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Spectrum of bronchoalveolar bacterial microbiota following hematopoietic stem cell transplantation: age dependence and microbiota shifts

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Summary

In healthy persons, lung microbiota shows close correlations with microbial landscape of upper respiratory tract. However, pronounced changes of lung microbiota are revealed by analysis of bronchoalveolar lavage (BAL) in severe pneumonias and other pulmonary complications, expecially, in immunocompromised patients. E.g., following intensive cytostatic therapy and hematopoietic stem cell transplantation (HSCT), severe impairement of lung microbiota is observed, due to transient cytopenia, immunocompromised state and massive antibiotic prophylaxis. BAL microbiology shows a number of commensal bacteria including potential pathogens from other infectious sites. Hence, the aim of our study was to evaluate the diversity of aerobic and facultative anaerobic microorganisms in BAL samples from the HSCT patients.

Patients and methods

Our study included 1123 BAL samples from 691 patients subjected to HSCT (1 to 71 years old). The patients were diagnosed, mainly, with myelo- and lymphoproliferative disorders. Myeloablative was carried out in 44% of cases. Stem cells were obtained from bone marrow or peripheral blood (497 *vs* 596 transplants).

The donor types were as follows: related compatible donors (19.2%); related haploidentical donors (21.6%); unrelated compatible donors (49.1%); autologous transplants (10.2%).

Prophylaxis of graft-versus-host disease (GVHD) was mainly performed by the posttransplant cyclophosphamide (PtCy). BAL samples were collected at diagnostic bronchoscopy within D-100 to D+180 post-HSCT, according to appropriate clinical indications. Microbiological cultures and isolation of aerobes and facultative anaerobic bacteria from BAL samples were made by classical bacteriological techniques. Clinical isolates were identified by commercial biochemical test systems, as well as with MALDI-TOF mass spectrometry. The sensitivity of clinical isolates to antibiotics was determined by means of disk diffusion test systems.

Results

Detection rates of the most common bacteria in BAL were as follows: *K.pneumoniae*, 19.1%; *P.aeruginosa*, 5%; *S. epidermidis*, 4.2%; *S. aureus*, 4.5%; *Acinetobacter spp.*, 3.7%; *E.faecium*, 7.0%; *E.faecalis*, 5.3%; *E.coli*, 2.5%; *Enterobacter spp.*, 2.3%; *Streptococcus pneumonia*, 1.5%; *Haemophilus spp.,* 0.9%, etc. The seeding rates for *S.viridans* and *S.epidermidis* tended to decrease with age, whereas the rates of *Klebsiella* detection, proved to be relatively high in all the studied age groups. Total bacterial numbers decreased during 1st month after HSCT, including those for *S.viridans*. Interestingly, the incidence of *Klebsiella spp.* showed sharp increase at

3-4 months posttransplant, due to selection of antibiotic-resistant strains.

Conclusion

The patients with oncohematological disease subjected to massive allogeneic HSCT exhibit sufficient changes of bronchoalveolar microbiota over first 6 months posttransplant. Decreased seeding levels are shown for *S.viridans* and *S.epidermidis* in adolescents over 15 years and adults. A sufficient suppression of BAL microbiota is revealed within 1st month posttransplant. At later terms after HSCT, high risk of *Klebsiella spp.* colonization is observed, due to selection of antibiotic-resistant strains. Higher incidence of *K.pneumoniae* and its high resistance rates suggest relevance of this pathogen for development of nosocomial infections in immunocompromised patients and other clinical settings.

Keywords

Hematopoietic stem cell transplantation, bacterial microbiota, bronchoalveolar lavage, age dependence, time dependence.

Introduction

Microbial, fungal and viral communities of bronchial and alveolar surfaces are closely related to microbiota of upper airways and oropharyngeal mucosa. Therefore, their microbiological variability shows sufficient intercorrelations [1]. In healthy persons, the microbial composition in lungs closely matches the upper respiratory tract (URT), especially in periglottic region. The total contents of bacteria are gradually decreasing from upper respiratory ways to the lower bronchoalveolar structures [2].

Meanwhile, in severe pneumonias, the clinically relevant changes of lung microbiota are generally, assessed by analysis of bronchoalveolar lavage (BAL) sampled during diagnostic bronchoscopy, thus representing a direct and efficient way to get a reliable etiological diagnosis in suggested infectious complications. Along with bacteriological studies, a number of viral pathogens are routinely detected, mainly, by PCR techniques or immunohistochemistry, whereas fungal infections could be suggested by specific polysaccharide antigens, or direct cultures. The bacterial diversity may be assayed both by classical cultural methods, and by modern DNA deep sequencing methods.

