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Biodiversity screening of gut microbiome during autologous stem cell transplantation as a predictor of bloodstream infections in oncohematological patients

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Summary

Allogeneic HSCT is frequently associated with severe systemic infectious complications. Endogenous infections of intestinal origin are known to play a leading role in their occurence. Despite abundant data confirming the key role of gut microbiome in allogeneic HSCT, less is known about its role in autologous HSCT (ASCT). In this study, we aimed to investigate the characteristics of the gut microbiome that contribute to the development of bloodstream infections (BSI) in oncohematological patients during high-dose chemotherapy and AHSCT. We conducted a study on the microbial diversity of the gut microbiome during AHSCT in 30 patients with multiple myeloma (MM). The protocol involved stool sampling prior to AHSCT and during the post-transplant period. Our study revealed a significant decrease of the bacterial diversity index during AHSCT (p=0.02). The dominance of *Proteobacteria* in the intestinal microbiome proved to be an independent factor in the development of Gram-negative bloodstream infections in the patients. An increased ratio of *Proteobacteria* in the spectrum of gut microbiome over 30% is a reliable predictor of systemic Gram-negative bloodstream infections in patients undergoing high-dose chemotherapy and AHSCT. Therefore, monitoring biodiversity of intestinal microbiome is crucial in both allogeneic and autologous HSCT to identify high-risk groups for developing bloodstream infections. The stool samples for such monitoring should be evaluated both prior to HSCT, and post-transplant.

Keywords

Autologous stem cell transplantation, multiple myeloma, gut microbiota, biodiversity index, bloodstream infections, predictors, oncohematological patients.

Introduction

Successful treatment options for hematological malignancies have significantly expanded over last decades. These advances are associated with the use of targeted therapy and HSCT. However, the use of modern therapy programs is associated with higher risks of infectious complications in oncohematological patients, with systemic infections of the bloodstream being the most dangerous events [1]. Infectious processes exacerbate the severity of the patient's condition sometimes leading to reduced dosage of specific therapy, treatment interruption and, ultimately, to progression of the underlying disease. Previously, severe bacterial infections were considered mostly of exogenous origin. However, some recent studies have shown that endogenous infections of intestinal origin may be primary source for the microbes entering the bloodstream in oncohematological patients.

These conditions are facilitated by immunosuppression and frequent development of mucositis in the patients [2]. Introduction of new methods for identifying microorganisms, such as real-time polymerase chain reaction (RT-PCR) and next-generation sequencing (NGS) of bacterial genome has revolutionized the approach to assessing the intestinal microbiota. Their use has allowed for a more comprehensive assessment of the species composition of the microbiota, which includes more than 1000 species of bacteria, 80% of which are unculturable microorganisms. This information has provided the basis for extensive studies of intestinal microbiota parameters as predictors of the development of bloodstream infections, particularly, in immunosuppressed oncohematological patients. In this study, we aimed to investigate the features of gut microbiome associated with development of bloodstream infections (BSI) in oncohematological patients during high-dose chemotherapy and autologous hematopoietic stem cell transplantation (AHSCT).

Patients and methods

Patients under study

The patient cohort consisted of 30 hospitalized patients with multiple myeloma, aged 48-67 years (median 60 years), who underwent autologous HSCT at the Russian Research Institute of Hematology and Transfusiology in St. Petersburg, Russia, from 2020 to 2022. The basic clinical characteristics of patients included in the study are depicted in Table 1.

Table 1. Clinical baseline characteristics of multiple myeloma patients included in the study

Baseline characteristics	No (%)
Age (years)	60 (48-67)
Gender	
Male	15
Female	15
The pre-transplant conditioning regimen	melphalan at a dose of 200 mg/m^2
Type of transplant	hemapoietic stem cells of blood
Renal injury	
Yes	3
No	27
Primary disease	Multiple myeloma
ISS Durie–Salmon staging	
IA	2
IIA	5
IIB	1
IIIA	18
IIIB	4
Febrile neutropenia	4
Infections	
Sepsis, septic shock	1
Pulmonary complications	0
Urinary tract infections	1

Study protocol

The study protocol included collection and processing of feces obtained from patients before AHSCT and at different times from 7 to 35 days after it. All patients were treated with fluoroquinolones to prevent infections. In case of infectious complications, antibiotic treatment was performed as based on bacteriological and clinical data. The study protocol included collection and deep freezing of stool samples obtained before transplantation and at various time points ranging from D+7 to D+35 post-transplant. The patients taken into the study had at least ≥3 consecutive sequenced samples. After extraction and purification of DNA from each biological sample, PCR amplification of the V5 region of the 16S rRNA gene was performed using modified universal bacterial primers. The purified PCR products were sequenced using the MiSeq Illumina platform according to instructions from manufacturer. Phylogenetic classification from phyla to the species level was performed using the Illumina database. For the rapid identification of pathogens, automatic bacteriological systems (BacT/ALERT 3D) were used in combination with our method based on real-time PCR [3]. Clinical and demographic parameters, i.e., patient's age, sex, underlying disease, conditioning regimen, source of hematopoietic stem cells, duration and choice of antibiotics, were included in the analysis.

