REVIEW

written by Prof. Valeriya Ignatova Kaleva, MD, PhD

of the dissertation for awarding the educational and scientific degree PhD,

professional area 7.1. Medicine, doctoral program 03.01.39

"Hematology and blood transfusion"

Author: **Dr Vladimir Todorov Gerov**

Topic: **Biomarkers for evaluation of bone disease in multiple myeloma**

Form of doctoral degree: self-study

Department: Internal diseases II, ES „Hematology", Medical University – Varna

Scientific advisor: prof. Ilina Dimitrova Micheva, MD, PhD

General presentation of the procedure

Following order № P-109-141 as of 09.04.2024 issued by Prof. Dimitar Raykov, MD, PhD, Rector of the Medical University in Varna, based on a decision taken by the Faculty council of the Faculty of Medicine (Protocol №19/01.04.2024) I have been selected as an internal member of a Scientific jury evaluating the dissertation work written by Dr Vladimir Todorov Gerov on the topic: Biomarkers for evaluation of bone disease in multiple myeloma. According to a decision taken at the first session of the scientific jury (Protocol №1 / 12.04.2024) I was appointed to write a review.

The presented set of materials according to the procedure for awarding the educational scientific degree "Doctor of Philosophy" (PhD) is complete, and is available both online and in a print version. It meets the requirements of the Act for the development of the academic staff in the Republic of Bulgaria and of the Guidelines for the development of the academic staff of MU, Varna.

Brief biographical data about the PhD candidate

Dr Vladimir Todorov Gerov graduated from the 4th language medium school "Frederic Joliot Curie|" with intensive teaching of French in 1980 and in 1988 he graduated from the Medical University in Varna. That same year he started his professional career as a doctor and therapist at the Emergency Medical Service Centre in Shumen (1988 – 1989) and a family doctor at DCC/Diagnostic and Consulting Centre/, Shumen (1989 – 1991). Since 1992 up until the present he has been working as a doctor at the Hematology Clinic at "St. Marina" University multiprofile hospital for active treatment, Varna and a part-time lecturer at the Second

department of Internal diseases, ES in Hematology at the Medical University in Varna. In 2014 he was reappointed for a doctor in the stem cell transplantation unit at the Clinical Hematology Clinic. He has obtained specialties in Internal diseases (1995) and Clinical hematology (1997). In 2006 he completed the Master's program in Healthcare Management at the Medical University in Varna. He specialized in the area of Clinical hematology and transplantation of hematopoietic stem cells in Colmar (France, 2015), Zagreb (Croatia in 2016) and Hannover (Germany, 2017).

Dr Vladimir Gerov has taken part in a number of national and international scientific forums and hematology training courses. He participated in research teams of joint scientific projects with the department of Biochemistry (2001-2002) and with the department of Biochemistry, Molecular medicine and Nutrigenomics, as well as with the department of Clinical laboratory (2019 – 2023). His scientific interests are in the area of diagnosis and treatment of acute leukemias and multiple myeloma, oxidative stress in oncohematological diseases, new biomarkers for the evaluation of bone disease in multiple myeloma, transplantation in oncohematological diseases. He has been a participant in the teams of international clinical trials and research. He is a member of the Bulgarian Medical Association, the Bulgarian Medical Society of Hematology and the European Society for Blood and Marrow Transplantation. He is fluent both in French and English.

Relevance, originality, and significance of the topic

The myeloma-induced bone disease (MBD) and the osteolytic lesions associated with it are distinctive biochemical and clinical expressions of multiple myeloma (MM). It is found in approximately 70% of the patients at the initial diagnosis and develops even when there is no evidence of an active disease. This defines MM as a main reason for the disease and mortality of patients suffering from MM. Despite the new therapeutic opportunities, in the course of the disease, skeletal events are observed in 80-90% of the patients such as aches in the bones (70-80%), spontaneous fractures (50-60%), hypercalcemia (15%) and bone marrow compression (2-3%). Besides, the daily physical impact of the bone disease poses additional threats to their safety and quality of life, even when they are in remission.

In everyday clinical practice various biomarkers are used to evaluate the tumor load or assess the risk but there are no such biomarkers that determine the scale of bone loss. In this respect, the MM patients are an ongoing therapeutic challenge which necessitates both identification of new markers participating in the bone remodeling in MM and development of new pharmaceutical molecules and approaches.

In the context of the motives thus formulated, I think that the suggested topic is interesting and with its relevance, topicality and unsolved problems could serve as a basis for new scientific research for evaluation of bone disease in MM and creation of a laboratory algorithm for its monitoring.