In particular, a range of techniques could be used for the search of opportunistic pathogenic bacteria in bronchoalveolar samples. E.g., Gaibani et al. [3] examined the BAL specimens from 24 patients with post-COVID pneumonia. The samples were cultured on conventional selective agar plates. The species of bacterial isolates were identified by MALDI-TOF mass-spectrometry, and *in vitro* antimicrobial susceptibility was tested by routine methods. Moreover, V3 to V4 region of the standard 16S rRNA gene was subjected to PCR, the resulting amplicon libraries were sequenced using MySec platform by means of next-generation sequencing (NGS). In summary, the lung microbiome of these patients showed predominance of *Pseudomonas spp.* (25%), *Enterobacteriaceae* (19%), *Streptococcaceae* (12%), *Staphylococcaceae* (11%). In particular, the DNA sequences of *Klebsiella spp.* (7%), *Enterococci* (5%), and *Prevotella* (4%) were also detected.

A number of patients with systemic malignant disorders present with polymicrobial airway colonization caused by preceding cytostatic therapy resulting into severe immune deficiency. Bronchoscopic examination of 436 consecutive adult patients with hematological malignancies and pulmonary infiltrates had revealed infectious agents in BAL of 219 patients of them 45 (20.5%), with microbial colonization, 39 of them with two pathogens, and 6 with three agents [4]. *Aspergillus spp.* was the most common co-pathogen identified. The authors have confirmed a more severe clinical course and higher hospital mortality in the patients with polymicrobial pulmonary infections.

A sufficient issue concerns relative diagnostic value of classical bacterial cultures and DNA-based NGS diagnostics. Such studies are now underway. E.g., direct comparisons between the diagnostic significance of was performed by a Chinese team who have enrolled a group of severe community-acquired pneumonia (SCAP) patients admitted to intensive care unit (ICU). BAL samples were taken by bronchoscopy within 48 h of ICU admission [5]. The isolated DNA was sequenced in the V3-V4 hypervariable region of the 16S rRNA gene of all PCR-amplified samples by means of Illumina Miseq platform. The multivariate analysis of variance has shown that positive bacteria lab test results had the strongest independent association with lung microbiota (P=0.018), thus confirming permanent diagnostic value of standard clinical culture of bacterial microbiota.

A number of studies concerned total contents and species diversity of lung microbiota in HSCT patients. Currently, the pre-transplant diagnostics of infectious respiratory disorders in the patients planned for HSCT is based on complex examination including pulmonary function tests (PFT), chest high-resolution computed tomography (HRCT), and laboratory examination of available BAL samples [6]. The authors have examined 142 children that should be subjected to HSCT. Different abnormalities were revealed in 74% of patients, mostly, for subnormal PFT tests. Chest HRCT showed clinically significant disturbances in 19% of the cases. BAL microbiota was abnormal in 43% of patients; respiratory viruses (PCR) were found in 35 patients, fungi (antigen or culture) in 21, and bacteria (culture) in 22. Prognostic value of these disorders could influence subsequent treatment approaches.

In the patients undergoing HSCT, severe impairement of lung microbiota is observed, due to transient cytopenia, immunocompromised state and massive antibiotic prophylaxis at early terms posttransplant. Early and late pulmonary infections post-HSCT are well known and described in details as reviewed by Astaschanka et al. [7].

The BAL microbiology, may yield a variety of common bacterial agents *S. aureus, E.coli, coagulase-negative staphylococci, Enterococci spp., P.aeruginosa, Klebsiella pneumonia* etc. Additional PCR and antigen assays revealed multiple viral and fungal species. E.g., BAL samples taken after hematopoietic transplants may often contain a number of different bacterial, fungal and viral species, sometimes, presenting mixed infection [8]. Fungal species, especially, *Aspergillus*, is a common finding in BAL samples taken from HSCT patients [9].

Similar study was carried out in 193 children and adolescents who underwent myeloablative conditioning and HSCT has revealed mixed microbiota [10]. Of them, 34% underwent bronchoscopy with a total of 101 BAL samples, mostly after allogeneic HSCT (allo-HSCT). The lung-derived samples were tested for bacterial, fungal and viral infectious pathogens using staining and culture methods. 40% of samples proved to be positive, with a majority showing bacterial pathogen as well as fungal and viral agents. In particular, the diagnostic assays revealed *Mycobacteria, S. epidermidis, vancomycin-resistant enterococci, coagulase-negative staphylococci, P.aeruginosa, Legionells, Serratia, Streptococcus spp., Enterococcus faecium, Lactobacilli spp*.

Despite recent advent of novel DNA-sequencing approaches, the classical bacteriological evaluation of biological samples retains its diagnostic value, in particular due to its ability to assess antibiotic resistance of microbial isolates. Hence, the aim of this study was a comparative evaluation of aerobic and facultative anaerobic microbiota components in bronchoalveolar lavage samples taken in immunocompromised patients with infectious lung complications which developed after intensive chemotherapy, antibiotic therapy and subsequent hematopoietic stem cell transplantation (HSCT).

Materials and methods

Our study included clinical and laboratory data of 691 patients subjected to hematopoietic stem cell transplantation (HSCT) 2013 through 2020, aged from 1 to 71 years (a mean of 38.5+ 23.9).

