

**TO
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OF THE RECTOR OF THE MEDICAL UNIVERSITY
“PROF. DR. PARASKEV STOYANOV” - VARNA**

REVIEW

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Of a dissertation on the award of educational and scientific degree “Doctor”
in the field of higher education: 4. Natural sciences, mathematics and informatics;
Professional drive: 4.3. Biological Sciences;
Scientific specialty “Microbiology”

**On the subject: “Investigation of bacteremia and invasive fungal infections in
patients following autologous and allogeneic hematopoietic stem cell
transplantation”**

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I declare that I have no scientific works in common with the PhD student.

Relevance of the dissertation work

Transplantation of hematopoietic stem cells is one of the revolutionary discoveries in medicine, which led to the treatment of many incurable diseases until the middle of the last century – malignant hematological, as well as non-malignant, which lead to bone marrow failure. However, due to the many risk factors and the impaired condition of the patients, hematopoietic stem cell transplantation is accompanied by the development of non-infectious and infectious complications. Infectious complications are caused by bacteria, fungi, viruses and parasites, with bacterial and fungal infections being among the leading causes of mortality in these patients.

All this determines the relevance of the dissertation work and the need for the conducted clinical-microbiological and epidemiological studies on bacteraemia and invasive fungal infections in patients after hematopoietic stem cell transplantation (HSCT).

Structure of the dissertation

The dissertation is written on 187 pages and is structured according to the generally accepted scheme with a proportional distribution of the text in the separate sections, as follows: introduction - 1 page, literature review - 47 pages, purpose and goals - 1 page, materials and methods - 19 pp., results and discussion – 69 pp., conclusions – 2 pp., contributions – 2 pp., and literature – 40 pp. A list of publications and participation in scientific forums related to the dissertation is attached to the dissertation. The dissertation work is optimally illustrated with 39 precisely made visual materials (19 figures and 20 tables). 430 literary sources are cited in the dissertation: 8 are in Cyrillic and 422 are in Latin, with 240 from the last 10 years - proof of the relevance of the dissertation being developed.

The introduction substantiates the relevance of the research problem and emphasizes the importance of modern microbiological diagnostics of infectious complications, which are among the leading causes of mortality in patients after hematopoietic stem cell transplantation.

Evaluation of the literature review

The literature review is logically structured and examines in full the risk factors for infectious complications in HSCT patients and the clinical significance of pre-transplantation screening of the donor and the recipient. Bacterial infections, which are among the most common infectious complications with fatal outcome, their prophylaxis and etiologic therapy are presented in detail. The infectious complications associated with multidrug-resistant (MDR) bacteria and the therapeutic options according to the resistance mechanisms detected are discussed in detail. Fungal complications after HSCT, which like bacterial infections are responsible for high mortality (40% - 90%), their prevention and etiologic therapy are described in detail. A special place in the review is occupied by colonization with MDR microorganisms - most often bacteria and fungi, as a result of prolonged use of antimicrobials. It is explicitly emphasized that colonization with MDR microorganisms does not necessarily imply the development of an invasive infection. But with the risk factors present and the impaired state of HSCT patients, detection of this carrier is advisable with readiness for adequate therapy when infectious complications arise.

The review concludes by examining the situation in Bulgaria in the light of the fact that in recent years the number of HSCTs has increased from a few in one calendar year to several in one month. And since no comprehensive studies including risk factors, etiological spectrum and therapeutic options of bacterial and fungal infections in HSCT have been conducted in Bulgaria, given the high mortality rate, the need for a study on bacteremia and invasive fungal infections in patients after hematopoietic stem cell transplantation is logically deduced.

Purpose and Goals

The author systematically and thoroughly approaches the set goals in accordance with the purpose of the dissertation: to perform a clinical-microbiological study on bacteremia and invasive fungal infections in patients after autologous and allogeneic hematopoietic stem cell transplantation, performed in the period 2019 - 2021 in the Department of Transplantation at the Clinic of Clinical Hematology of St. Marina University Hospital.

