, Sulfamethoxazole, Trimethoprim, Tobramycin, Vancomycin

Table 1. Classification of antibiotic groups based on the target spectrum and anti-anaerobic activity.

Statistical Analysis

We examined the ability of high-risk antibiotic administration and pre-treatment conditioning intensity to predict clinical outcomes. Analysis began at the time of transplantation and continued for 5 months or until death or first incidence of CDI infection. There were no recorded dates for GvHD onset, so this could not be utilized as an early end of study date. Relative risks were estimated by Poisson regression analyses, adjusted for gender and age at transplant. Incidence rates (per 1000 patient years) and incidence rate ratios were calculated for death, GvHD, and first post-transplantation CDI. Other clinical variables that were examined as predictors of clinical outcomes were the number of days on low risk antibiotics, the number of days on high risk antibiotics, and whether radiation was administered before transplant. Days of antibiotics were counted per individual medication treatment (i.e. overlapping treatment days were counted twice). A t-test or chi-test was performed to determine whether high-risk antibiotic administration, low risk antibiotic administration, median days on high or low risk antibiotics, radiation, age, and gender differed between MAC HSCT and mini HSCT patients.

Results

Clinical characteristics of the 59 allogenic HSCT recipients are listed by conditioning group in Table 2. All subjects had at least 5 months follow-up and were administered antibiotics within 6 months prior to transplantation. We found that MAC HSCT and mini HSCT recipients differed significantly in several clinical characteristics. MAC HSCT patients were younger than mini HSCT patients at the time of transplant, with the MAC HSCT group having a median age of 50 (IQR 39-59), while the mini HSCT group had a median age of 63 (IQR 60-67) (p-value=0.001). The two groups also differed in whether radiation was administered prior to transplantation, which occurred in 100% of the mini HSCT patients but only 41% of the MAC HSCT patients (p-value<0.0001). Additionally, high risk antibiotics were administered in 83% of MAC HSCT patients compared to 50% of mini HSCT patients (p-value=0.0073). There were no differences between the groups in gender or median days on high or low risk antibiotics. All MAC HSCT and mini HSCT patients received low risk antibiotics within 6 months prior to transplantation. There were no statistically significant differences between MAC and mini HSCT patient groups in the number of patients that developed GvHD (p-value=0.176), post-transplantation CDI (p-value=0.148), or died (p-value=0.142).

Table 2				
Clinical Characteristics of HSCT Patients				
Characteristics	Mini	MAC	Total	p value
	N=30	N=29	N=59	
Pre-transplant characteristics				
Median age (IQR) years	63 (60-67)	50 (36-59)	60 (46-64)	0.001
Male gender (%)	14 (47)	18 (62)	32 (54)	0.235
Received radiation (%)	30 (100)	12 (41)	42 (71)	<0.0001
Received high-risk AB (%)*	15 (50)	24 (83)	39 (66)	0.0073
Median days on high-risk AB (IQR)	0.5 (0-21)	7 (1-23)	3 (0-23)	0.452
Median days on low-risk AB (IQR)	22 (10-141)	28 (13-173)	28 (11-151)	0.226
Clinical outcomes				
GvHD (%)	11 (37)	6 (21)	17 (29)	0.176
CDI post-transplantation (%)	3 (10)	7 (24)	10 (17)	0.148
Death (%)	6 (20)	2 (7)	8 (14)	0.142
(*All patients received low-risk AB)				

Table 2. Pre-transplant characteristics and clinical outcomes of subjects grouped by pre-transplantation conditioning.

Incidence rates per 1000 patient years and incidence rate ratios for death, first post-transplantation CDI, and GvHD are shown by patient group in Table 3. Incidence rate ratios were adjusted for age at time of transplant and gender. Out of all 59 patients, 8 patients died (14%), 10 developed post-transplantation CDI (17%), and 17 developed GvHD (29%). Between the MAC HSCT and mini HSCT patients, there were no statistically significant differences in rates of death, CDI, or GvHD. A total of 6 mini HSCT patients (21%) died compared to 2 MAC HSCT patients (7%), but differences in mortality were not statistically significant (IRR 0.24, p-value=0.097, 95% CI 0.04-1.3). CDI developed in 7 MAC HSCT patients (23%) compared to 3 mini HSCT patients (10%), but MAC HSCT patients were not significantly associated with a higher likelihood to develop CDI (p-value=0.405). GvHD developed in 11 mini patients (38%) compared to 6 MAC patients (20%), but this difference was not statistically significant (p-value=0.506).