Distribution of BAL samples by distinct disorders was as follows: acute lymphoid leukemia (ALL, n=232 samples); acute myeloid leukemia (AML, n=331); aplastic and refractory anemias (AA, n=104); Hodgkin disease (HD, n=163); chronic myeloid leukemia (CML, n=79); non-Hodgkin lymphomas (NHL, n=63), other malignant and inherited disorders (n=58).

Myeloablative and non-myeloablative conditioning regimens for HSCT were carried out in 44% and 56% of cases, respectively. Stem cells were obtained from bone marrow or peripheral blood 497 : 596).

The types of HSCT were as follows: related compatible donors (19.2%); related haploidentical donors (21.6%); unrelated compatible donors (49.1%); autologous transplants (10.2%).

The doses of transfused CD34+ cells widely varied from 0.8 to 18×10^6 cells /kg body weight.

Prophylaxis of graft-versus-host disease was mostly, performed by the posttransplant cyclophosphamide (PtCy) as well cyclosporin A, tacrolimus, sirolimus and glucocorticosteroids.

For statistical analysis, the groups of patients were also divided by age: 0-5 years (group 1, n=79); 6-14 children (group 2, n=129); 15-21 years old (group 3, n=194); >22 years (group 4, n=720). Moreover, the results of bacteriological examination were classified by terms post-transplant, starting from <100 days before HSCT (point 0); during the 1st month (point 1); 2nd month (point 2); 3rd month (point 3), etc., up to 6 months after HSCT (point 6).

In the course of conditioning treatment and HSCT, the patients obtained antibiotic prophylaxis including administration of fluoroquinolones from D+1 to D+60. Amoxicillin was also administered, especially, to children. In cases of posttransplant febrile neutropenia, empirical therapy with broad-spectrum antibiotics was prescribed. Upon isolation of antibiotic-resistant microbial strains, the treatment was changed to other antibiotics (per os or intravenously) as guided by the *in vitro* testing of microbial sensitivity.

A total of 1123 samples of bronchoalveolar lavage were collected by means of diagnostic bronchoscopy within D-100 to D+180 post-HSCT. The endoscopy was performed according to appropriate clinical indications as prescribed by the attending doctor and intensive care specialist. Written informed consent for the procedure was obtained from the patients or their guardians.

Inoculation of laboratory cultures and isolation of bacteria from the BAL samples were made to differential culture media by classical bacteriological techniques. The isolated microorganisms were identified by means of commercial biochemical test systems (BBL Crystal), as well as with MALDI-TOF mass spectrometry using VITEK MS instrument. The sensitivity of clinical isolates to antibiotics was determined by means of disk diffusion test systems. The results of microbial sensitivity tests were interpreted according to the Guidelines of European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria.

Statistical evaluation of results was performed using parametric and nonparametric statistic criteria, to analyze time- and age-dependent course of the bacterial landscape revealed by microbial cultures, and for distinct microbial associations after HSCT using the STATISTICA 10 program.

Results

The detection rates for distinct cultured bacterial species among the total BAL sample massive was as follows: bacteria of *Enterobacterales* order were revealed in 25% of the samples (280/1123), with *Klebsiella pneumoniae* (19.1%, 215/1123) being prevailing species, whereas *Escherichia coli* (2.5%) and *Enterobacter spp.* (2.3%) were detected at similarly low rates (28/1123 and 26/1123, respectively. The incidence of *Citrobacter spp., Proteus mirabilis, Serratia marcescens* did not exceed 1%. Among Gram-negative, non-fermenting bacteria, *Pseudomonas aeruginosa* was detected in 5% of BAL specimens (56/1123). In particular,

P. aeruginosa v. non mucosa, and v. mucosa were found at similar rates (29/1123 и 27/1123). Other *Pseudomonas* species were isolated in 4.5% (51/1123); *Acinetobacter spp.*, 3.7% (41/1123); *Stenotrophomonas maltophilia*, 2.1% (24/1123). The ratio of coagulase-negative staphylococci was 5.6% (63/1123), of them *Staphylococcus epidermidis* was found in 4.2% (47/1123). *Staphylococcus aureus* was isolated in 4.5% of the samples (51/1123). *Streptococcus spp.* was revealed in 20.7% (233/1123 samples) including *Streptococcus viridans group* (15.7%, 176/1123); *Streptococcus pneumoniae* (1.5%, 17/1123). *Enterococci* encountered in 12.4% of BAL samples (139/1123), of them *Enterococcus faecium* was identified in 7.0% (79/1123); *Enterococcus faecalis*, in 5.3% (60/1123). Different *Candida* species were found in 14% of the BAL specimens (157/1123), as a rule, within microbial associations; the incidence of *Haemophilus spp.* was 0.9% (10/1123).

Detection rates for the most common bacterial species as classified by the age of patients are shown in Fig. 1. Of note, the seeding rates for *S.viridans*, a common member of normal mucosal microbiota, tended to decrease with age, reaching a significant decline in adolescents of >15 years old and adult patients ($p<0.01$). Meanwhile, the rates of *Klebsiella* detection, in our experience, proved to be relatively high in all studied age groups.

To prove the trends of posttransplant microbial colonization, we have assessed time dynamics for the dominant bacterial species within 6 months after HSCT (Fig. 2).

