

To: Scientific Jury

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Of the Rector of the Medical University – Varna

REVIEW

From Assoc. Prof. Milena Krasimirova Bozhkova, MD,PhD

Internal member of the Scientific Jury

Medical University - Varna

Subject: Dissertation for the acquisition of educational and scientific degree "Doctor" under doctoral program "Microbiology" in professional field 4.3. Biological sciences and Scientific specialty "Microbiology"

Title of the dissertation:

"INVESTIGATION ON BACTERIEMIA AND INVASIVE MYCOTIC INFECTIONS IN PATIENTS FOLLOWING AUTOLOGOUS AND ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION"

Scientific consultants:

Prof. Temenuga Stoeva MD,PhD

Assoc.Prof. Ilina Micheva MD,PhD

Author of the dissertation:

Denis Sunai Niazi, MD

I declare that I have no conflict of interest of a scientific or other nature.

The dissertation meets the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria (HRSRB) and its implementing rules at MU-Varna.

Dr. Dennis Sunai Niazi has been working since 2018 as an assistant professor in the Department of Microbiology and Virology at MU-Varna and as a medical doctor in the Laboratory of Microbiology at University Hospital “St. Marina” Varna.

Relevance of the topic:

Hematopoietic stem cell transplantation (HSCT) is one of the most revolutionary achievements of modern medical practice. It is clear that among the main risks for an adverse outcome among patients are the infectious complications of any origin – bacterial, viral and mycotic. Early identification of potential risk factors for the development of invasive bacterial and mycotic infections in patients after autologous and allogeneic HSCT and the introduction into clinical practice of screening tests for timely prevention and rapid and accurate detection of this type of complications significantly improve the long-term prognosis and today emerge as one of the most important strands in the complex approach in the treatment of patients with bone marrow failure.

The dissertation addresses important problems at the local level, an essential part of the global problem with the success of HSCT. Knowledge of the etiological spectrum of the invasive bacterial and mycotic infections, identifying the main risk factors for their occurrence, revealing the mechanisms of antibiotic resistance and epidemiological typing of isolates by patients after HSCT provide valuable guidance for the treatment of these potentially life-threatening complications. In this sense, I consider the actuality of the present dissertation work to be undeniable.

Sections of the dissertation:

The dissertation of Dr. Niazi is properly structured, with all the necessary sections and consists of 187 pages. 19 figures and 20 tables are used for illustration. The cited literary sources are a total of 430, of which 8 are in Bulgarian and 422 are in English.

The literature review is written competently and comprehensively. It thoroughly presents the main risk factors for the occurrence of infectious complications in patients after HCCT. Special attention is paid to pre-transplant screening tests of a donor and recipient as an important condition for the success of the procedure. Following the main line of his dissertation, Dr. Niazi presents in depth the basic characteristics of bacterial and mycotic infections in this target patients' group. Numerous up-to-date data on the frequency and etiological spectrum of invasive infections and the main sources of infection are presented. The leading approaches for the etiotropic treatment of bacterial infections associated with the most common causative agents – staphylococci, streptococci, enterococci, enteric bacteria and non-fermentative gram-negative bacteria are characterized in detail. Dr. Niazi logically focuses on the treatment options for infections caused by multiple-resistant bacteria (MDR) – one of the most serious challenges in modern medical practice in the era of global antibiotic resistance. Fungi as causative agents of invasive infections in patients after HSCT are also examined in detail and in depth. Dr. Niazi focuses on main prevention algorithms and modern therapy strategies with already established in clinical practice antimycotic agents such as amphotericin B and fluconazole and newer antifungal antibiotics from the group of echinocandins and azoles (voriconazole, isavuconazole, posaconazole, anidulafungin, caspofungin). On the basis of the review, the main objective of the dissertation is set out, with seven specific tasks, clearly formulated and set in this regard.

Materials and methods: Dr. Niazi's dissertation is a clinical – microbiological prospective study on 74 patients who have undergone autologous

and allogeneic HSCT at the Clinic of Clinical Hematology of University Hospital "St. Marina" in the period 2019-2021. The study of the 107 microbial isolates of the patients covered was carried out with a variety of classical and modern methods, explained in details and therefore enabling the protocols to be used in subsequent studies. The identification of all bacterial isolates was made with automated systems - Phoenix 100 (BD, USA) and MALDI Biotyper Sirius (Bruker, Germany). The determination of susceptibility to antimicrobial agents was performed by different methods: an automated system - Phoenix 100 (BD, USA), broth microdilution method and E-test. Modern molecular-genetic methods for the detection of genes, encoding resistance to beta-lactams and glycopeptides, based on polymerase chain reaction (PCR) as well as DNA sequencing of the resulting genes have also been applied. In addition to the classic tests for detection of slime – production in staphylococci such as inoculation on Congo red agar and Christensen method, methods based on PCR (detection of genes *icaA* and *icaD*) were used.

Galactomannan Ag test - Platella™ *Aspergillus* Ag (Bio-Rad, France) in serum and/or BAL has been used as diagnostic test for invasive aspergillosis. Epidemiological analysis was performed by two alternative methods based on PCR – RAPD and ERIC.