Effects of antibiotic administration of clinical outcomes were also analyzed. The administration of high risk antibiotics within 6 months before treatment did not significantly impact rates of death, CDI, or GvHD. A total of 7 patients (18%) that received high risk antibiotics died compared to 1 patient (5%) in the control group, but these differences were not statistically

significant (IRR 4.67, p-value=0.167, 95% CI 0.52-41.8). There were similar percentages of CDI incidences in patients who had received high risk antibiotics (18%) and those that had not (15%), and neither group was associated with greater rates of CDI (p-value=0.554). GvHD rates also did not differ between the high risk antibiotic group and the control group (IRR 1.13, p-value=0.825, 95% CI 0.38-3.39). Likewise, the total number of days of treatment with high risk antibiotics within 6 months before transplantation did not affect CDI (p-value=0.475), death (p-value=0.0118), or GvHD (p-value=0.741).

Additionally, all patients received low risk antibiotics within 6 months of transplantation, ranging from 5 to 615 days of treatment with a median of 28 days. The total number of days on low risk antibiotics did not affect CDI (p-value=0.287), death (p-value=0.910), or GvHD (p-value=0.611). A total of 42 patients (72%) received radiation prior to transplantation. Receiving radiation was not associated with increased rates of CDI (IRR 0.475, p-value=0.261, 95% CI 0.13-1.74), death (IRR 2.80, p-value=0.340, 95% CI 0.34-23.2), or GvHD (IRR 1.22, p-value=0.730, 95% CI 0.39-3.79), when adjusted for age and gender.

Table 3

Incidence rates and incidence rate ratios of death, first post-transplantation CDI, and GvHD for HSCT patients

	Death		CDI		GvHD	
	IR/1000 PY	IRR	IR/1000 PY	IRR	IR/1000 PY	IRR
	(n death/ n PY)	(95% CI.)	(n CDI/ n PY)	(95% CI.)	(n GvHD/ n PY)	(95% CI.)
Treatment group						
Mini	504 (6/29)	Ref. group	252 (3/29)	Ref. group	925 (11/29)	Ref. group
MAC	203 (2/30)	0.24 (0.04-1.3, p=0.097)	712 (7/30)	1.85 (0.48-7.9, p=0.405)	611 (6/30)	0.69 (0.23-2.07, p=0.506)
Received high risk AB						
No	131 (1/20)	Ref. group	393 (3/20)	Ref. group	786 (6/20)	Ref. group
Yes	497 (7/39)	4.67 (0.52-41.8, p=0.167)	497 (7/39)	0.60 (0.11-3.3, p=0.554)	781 (11/39)	1.13 (0.38-3.39, p=0.825)
Total	368 (8/59)		461 (10/59)		487 (17/59)	

Table 3. Incidence rates and incidence rate ratios of death, first post-transplantation CDI, and GvHD for HSCT patients, adjusted for gender and age at transplant.

Discussion

In several studies prior to the development of advanced sequencing, the use of broad-spectrum antibiotics with the goal of decontaminating the gut had been thought to prevent GvHD and death. In contrast, more recent studies have revealed that dysbiosis and reduced diversity of the gut microbiome is associated with severe GvHD, infection, and death. Antibiotics are routinely administered in immunocompromised HSCT patients and they have a significant impact on the gut microbiome. In this study, we aimed to more specifically analyze whether the effects of antibiotics on clinical outcomes could be differentiated based on the target-spectrum of the antibiotics administered.

The patients in this study received one of two different pre-transplantation conditioning regimens based on the appropriate level of conditioning intensity. MAC HSCT patients had received high intensity pre-transplantation conditioning while mini HSCT patients had received reduced intensity conditioning. We determined that there were several significantly different clinical characteristics between these groups. MAC HSCT patients were younger at time of transplantation, which was expected since younger patients are often more suitable to receive intense conditioning. MAC HSCT patients also less often received radiation prior to transplantation, likely because patients that receive increased chemotherapy do not always require additional radiation to destroy malignant cells. Interestingly, MAC HSCT patients were much more likely to receive high-risk antibiotics. Due to the harsher chemotherapy regimen prior to transplantation, MAC HSCT patients are at higher risk of infection or febrile neutropenia which may cause them to receive more broad-spectrum antibiotics. Additionally, MAC HSCT patients are generally younger and therefore potentially more able to tolerate the side effects associated with broad-spectrum antibiotics. The clinical outcomes between MAC HSCT and mini HSCT patients were not significantly different which was likely due to the small sample size of 59 patients.