As seen from Fig.2A, the *S.epidermidis* seeding frequency did not sufficiently change over time posttransplant (a mean of 10-18%). By contrary, *S.viridans* detection rates dropped ca. 2-fold during 1st month after HSCT (approx. from 30% to 10%), probably, due to intensive antibiotic prophylaxis over the period of transient cytopenia ($p<0.02$). Meanwhile, the most interesting finding concerned posttransplant changes in *Klebsiella spp.* detection. Its low seeding rates before HSCT and over 1st month posttransplant (3 to 5%) were followed by sharply increased emergence of this commensal bacteria at later terms after HSCT (2 to 6 months), as seen from Fig. 2C ($p<0.001$). Interestingly, its detection was not affected by antibiotic treatment during early posttransplant period and additional antibacterial treatment due to presumed infectious complications requiring bronchoscopy and BAL sampling.

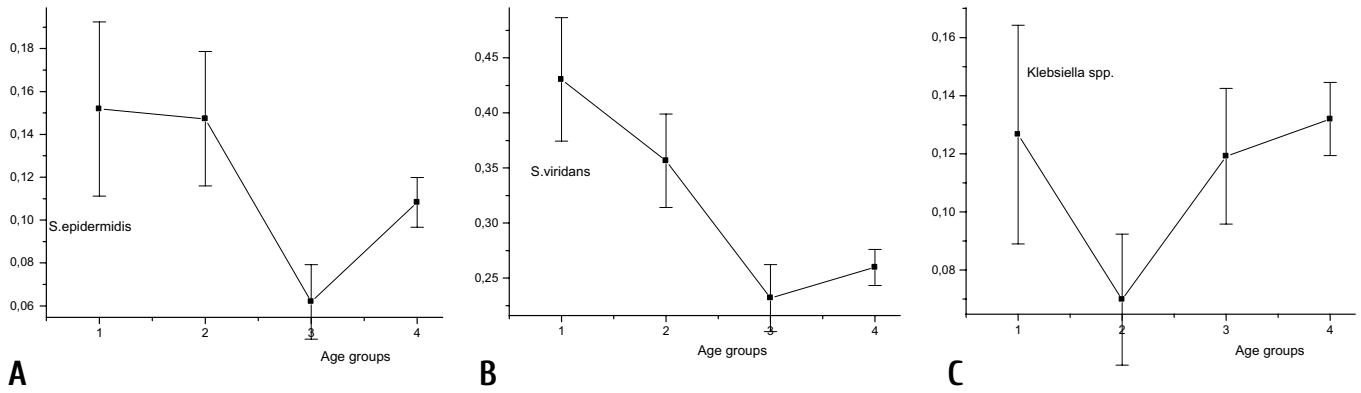

Figure 1. Age dependence of bacterial seeding rates for the dominant bacterial species in bronchoalveolar lavage of oncohematological patients both before and after HSCT (group 1: 0-5 years; group 2: 6-14 years; group 3: 15-21 years; group 4: >22 years. A, *S.epidermidis*; B, *S.viridans*; C, *Klebsiella spp.* Abscissa, age groups (group 1, 0-5 y.o.; group 2, 6-14 y.o.; group 3, 15-21 y.o.; group 4, >22 y.o. Ordinate, ratio of positive microbial findings.

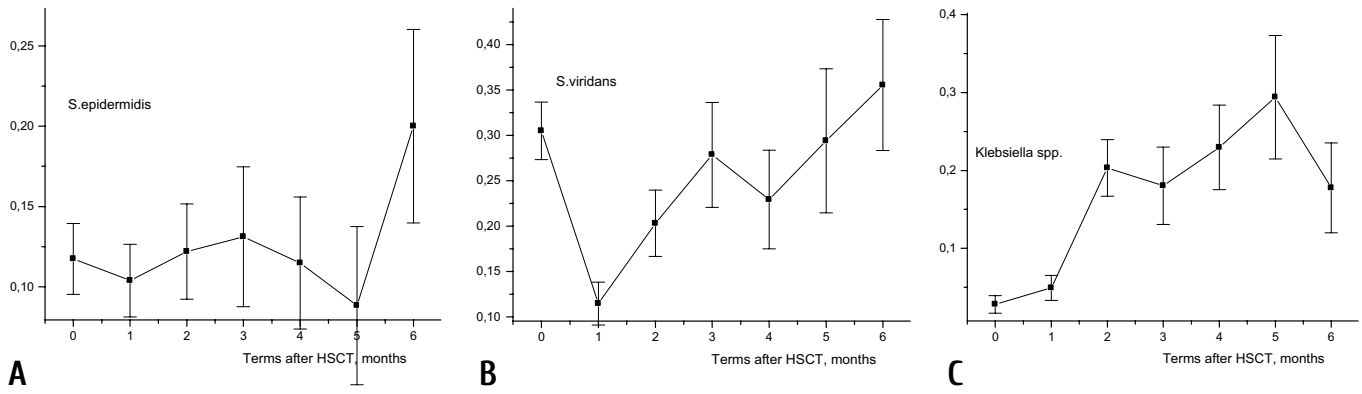

Figure 2. Frequency of *S.epidermidis* (A); *S.viridans* (B), and *Klebsiella spp.* (C) detection in bronchoalveolar lavage of oncohematololgical patients collected at various times after HSCT. Abscissa, terms post-HSCT (months); ordinate, ratio of positive microbial findings.