General characteristics and structure of the dissertation

The dissertation work thus presented contains 184 standard pages and has been illustrated with 50 tables and 52 figures. It has been structured according to the generally accepted requirements and includes: 2 pages – table of contents, 1 page of abbreviations, 2 pages of introduction, literature review spread on 40 pages, 1 page of aims and objectives, 9 pages of materials and methods, 68 pages of results, 30 pages of discussion of the outcomes, 2 pages of conclusions, 1 page of contributions, a list of publications and participation in scientific forums related to the dissertation work in 1 page, and 25 pages of references, including 424 literature sources, of which one page in Cyrillic and 423 in Latin.

Literature review

The literature review is exhaustive and demonstrates excellent knowledge and understanding of the problem. The epidemiology of MM and the modern understanding of MBD pathogenesis have been elaborated in great details. The peculiarities of different cellular components of the bone marrow and the molecular mechanisms of their interaction with the myeloma plasma cells have been demonstrated. The role of the bone marrow microenvironment has been emphasized as it is crucial to the progression of MM and the development of MBD. The most frequently activated signaling pathways have been pointed out. They support the growth, survival, migration and drug resistance of the myeloma cells. Approximately two thirds of the literature review are dedicated to 5 pathogenic factors selected by the author that are related to the imbalance of the processes in bone remodeling: Dkk-1 and sclerostin, as osteoblast activity inhibitors, sRANKL, as a major osteoclast activating factor, as well as osteopontin and periostin as important proteins of the extracellular matrix, providing favorable conditions in the bone marrow microenvironment for the settlement, proliferation and expansion of myeloma clonal plasma cells. Detailed information has been provided for each one of them on their cellular structure, gene expression, mechanism of action and biological role in the processes of bone resorption and bone formation. Special attention is paid to the facts, well known up to now, related to their role in the onset and progression of MBD.

After summarizing the available literature data, the author draws the conclusion that for one part of the investigated factors (sclerostin, osteopontin and periostin) the clinical

investigations are too limited and do not allow for recommendations to be formulated for clinical application, while for the remaining factors controversial results have been shared that lead to different conclusions as regards to their role in the evaluation of bone disease in patients with MM. The author formulated a summary according to which additional research needs to be carried out and with its help the role of Dkk-1, sclerostin, sRANKL, osteopontin and periostin at the onset, maintaining an impaired balance between the main processes of bone remodeling such as osteoclastogenesis and osteoblastogenesis will be clarified.

The aim is precisely and briefly defined.

The objectives for its completion are clearly formulated, they follow the logic of the literature review, and their solutions are in harmony with the diagnostic methods used.

The section on "***Materials and Methods***" is spread over 9 pages. This is a single-centre, prospective study that is carried out in the Hematology Clinic at "St. Marina" University multiprofile hospital for active treatment, Varna after obtaining approval by the Committee for Ethics and Academic Unity at the Varna Medical University. 74 individuals are included in total – 41 newly diagnosed patients with MM and 33 healthy people who form the control group. They were selected according to clearly defined inclusion and exclusion criteria. The research design has been described briefly and demonstrates the real opportunity for dynamic tracing and monitoring of the levels of the bone biomarkers investigated in relation to the stage and severity of MM, the level of bone marrow plasma cell infiltration and the routine hematological, morphological, and biochemical indicators. The bone biomarkers of the individuals from the control group were investigated just once while the patients' biomarkers were investigated in three stages depending on the treatment carried out: before the beginning of the treatment (stage T0), after finishing 4 courses of chemotherapy (stage T1), after completion of 4 additional courses of chemotherapy for non-transplant patients (stage T2) and three months after the carrying out of autologous stem cell transplantation (ASCT) in transplant patients (TA). At each of the stages, the patients are routinely investigated with all indicators used in the diagnostic clinical practice and an additional quantity of venous blood is drawn to determine the serum levels of sclerostin, Dkk-1, sRANKL, osteopontin and periostin. The measuring is carried out with the help of highly sensitive ELISA-methods with kits of Shanghai Sunred Biological Technology Co., Ltd, China. The bone disease is investigated with a low-dose whole-body computed tomography (CT). All methods applied are modern and relevant for completing the tasks set. The statistical methods together with the software programs for processing the data are presented in details and in a competent way.

The presentation of the ***results*** is spread over 68 pages and includes 7 subsections. In the first subsection demographic and clinical laboratory characteristics of the individuals investigated are presented. The lack of heterogeneity in relation to the sex and age in the two groups investigated (patients and healthy controls) proves a well-balanced selection that guarantees the quality of the comparative analysis. In the following five subsections the results of each of the biomarkers investigated (Dkk-1, sclerostin, sRANKL, osteopontin and periostin) are demonstrated. Complying with the plan of the scientific research, they are described and visualized correctly and follow the sequence of the tasks.