Bioinformatic analysis

Primer sequences (region V3-V4) were excluded from analysis using the pre-process 16S program. Trimming of low-quality reads was performed using the Trimmomatic program [4]. Taxonomic classification to the species level was carried out using the Kraken taxonomic classification system with the Kraken standard database [5, 6]. Re-estimation of microbial abundance was conducted using the Bayesian Reestimation of Abundance with Kraken algorithm [7]. The sequences were grouped into operating taxonomic units (OTUs) based on 97% identity. The total number of 16SrRNA genes *per* 1 g of biological material served as a measure of bacterial density being calculated by means of quantitative PCR, based on the total DNA amount extracted from each sample.

Biodiversity indices used in this study included Shannon, Simpson, Chao 1, Simpson's inverse index. Processing and analysis of clinical, laboratory and phylogenetic data was performed using R software (R Development Core Team, Vienna, Austria, version 4.2.1) [8, 9], along with the Phyloseq package (version 1.41.0) [10], Tidyverse (version 1.3.1) [11], rstatix package (version 0.7.0) [12] and ggstatsplot package (0.9.3) [13]. To assess the independent nature of differences in microbiome biodiversity following HSCT, we employed Bayesian nonparametric statistics, including the Dirichlet distribution (Dir (α)) from the HMP package (version 2.0.1) 14. The Bray-Curtis index was used as a measure of compositional dissimilarity between two different sites based on the counts from each site.

Results

We have found a significant decrease in the alpha diversity indexes of intestinal microbiome in patients after AHSCT.

The results of assessing changes in the alpha diversity index of MM patients examined in different periods before and after HSCT are presented in Fig. 1. Moreover, a decrease in the Shannon diversity index was detected up to 7 days after transplantation in 26 out of 30 patients. However, the extent of this decline proved to be variable. Of note, the greatest decrease in the biodiversity index (from 2.527 to 1.17) was found in one case (patient K.).

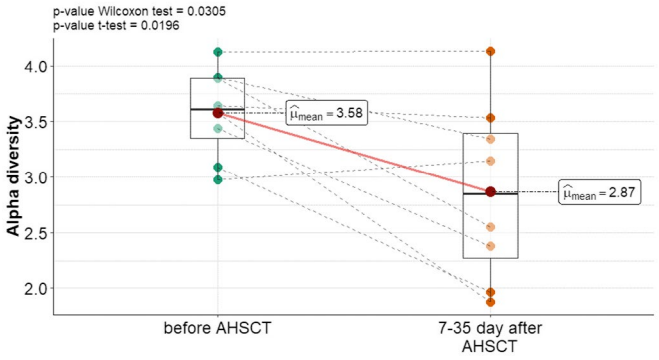

Figure 1. Alpha diversity index of gut microbiome in myeloma patients before and after AHSCT

A significant decrease in the microbiota diversity index after AHSCT was shown, regardless of the stage of multiple myeloma or renal failure (p = 0.0305). One should note that a significant decrease in the microbiota diversity index was established in patients over time following AHSCT, along with negative effect of antibiotics and cytostatic chemotherapy on the diversity of the microbiota. The data of individual patients has demonstrated a decrease in the Shannon index in six cases over the period of 7 to 35 days post-transplant. In three cases, this index did not change, and only in one patient an increase in the diversity index was observed after HSCT.

Furthermore, we studied the dynamics of the composition of the intestinal microbiome at the phylum level. To simplify the analysis of changes in the intestinal microbiome during AHSCT, the most common phyla of bacteria (*Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria*) were chosen. The time changes of intestinal microbiome composition at the level of phyla (Fig. 2) showed that the *Bacteroides* phylum formed the basis of the microbiota in a significant part of the patients. *Firmicutes*, another dominant phylum, was found somewhat less frequently.