Antibiotic resistance was tested for all bacterial isolates derived from the BAL samples. Figures 3 to 8 depict the resistance patterns of the leading BAL microbiota members obtained from the subjected to HSCT between 2013 and 2020. Antibiotic resistance of *Klebsiella pneumoniae ssp pneumoniae*, was equally high during the entire tine period. It comprised over 80% for III generation cephalosporins, aztreonam, fluoroquinolones and protected aminopenicillins. Carbapenem resistance varied from 33% to imipenem to 54% for meropenem. 96% of the tested isolates were colistin-sensitive. Colistin sensitivity was determined by serial dilutions technique in liquid nutrient medium.

Common detection of *K.pneumoniae* accomplished by increased resistance rate presume high relevance of this pathogen for development of nosocomial infections in various clinical settings, especially in immunocompromised patients. Within mentioned time period, the meticillin-resistant *S.aureus* (MRSA) was isolated in 6% of BAL samples, whereas the rate of MRCoNS was 71%. All isolates of *Staphylococci* proved to be tigecycline-sensitive. Sensitivity of staphylococci to vancomycin was assessed by means of gradient diffusion (E test). *S.aureus* showed 100% sensitivity to vancomycin and linezolid. Among the CoNS strains, 2% were resistant for vancomycin, and 5%, for linezolid. The isolates sensitive to linezolid were also considered tedizolid-sensitive. Erythromycin was used to determine sensitivity to azithromycin, clarithromycin, and roxythromycin. Fluorochinolone sensitivity of staphylococci was evaluated by their sensitivity to norfloxacine. The norfloxacin-sensitive strains were considered to be sensitive to moxyfloxacin and, at higher exposure, to ciprofloxacin and levofloxacin. Fluorochinolone sensitivity of *S.aureus* proved to be 97%, appropriate rate for CoNS was 35%.

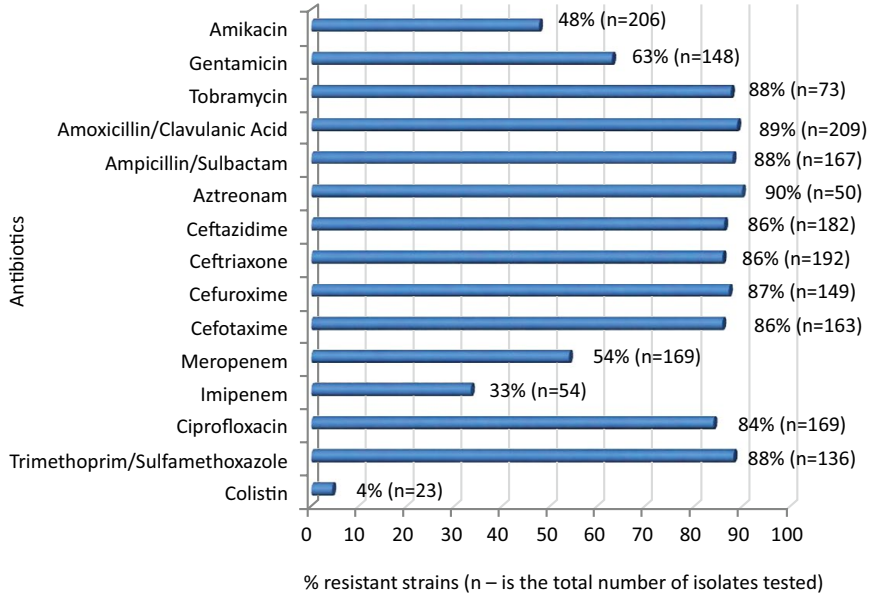

Figure 3. Resistance of *Klebsiella pneumoniae ssp pneumonia* to various antibiotics (biological samples: bronchoalveolar lavage, 2013 to 2020)

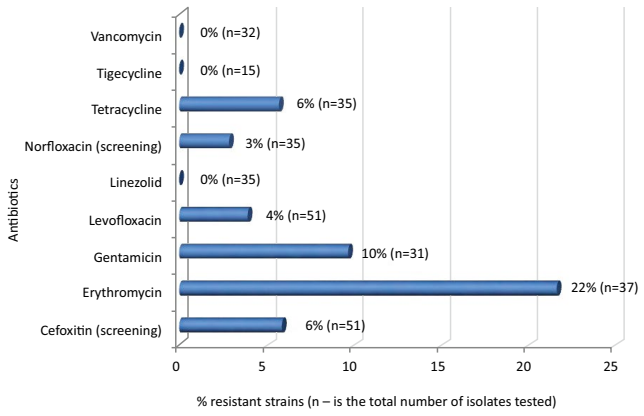

Figure 4. Resistance of *Staphylococcus aureus* to various antibiotics (biological samples: bronchoalveolar lavage, 2013 to 2020)