Starting with the completion of the first task, the PhD student detects statistically higher levels of the proteins investigated in newly diagnosed patients in comparison with the control group and in relation to their sex, the significant differences are only for sclerostin and periostin in healthy individuals. On evaluating the influence of the kidney function on the serum levels of the biomarkers thus investigated, a statistical significance is observed only for osteopontin in patients with impaired renal function. At stage T0 the dependence of the investigated proteins on the stage of the disease, the extent of the bone disease and bone marrow infiltration with plasma cells, as well as the correlational relationship with the routine hematological, morphological and biochemical indicators is evaluated. The proteins thus selected adequately reflect the stage and the severity of the bone disease and correlate significantly with the approved for MM beta-2 microglobulin. For each of the investigated biomarkers the dynamics of their serum levels is monitored in the course of the therapy and depending on the treatment response. For all indicators a decrease in the values is detected which depends on the number of chemotherapy courses carried out and has a great effect on the transplant patients.

In the last subsection a ROC-analysis is carried out to determine the diagnostic value of the investigated proteins. For all the investigated indicators AUC has the value of 0.8 at $p<0.0001$, and for two of them (sclerostin and periostin) a value is reached even close to 0.9, which confirms their excellent diagnostic value. With the help of the same analysis, the PhD student investigates the predictive value of each one of the proteins and their capability to correctly select patients with light or severe injury of the skeletal system. All investigated proteins with the exception of sRANKL are characterized by a high value of AUC which gives reason to the author to apply a logistic regression analysis as well in order to evaluate and assess the individual effect of the investigated biomarkers on the MBD progression. It has been established that the sclerostin and periostin parameters form the most significant combination in relation to a prognosis for the bone disease progression.

The discussion of the outcomes takes place in a separate section ***Discussion*** which is

developed in 30 pages and follows the logic of the research carried out. The doctoral student demonstrates in it his excellent knowledge of the pathogenesis of MBD and profound communication skills and conclusions about his own results obtained. Parallels have been drawn with the results obtained by other foreign investigations for all parameters studied, proportionally distributed in the volume of the contents. As a whole the discussion is elaborate and in-depth, it shows excellent knowledge and understanding of the peculiarities of the pathogenesis of MBD. The personal interpretation of the PhD student has been supported by arguments in a convincing and justified manner when it comes to investigating the reasons for the available correspondences and differences.

The conclusions of the dissertation are 12 and have been formulated correctly extracting the results of the eight objectives set.

Evaluation of the dissertation work contributions

The dissertation ends with the presentation of ***8 contributions,*** the merit of which lies in their originality both in terms of science and its implementation in practice. I fully accept them.

Evaluation of the publications related to the dissertation

The PhD student presents ***4 publications***, two of which are in Bulgarian periodicals and two – in international journals with high impact factor. Dr Vladimir Gerov is the first author in all these publications which is circumstantial evidence of his personal contribution to the development of the scientific concept, data collection, study design, summary of the results and their publication. All this is supported also by the presentation of part of the results at five national and international scientific forums, in four of which the doctoral student is the lead author.

Abstract

The abstract is spread over 115 pages and is structured analogously to the dissertation. It is illustrated with 50 tables and 52 figures which gives a very good idea of the aims and objectives set, achieved results and discussion, conclusions, and self-evaluation of the contributions.

Conclusion

Dr Vladimir Gerov's dissertation is dedicated to a topical medical problem and contributes to the detection and introduction in the clinical practice of new biomarkers for

evaluating the bone disease in MM patients. In the long term the results thus obtained could be a prerequisite for developing new biological agents for target therapy of MM, which is a kind of additional contribution of the dissertation.

In terms of structure, volume and content, the dissertation thus submitted meets all the requirements of the Act for the development of the academic staff in the Republic of Bulgaria (ADASRB), the Guidelines for the implementation of ADASRB and the corresponding Guidelines of MU, Varna. The dissertation is an original work written by the PhD candidate and demonstrates his theoretical competence and professional skills in the field of the scientific specialty "Hematology and blood transfusion", including academic analyticity for scientific information and conceptuality for carrying out scientific research.

Everything formulated above is a reason to give a positive assessment and evaluation and to recommend before the esteemed Scientific jury for awarding Dr Vladimir Todorov Gerov the educational and scientific degree **"Doctor of Philosophy"/PhD** according to the doctoral program "Hematology and blood transfusion".

Date: 07.05.2024 Author of review: ……

(Prof. Valeriya Kaleva, MD, PhD)