We also analyzed patient outcomes based on whether they were administered high risk antibiotics within 6 months prior to transplantation. We divided the 36 antibiotics into a high risk and low risk group based on their target spectrum. Although more patients receiving high risk antibiotics died and developed CDI, these differences were not statistically significant. This is likely due to the relatively small sample size. We would need to collect additional patient records

to determine whether broad-spectrum, anti-anaerobic antibiotics increase rates of CDI and death. This would support similar data collected by Taur et al. that β-lactams and metronidazole were associated with decreased gut microbiome diversity which was then associated with higher rates of death and CDI.[6] Interestingly, there was also no difference between the percentages and incidence rates of GvHD amongst patients who had and had not received high risk antibiotics. We expected more patients who had received high risk antibiotics to develop GvHD based on results by Jenq et al. that found GvHD to be associated with a significant loss of gut microbiome diversity in mice.[5] Similarly Shono et al. found that in 857 allogenic HSCT patients, treatment with piperacillin and tazobactam, one of the antibiotic combinations in our high-risk group, was associated with increased GvHD-related mortality at 5 years.[7] However, it is possible that we would have seen similar trends with a larger patient cohort and longer follow-up time. Overall, the 95% confidence intervals for all rate calculations were relatively large which indicates that the sample size was too small to conclude any strong correlations.

There were several possible confounding factors in this study. There were many factors that determine which antibiotics a patient receives. It is possible that certain patients may have been given different antibiotics based on differences in initial health. Therefore, clinical outcomes may be based somewhat based on the original patient conditions that lead them to be given certain antibiotics, rather than solely the effect of the antibiotics. However, the multivariate analysis accounted for some of these outside variables that may lead certain patients to more often receive certain antibiotics, such as age.

Conclusion/Future Perspectives

Overall, we speculate that the administration of high-risk antibiotics may possibly be associated with increased mortality, CDI, and GvHD, but the sample size in this study was too small to yield any statistically significant support for this hypothesis. These findings suggest that further investigation should be completed with more patients and a longer follow up time to see whether there is stronger evidence for trends in clinical outcomes of HSCT patients with different pre-transplantation conditions. Additionally, other factors could also be added to the baseline patient analysis such as underlying disease, occurrence of pre-transplant CDI, method of antibiotic administration (intravenous or oral), and specific stem cell source. Furthermore, some patients were prescribed antibiotics to take as needed, most often being the low risk antibiotics

ciprofloxacin and phenoxymethylpenicillin. These antibiotics were included in our analysis, but there is no record for how many days the patients actually took them. It could be interesting to examine whether the administration of antibiotics that were prescribed to be taken as needed has any effect on clinical outcomes. Additional investigations that could be conducted include a gut microbiome analysis to examine whether antibiotics administered within one half year before HSCT produce a significant difference in the composition of the gut microbiome at transplantation and whether this is associated with specific clinical outcomes. Another option for further research could be testing different antibiotics as a part of the high risk group, to see whether this creates any difference in the data. Effects of antibiotics could also be analyzed on an individual basis rather than by groupings. HSCT is a potentially lifesaving procedure but patients still face common incidences of infection, GvHD, and death. Once accomplished, these future perspectives could lead to a possibility of more specific pre-transplantation conditioning and antibiotic regimens to lower the risk of unfavorable clinical outcomes.

Limitations

This study had several limitations that may have influenced the data. The original patient database was lacking 5 antibiotic treatment end dates. To address this issue, we input the study end date as the antibiotic end date. This likely did not affect the data since there were only 5 out of 573 of these missing entries, most of which were low risk antibiotics and resembled the average administration length when we entered the artificial end date. Still, it is difficult to estimate whether this had any effect on the results. There were also no recorded dates for GvHD onset, which may have prolonged the study length for some patients since we could not include these dates as early end of study dates. Additionally, the patient records contained some contradictory information due to overlapping inpatient and outpatient records which were resolved by referencing the original database, but there still may have been the possibility of errors in the clinical records. We were also limited by the relatively small sample size which caused high confidence intervals and may have occluded the significance of our results.

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