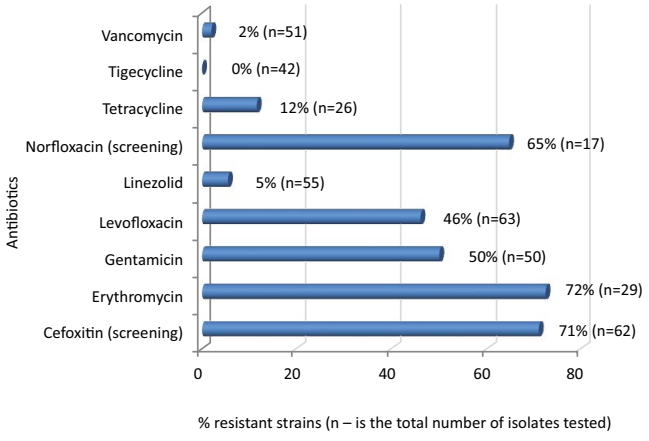

Figure 5. Resistance of coagulase-negative *Staphylococci* (CoNS) to various antibiotics (biological samples: bronchoalveolar lavage, 2013 to 2020)

The members of Gram-negative non-fermenting bacteria exhibited high resistance levels to carbapenems, i.e., >70% of resistant isolates among *Acinetobacter spp.*, over 50% resistant strains were documented for *P.aeruginosa*. Colistin sensitivity was as high as 96% for *Pseudomonas spp.* (two resistant strains from 52 isolates) and 93% for *Acinetobacter spp.* (one case of resistance of 15).

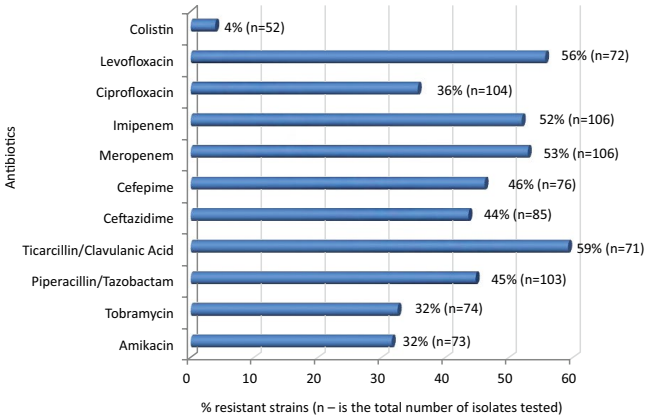

Figure 6. Resistance of *Pseudomonas spp.* to various antibiotics (biological samples: bronchoalveolar lavage, 2013 to 2020)

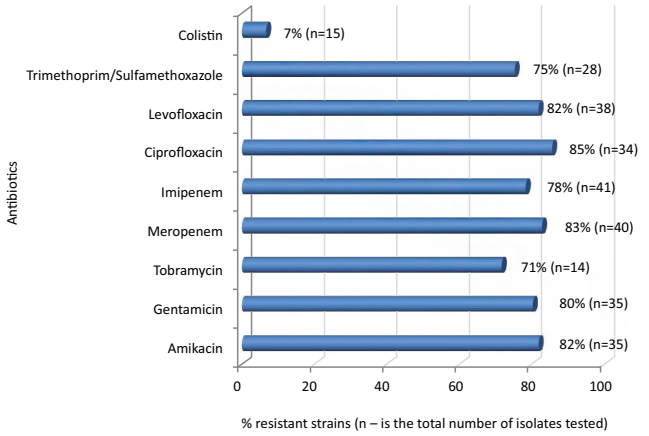

Figure 7. Resistance of *Acinetobacter spp.* to various antibiotics (biological samples: bronchoalveolar lavage, 2013 to 2020)

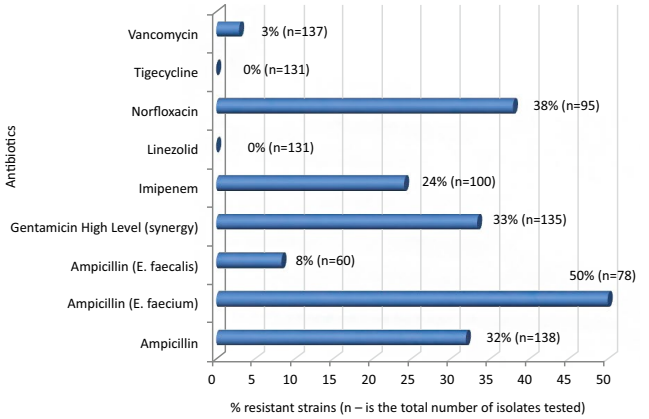

Figure 8. Resistance of *Enterococcus spp.* to various antibiotics (biological samples: bronchoalveolar lavage, 2013 to 2020)

The *Enterococcus* isolates from BAL samples have shown 100% resistance rates for linezolid and tigecycline; resistance rate was 3% for vancomycin (4 isolates of 137). 50% of *E.faecium* isolates proved to be ampicillin-resistant, whereas 92% of *E.faecalis* isolates remained sensitive to this drug.