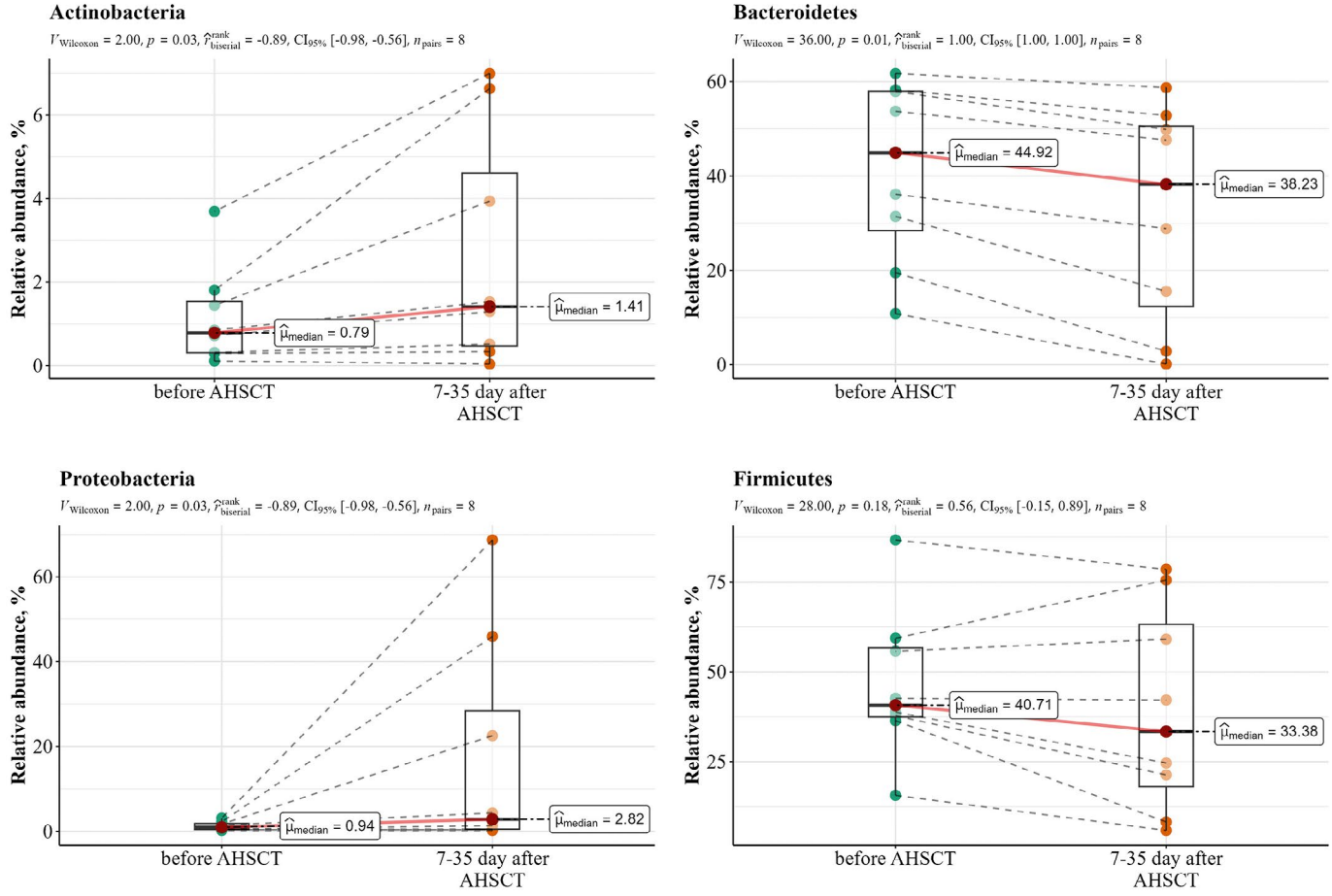

Figure 2. Time changes of intestinal microbiome composition at the level of major bacterial phyla before AHSCT and on day +7 to D+35 post-transplant

In our study, the median frequency of *Proteobacteria* detection in gut microbiota ranged from 1% to 6%, and only in one case (patient K.), the proportion of the *Proteobacteria* type was 38.9%, being detected on the day +2 after transplantation (Fig. 3).

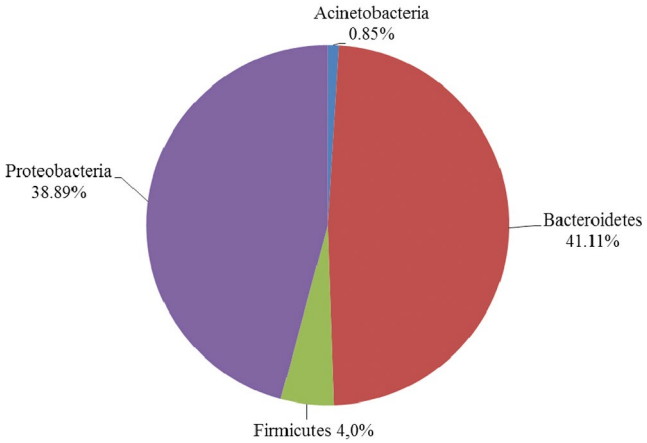

Figure 3. Top phylum classification results (patient K. day +2 after transplantation)

Of note, the post-transplant period in the patient K was complicated by sepsis with the development of infectious-toxic shock. It should be emphasized that no serious clinical manifestations of infectious complications were observed in other patients.