In order to achieve the purpose of the study, 7 goals were set, which follow the sequence of the planned research, namely: (1) to investigate the incidence and risk factors for bacteremia and fungemia in patients after autologous and allogeneic hematopoietic stem cell transplantation, as well as the 4-month survival rate in the entire study group of patients, and to analyze the factors that affect it; (2) to investigate the etiological spectrum of bacteremia and fungemia and to determine the susceptibility of microbial isolates to antimicrobial agents; (3) to investigate by molecular methods the mechanisms of methicillin resistance in isolated staphylococci and resistance to cephalosporins and carbapenems in Gram-negative blood isolates; (4) to investigate by phenotypic and molecular methods the capacity for slime production in isolated staphylococci as their important virulence factor; (5) to study the etiological spectrum of invasive fungal infections; (6) to study the level of fecal carriage of *Enterobacterales* isolates resistant to 3rd generation cephalosporins and carbapenems, of carbapenem-resistant *Pseudomonas* spp., of vancomycin-resistant *Enterococci*, *Stenotrophomonas maltophilia* and fungi in the study group of transplanted patients and identify the genetic mechanisms of resistance to these antibiotics; (7) to investigate the epidemiological relationship between faecal isolates and blood isolates showing resistance to 3rd generation cephalosporins, carbapenems and glycopeptide antibiotics.

Evaluation of the Materials and Methods section

A large set of classical microbiological and modern molecular methods were used to phenotypically and genetically characterize 107 non-repetitive microbial isolates (89 bacterial and 18 fungal) obtained from blood (42) in septic episodes and from feces (65) in routine fecal screening of 74 patients undergoing hematopoietic stem cell transplantation from January 2019 to December 2021. The methods used were highly informative and suitable for the set goals.

Evaluation of the Results and Discussion section

A considerable amount and variety of research work has been carried out. In accordance with the design of the prospective clinical-microbiological and epidemiological study, the results of the studied patients and their microbial

isolates over a three-year period are presented and analysed. The results obtained and their discussion strictly follow the set objectives as follows:

1) A high cumulative incidence of bloodstream infections was found in the study group of transplanted patients (32%-38.5%), with a mean time of onset of infectious complication of 47 days after the procedure. Fecal colonization and bloodstream infection preceding HSCT were independent risk factors for bloodstream infection in patients after HSCT. A high 4-month survival rate was found among the entire group of patients followed (86.5%). A statistically significant correlation was found between 4-month survival and the parameters - type of transplant, underlying disease, absence or presence of previous transplantation, with patients with allogeneic HSCT, previous HSCT and underlying disease leukemia or lymphoma having lower odds of survival for the first 4 months after transplantation.

2) In the established etiological spectrum of bloodstream infections in patients after HSCT, Gram-positive bacteria lead the way, with coagulase-negative staphylococci (CoNS) being the most common causative organism. *E. coli* predominates among Gram-negative microorganisms. A low relative proportion of fungemias has been demonstrated. The CoNS associated with bloodstream infections in the present study demonstrated very high levels of methicillin resistance, and therefore glycopeptide therapy is recommended in cases of staged CVC and suspected catheter-associated infection. Ampicillin, ciprofloxacin and trimethoprim/sulfamethoxazole have significantly reduced activity against Gram-negative bacteria isolated from blood.

3) The main mechanism of resistance to 3rd generation cephalosporins in blood isolates of the *Enterobacteriaceae* family - production of CTX-M-15 ESBL - has been established. Carbapenem resistance in *A. baumannii* is associated with four *bla* genes encoding carbapenemases of two different classes - class B (*bla_{VIM}*) and class D (*bla_{OXA-48}*, *bla_{OXA-23}*, *bla_{OXA-24/40}*). Methicillin resistance, demonstrated in all CoNS, is entirely associated with the *mecA* gene. The proportion of ESBL producers among *Enterobacteriaceae* intestinal bacteria is 20%. Imipenem/meropenem, piperacillin/tazobactam and amikacin are the drugs with the best activity, making them

suitable for initial empirical treatment in cases of febrile neutropenia and septicemia.

4) A high relative proportion of blood-borne slime-producing CoNS, among which *Staphylococcus epidermidis* species is the most common, has been found to be associated with the presence of *ica* genes. The association between *ica* gene carriage and methicillin resistance was statistically significant.

5) A low incidence of invasive pulmonary aspergillosis but a high mortality among proven cases was found in the studied group of patients.

6) The study group of transplanted patients was characterized by a high incidence of intestinal colonization with fungi and polyresistant bacteria (49%), with 33.8% relative proportion of ESBL producers (mainly CTX-M-15 ESBL); 13.8% of carbapenem-resistant bacteria (*Pseudomonas* spp. with VIM-2 and *E. cloacae* with VIM-1 metallo-carbapenemases) and 23% to VRE, all carriers of the *vanA* gene. Among the fungal isolates, *Candida nonalbicans* species dominated the representatives, and species with concurrent resistance to echinocandins and the azole group of antimycotics were demonstrated.