Discussion

The significance of bacterial findings in BAL was considered in different transplantological aspects. E.g., a review article by Gudiol et al. [11] was dedicated to Hospital-acquired pneumonia (HAP) which present serious complications in transplant patients. Bacteria are the leading cause of nosocomial pneumonia for both immunocompetent and transplant recipients caused by Gram-negative organisms, and, especially, highly prevalent *Pseudomonas aeruginosa*. In addition to the usual colonizing microorganisms of the respiratory tract, such as *Streptococcus pneumoniae, Branhamella catharralis* and *Staphylococcus aureus*, and various Gram-negative bacilli are an important cause of HAP/VAP in both populations. The most relevant opportunistic pathogens are *Aspergillus fumigatus, Pneumocystis jirovecii* and cytomegalovirus.

Streptococcus viridians is nearly absent from normal lungs, but it proved to be a common commensal microbe cultured from many BAL samples in the patients with chronic obstructive pulmonary disease, bronchial carcinoma, as well as following tracheostomy [12], thus presuming massive colonization with this species in various chronic respiratory disorders. Rapid bacterial colonization, especially, with *S.viridans, S.aureus, P. aeruginosa* was observed after bronchial valve implantation in lung emphysema patients [13]. Therefore, predominance of *S.viridans* in BAL samples both before and after HSCT seems to be a useful marker of chronic lung damage due to massive cytotoxic therapies in oncohematological patients. Increased frequency of *S.viridans* in younger patients (<15 years old) revealed in our study could be also explained by higher susceptibility of respiratory mucosa in children to previous anticancer treatment.

To our knowledge, there are no published data on the time-dependent changes of *Klebsiella spp.* in bronchoalveolar lavage following hematopoietic stem cell transplantation. Rather high prevalence of this bacteria was found among the HSCT patients with sinusitis and other disorders of upper respiratory ways at our BMT Center. Interestingly, higher rates of *Klebsiella* detection were found in the maxillary cavities which represent a common reservoir of pathogenic agents in immunocompromised persons [14].

One may presume that the late activation of *Klebsiella* on respiratory mucosa may be caused by antibiotic-resistant strains colonizing these areas.

To suggest possible origin of these strains, one may refer a study of 91 BAL specimens obtained from critically ill patients with acute respiratory disorder (ARDS). Next-generation sequencing of bacterial DNA fragments was performed by means of Illumina MiSeq platform [15]. The ARDS-associated sequencing reads were similar to *Enterobacteriaceae*, including *Escherichia coli, Enterobacter spp.*, and *Klebsiella pneumoniae*. The authors conclude that lung microbiota in ARDS patients is characterized by relative enrichment with

gut-associated species of the *Enterobacteriaceae* family. Our findings concerning *Klebsiella* colonization of lower airways confirm these observations and provide evidence for higher risk of gut bacteria migration to other anatomic sites, due to extended septic process. This suggestion was made in our previous review [16].

A complex study of general cytokine responses and NGS-based evaluation of BAL bacterial microbiota in HSCT patients with pneumonias has shown reactive and non-reactive microbiota phenotypes [17, 18]. In the reactive phenotype, *Pseudomonas Aeruginosa* was the most abundant species, while in the nonreactive phenotype, cytomegalovirus (CMV) predominated. Moreover, other bacteria, *S.pneumoniae, S.aureus, Acinetobacter spp., Klebsiella spp., Stenotrophomonas spp.* were found in different quantities.

The new sequencing methods are recently applied for analysis of BAL microbiota. When comparing relative diagnostic efficiency of conventional bacterial cultures and novel metagenomic next-generation sequencing (mNGS) of BAL samples, a much higher sensitivity was revealed by analysis of different biological samples from pediatric HSCT patients by means of NGS approach, due to versatility of DNA reading technique and larger number of potentially pathogenic agents found by the multiple DNA sequencing [19]. The sensitivity of mNGS for diagnosing pulmonary infections post-transplant was 91.7 *vs* 22.9% by conventional testing. However, mNGS proved to be less specific (78.5%) than traditional methods (92.9%).

Later recolonization of pathogenic microorganisms post-HSCT is possible, including *Klebsiella spp., S. aureus, S.pneumoniae*, at a high risk of resistant strain selection, which was confirmed by us in the present work. One should note, however, that these 3 types of pathogenic bacteria were detected in a total of 13% of patients with sinusitis, i.e. the pathogen remained unknown in most cases. For additional diagnostics, along with search for pathogenic fungi and viruses, the extended diagnostics, e.g., of strictly anaerobic microbiota, are needed. In this aspect, implementation of advanced sequencing (NGS technique) will be of great importance, thus making it possible to assess biological diversity and the ratio of main microbiota classes in complex clinical samples, e.g., from mucosal surfaces.