These results, in our opinion, suggest a significant diagnostic value of assessing the microbiota changes. The identification of the dominance of the *Proteobacteria* type made it possible to predict the occurrence of sepsis and prescribe timely antibacterial therapy, which led to the resolution of the infectious process.

Discussion

Bloodstream infections and sepsis are serious life-threatening complications in patients with hematological malignancies during high-dose chemotherapy and HSCT. Routine bacteriological analysis is often not able to predict the risk of its occurrence. It has been shown that the main route of penetration of microorganisms into the bloodstream is endogenous infection originating from intestines [2]. Extensive studies have been conducted to investigate the pathogenesis of bloodstream infections (BSI) during endogenous microbial dissemination. The opportunity of using microbiome changes as a predictor of developing systemic BSI and septicemia was suggested. It is known that endogenous infection from the intestine plays a leading role in the development of Gram-negative bloodstream infections [2]. The composition of intestinal microbiota plays an important role in this process. In addition to decreased microbial biodiversity, HSCT may result into massive replacement of normal microbiome by a single type of bacteria. A decade ago, Taur Y. et al. [15] suggested a phenomenon "domination" (replacement of more than 30% of the relative abundance of the microbiome with one taxonomic type). It was demonstrated that the dominance of the *Proteobacteria* type with more than 30% in gut microbiome is a reliable risk predictor of a developing Gram-negative BSI in allogeneic HSCT [15]. Recently, it was confirmed the possibility of using this criterion (*Proteobacteria* dominance) in clinical practice as a BSI predictor in patients with allogeneic HSCT [16]. Our present results show that this phenomenon is also observed in autologous HSCT.

Conclusions

Our results confirm the view that a significant decrease in the microbiota biological diversity index is observed in AHSCT. It has been demonstrated that endogenous infection originating intestines plays a leading role in the development of Gram-negative bloodstream infections. The composition of the intestinal microbiota plays a crucial role in this process. In addition to a decreased biodiversity during HSCT, an almost complete replacement of gut microbiome by one type of bacteria may be observed. Dominance of the *Proteobacteria* phylum has been shown to be an independent risk factor for the development of Gram-negative bloodstream infections. The possibility of using this phenomenon (dominance of the *Proteobacteria* type above 30%) in clinical practice for the diagnosis of systemic BSI in patients after AHSCT has been demonstrated. The composition of the intestinal microbiome significantly influenced the infections incidence in patients subjected to AHSCT.

A sufficiently increased proportion of *Proteobacteria* in the spectrum of the intestinal microbiome may be a reliable risk predictor of systemic Gram-negative BSI after high-dose therapy and AHSCT. The stool samples should be assessed by NGS both before and after HSCT, when monitoring the gut microbiota composition.

Conflict of interest

No potential conflict of interest is reported.

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Скрининг биоразнообразия микробиома кишечника при трансплантации аутологичных стволовых клеток как предиктор развития инфекций кровотока у онкогематологических больных

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Резюме

Аллогенная ТГСК часто связана с тяжелыми системными инфекционными осложнениями. Известно, что ведущую роль в их развитии играют эндогенные инфекции из кишечника. Несмотря на множество фактов, подтверждающих, что микробиом кишечника играет ключевую роль при аллогенной ТГСК, меньше известно о его роли в контексте аутологичной ТГСК. Мы исследовали 30 пациентов с множественной миеломой, перенесших аутологичную ТГСК (АТГСК). Целью нашей работы было изучение особенностей микробиома кишечника, способствующих развитию инфекций у онкогематологических больных на фоне высокодозной химиотерапии и АТГСК. Протокол включал сбор образцов стула до начала аутологичной ТГСК и в посттрансплантационном периоде. Показано, достоверное (p=0.02) снижение индекса разнообразия микробиоты после проведения аутологичной ТГСК. Доминирование типа *Proteobacteria* в спектре микробиома кишечника является независимым фактором развития грамотрицательных инфекций кровотока у пациентов. Увеличение типа *Proteobacteria* в спектре микробиома кишечника выше уровня 30% является надежным предиктором развития системных грамотрицательных инфекций кровотока у пациентов, получающих высокодозную терапию и АТГСК. Биоразнообразие кишечного микробиома необходимо контролировать как при аллогенной, так и при аутологичной ТГСК для выявления групп высокого риска развития инфекций кровотока. Образцы стула для мониторинга следует оценивать как до ТГСК, так и после нее.

Ключевые слова

Трансплантация аутологичных стволовых клеток, множественная миелома, микробиота кишечника, индекс разнообразия, инфекции кровотока, предикторы, онкогематологические пациенты.