Results and discussion: The results obtained are discussed and presented in seven subsections. The PhD candidate details the data obtained on the risk factors for the development of blood infections after HSCT, their cumulative frequency (31.5%), the average period for their development (approximately 47 days after transplantation) and the survival of patients, using for comparative analysis data from similar cohort studies. Of particular interest is the statistically reliable correlation established by Dr. Niazi between the colonization of GIT with multiple-resistant bacteria and fungi and the occurrence of invasive infections after HSCT. The study proves domination of Gram-positive bacteria (mainly

CoNS) as causative agents of bacteriemia, which the author associates with the use of CVC and the antibiotic prophylaxis during the period of neutropenia. There is relatively low frequency of fungemia (only 2.4%), but special attention is paid to the fact that the causative agent – *Candida krusei* belongs to "nonalbicans" group which is more resistant to antifungal antibiotics.

An analysis of data from the antibiotic sensitivity of hemoculture isolates shows a high incidence of methicillin -resistance among CoNS (85.7%), as well as high levels of resistance to macrolides, aminoglycosides and quinolones (71.4%, 62.0% and 52.4%, respectively).

The study of the mechanisms of resistance to strategic antibiotics among invasive bacterial pathogens is based on PCR and covers a total of 22 isolates – 18 u is phenotypically MRCoNS and 4 Gram-negative isolates, resistant to third and fourth generation cephalosporins. Dr. Niazi found that methicillin resistance among the staphylococci studied was associated only with *mecA* genes. The PCR experiments with the invasive isolates from family *Enterobacteriaceae* reveals as leading mechanism of resistance to beta-lactams the production of extended-spectrum beta-lactamases from the CTX group, and CTX-M-15 in particular, confirming their widespread geographical *distribution*. The only documented invasive isolate *A. baumannii* demonstrated multiple resistance and has been proven as a carrier of genes *bla*_{VIM-like}, *bla*_{OXA-48-like}, *bla*_{OXA-24/40-like}, and *bla*_{OXA-23-like}.

Slime production has long been known as an important virulence factor, with a leading role in the pathogenesis of staphylococcal infections. In this dissertation work, all hemoculture-isolated staphylococci were tested with phenotypic and molecular-genetic tests – PCR for detection of *icaA* or *icaD* genes. The analysis of the results indicates a high relative share of confirmed and potential slim-producing staphylococci - 71.4%. In addition, Dr. Niazi demonstrated a statistically reliable correlation between *mecA* and *ica* genes.

which additionally complicates the correct choice of appropriate antimicrobial therapy.

As a particularly important part of the dissertation, I evaluated the analysis of the results obtained from the detection of *Aspergillus* galactomanan antigen by ELISA in serum and/or BAL in order to prove potentially fatal infectious complications after HSCT as IPA. The incidence of the disease (11.5%) and the mortality (66.7%) of IPA patients were documented. The main clinical-microbiological criteria and algorithms in diagnosing the disease, the specifics taking into account the galactomanan test and possible sources of error are presented very thoroughly and comprehensively. The therapeutic approach and the final outcome of the disease is described in details for the three patients, categorized as "possible case of IPA". This study demonstrates indisputably the reliability of *Aspergillus* galactomanan Ag - ELISA test for IPA diagnosis and monitoring of therapeutic response.

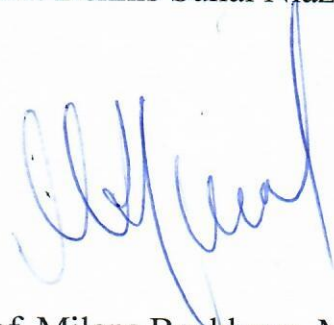
Data from fecal screening for multiple-resistant bacteria and fungi were analyzed, with about half of the patients studied (49%) had one or more "problematic" isolates. The resistance profiles of isolated bacteria and fungi have been thoroughly analyzed and the leading genetic mechanisms of resistance to third-generation cephalosporins and carbapenems among Gram-negative bacteria and to glycopeptides in enterococci have been established. In this dissertation it has been studied by molecular genetic methods (ERIC and RAPD) the epidemiology of 39 bacterial isolates derived from fecal samples and hemocultures. Small cluster groups have been found in *E. cloacae* and *E. faecium*, suggesting their ability to disseminate in hospital ecosystems. In one of the patients studied, a complete identity of two *E. cloacae* isolates from feces and hemoculture was demonstrated as confirmation of the invasive potential of intestinal microflora in patients after HSCT.

Dr. Niazi presents an in-depth overview study and a large volume of valuable experimental data, on the basis of which he logically draws 11

conclusions, with which I fully agree. I highly appreciate the PhD candidate's contributions, which are clear and correctly formulated.

In relationship with the dissertation, three publications and four participations in scientific forums were presented, the first author of which is Dr. Niazi, which clearly testifies that the dissertation is his personal work.

In conclusion, the dissertation submitted to me for review is a thorough and comprehensive clinical-microbiological study of an up-to-date medical problem carried out with classical and modern molecular and genetic methods. This gives me reason to give my personal positive assessment and to propose to the members of the scientific jury to vote for the award of an educational and scientific degree "Doctor" in the scientific specialty "Microbiology" to Dr. Dennis Sunai Niazi.

A handwritten signature in blue ink, appearing to read 'Milena Bozhkova', is written over the printed name below.

Assoc. Prof. Milena Bozhkova, M.D.