Conclusion

1. In summary, the patients with oncohematological disease subjected to massive cytostatic therapy and allogeneic HSCT exhibit sufficient evolution of bronchoalveolar microbiota over the first 6 months posttransplant.

2. Follow-up of the BAL bacterial microbiota has revealed early exhaustion of *S.viridans* pool post-transplant, more likely, due to intensive anti-infectious treatment over the cytopenic period.

3. Decreased seeding levels are shown for *S.viridans* and *S.epidermidis* in adolescents over 15 years and adult patients.

4. Sharp increase of *Klebsiella spp.* detection rates at later terms (>2 months) after HSCT suggests airway colonization by the antibiotic-resistant microorganisms.

5. Future studies of BAL microbiota require combined diagnostic approaches, including mass spectrometry, PCR techniques for detection of strictly anaerobic bacteria, viral and fungal agents, as well as novel DNA-based technologies (e.g., next-generation DNA sequencing).

Conflict of Interest

None declared.

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Спектр бронхоальвеолярной бактериальной микробиоты после трансплантации гемопоэтических стволовых клеток: возрастная зависимость и нарушения микробиоты

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Резюме

Состав микробиоты бронхоальвеолярных отделов у здоровых детей и взрослых в целом коррелирует с составом популяций верхних дыхательных путей. Однако при развитии тяжелых пневмоний, особенно у пациентов с иммунодефицитами, отмечается существенное нарастание количества, частоты выявления и биоразнообразия бактерий в бронхоальвеолярных смывах (БАЛ), наряду с колонизацией бронхов условно патогенными микроорганизмами из других инфицированных сайтов. Так, у многих пациентов с онкологическими заболеваниями на фоне цитостатической терапии развиваются тяжелые пневмонии с полимикробной колонизаций нижних дыхательных путей. Целью нашей работы была сравнительная оценка аэробной и факультативно-анаэробной микробиоты в образцах БАЛ от детей и взрослых пациентов после трансплантации гемопоэтических клеток (ТГСК).

Пациенты и методы

Проведено обследование 691 пациента, главным образом – с онкогематологическими заболеваниями, леченными цитостатической терапией и ТГСК (алло-ТГСК в 90% случаев). Возраст пациентов составлял от 1 до 71 г. (медиана – 38,5 л.). Применяли миело- или немиелоаблативную кондиционирующую терапию (44% и 56% случаев). Для исследования проводили забор 1123 образцов биоматериала (БАЛ) при диагностической бронхоскопии по соответствующим клиническим показаниями в период от D-100 до D+180 после ТГСК. Культивирование бактерий на селективных средах проводили в аэробных условиях по стандартным методикам, виды бактерий в изолятах идентифицировали с помощью биохимических тестов (BBL Crystal), масс-спектрометрии (MALDI-TOF), чувствительность к антибиотикам – диск-диффузионными тестами.

Результаты

В целом, частота выявления отдельных бактериальных видов в образцах БАЛ была следующей: *K.pneumoniae* – 19,1%, *P.aeruginosa* – 5%, *S. epidermidis* – 4,2%, *S. aureus* – 4,5%, *Acinetobacter spp.* – 3,7%, *E.faecium* – 7,0%, *E.faecalis* – 5,3%, *E.coli* – 2,5%, *Enterobacter spp.* – 2,3%, *Streptococcus pneumoniae* – 1,5%, *Haemophilus spp.* – 0,9% и т.д. Другие микробы *Corynebacteria spp., Neisseria spp.* и др. встречались реже. Отмечены значительная возрастная динамика состава и частоты различных видов микробиоты в БАЛ после ТГСК. В частности частота высеваемости *S.viridans* была максимальной у детей младшего возраста (0-5 лет), снижаясь у подростков >15 лет. Та же закономерность, но менее выраженная, отмечена для *S.epidermidis*. Оба этих микробных вида часто выявляются в нормальной микробиоте. Напротив, частота выявления *Klebsiella spp., Pseudomonas spp.* и *S.aureus* в пробах БАЛ после интенсивной терапии и ТГСК повышается с возрастом пациентов, что говорит о большем риске жизнеопасных легочных инфекций после ТГСК, в том числе – резистентными к антибиотикам штаммами из кишечника у взрослых пациентов в период до 180 дней.

Выводы

У больных с онкогематологическими заболеваниями в течение 6 мес. после ТГСК отмечаются существенные сдвиги бронхоальвеолярной микробиоты. Показана сниженная высеваемость *S.viridans* и *S.epidermidis* у детей старше 15 лет и взрослых. Выявлено подавление микробиоты БАЛ в течение 1-го месяца после ТГСК. В более поздние сроки отмечен высокий риск колонизации *Klebsiella spp.* в связи с селекцией антибиотикорезистентных штаммов. Частота встречаемости *K.pneumoniae* и ее высокий уровень резистентности показывают актуальность данного патогена в развитии нозокомиальных инфекций у иммунокомпрометированных пациентов.

Ключевые слова

Трансплантация гемопоэтических клеток, бактериальная микробиота, бронхоальвеолярные смывы, возраст, временной фактор.