7) Clusters of identical and/or closely related isolates of *E. cloacae* and *E. faecium*, as well as the genetic identity between fecal and blood isolates of *E. cloacae* obtained from a single patient, confirm the gastrointestinal tract as an important reservoir for infectious complications in patients after HSCT, the invasive potential and the ability of these microorganisms to clonal dissemination in hospital settings.

Evaluation of the scientific contributions

Dr. Niyazi's dissertation work has made significant original and confirmatory contributions of a scientific and applied nature.

Among the original contributions, the following stand out: (1) the incidence and risk factors for bacteremia and invasive fungal infections in patients after hematopoietic stem cell transplantation, as well as 4-month survival in the entire group of patients studied, were studied and analyzed, and the factors influencing it were analyzed; (2) the etiological spectrum and antimicrobial drug susceptibility of microbial causative agents of bloodstream infections in the study patients were analyzed; (3) the colonization status

of the gastrointestinal tract with polyresistant bacteria (ESBL and carbapenemase-producing Gram-negative bacteria, vancomycin-resistant enterococci, *S. maltophilia*) and fungi in post-HSCT patients; (4) carbapenem-resistant isolates of *E. cloacae* with *bla*_{VIM-1}, carbapenem-resistant isolates of *Pseudomonas composti* and *Pseudomonas mendocina* with *bla*_{VIM-2}; (5) identified from haemoculture a carbapenem-resistant isolate of *A. baumannii* carrying *bla*_{OXA-48-like}, *bla*_{OXA-23-like}, *bla*_{OXA-24/40-like} and *bla*_{VIM-like} genes; (6) *Candida nonalbicans* isolates (*C. glabrata*, *C. krusei*, *C. tropicalis*) with multiple resistance were demonstrated from faecal screening.

The contributions of a confirmatory nature are distinguished by a clear concentration on problems of clinical microbiology in the following main areas: (1) the gastrointestinal tract is confirmed as an important source for infectious complications in post-HSCT patients, and intestinal colonization with multidrug-resistant bacteria is demonstrated as a significant risk factor for the development of bacteremia in this group of immunocompromised patients; (2) the leading etiologic role of coagulase-negative staphylococci in bacteremias of patients after HSCT and CVC placement, the widespread prevalence of methicillin resistance mediated by the *mecA* gene in these isolates, the association between *ica* operon carriage and slime production in these patients, and the association between *ica* gene carriage and methicillin resistance are confirmed; (3) the widespread prevalence of CTX-M-15 as a major mechanism of resistance to third-generation cephalosporins in *Enterobacteriaceae* isolates derived from blood and feces of post-HSCT patients is confirmed; (4) glycopeptide resistance in vancomycin-resistant isolates of *E. faecium* is mediated by the *vanA* gene; (5) the invasive potential of *E. cloacae* and the ability of clonal dissemination of isolates of *E. faecium* and *E. cloacae* have been confirmed; (6) invasive pulmonary aspergillosis in patients undergoing HSCT is associated with high mortality.

Among the contributions of a scientific and applied nature with great clinical relevance is the introduction into routine microbiological diagnostics of (1) the CRA test and the Christensen test as phenotypic methods for the detection of slime production by *Staphylococcus* spp. isolates; (2) the immunoenzymatic method Platelia *Aspergillus* Ag test (Bio-Rad, France) for the diagnosis of invasive pulmonary aspergillosis in patients after HSCT.

Publications related to the dissertation

The results of Dr Denis Niyazi's extensive research work are reflected in 3 publications in which he is first author. The publications are in our journals with an SJR of 0.101 to 0.203, two of which are in English. The dissertant has presented his achievements in 4 scientific forums, and one of his papers was awarded with the 1st place prize. These scientific metrics present the dissertant as an established researcher with significant contributions to the development of clinical microbiology and knowledge of the microbiological diagnosis of bacteremia and invasive fungal infections in patients after hematopoietic stem cell transplantation.

Conclusion

The presented dissertation work is on an extremely important problem for medical science and practice with significant scientific and applied contributions related to all clinical and microbiological aspects of bacteremias and invasive fungal infections in patients after autologous and allogeneic hematopoietic stem cell transplantation in Bulgaria. The dissertation repeatedly exceeds the requirements of the Academic Staff Development Act in the Republic of Bulgaria, the Regulations for its implementation and the Regulations of MU-Varna. This gives me reason to confidently support the awarding of the educational and scientific degree "Doctor" in the scientific specialty "Microbiology" to Dr. Denis Sunay Niyazi.

24.08.2022

Reviewer:



/ Prof. Stefana Sabcheva, MD, PhD /