REVIEW

by Prof Valeria Ignatova Kaleva, MD PhD

on a dissertation work for awarding educational and scientific degree "Doctor" in professional field 7.1 Medicine

doctoral program 03.01.39 "Hematology and blood transfusions"

titled: **STUDY OF THE IMPACT OF LOW FVIII LEVEL ON BONE MINERAL DENSITY IN ADULT PATIENTS WITH SEVERE HAEMOPHILIA A IN CORRELATION WITH MARKERS OF BONE METABOLISM**

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1. General presentation of the procedure and Doctoral candidate

The presented materials are in accordance with art. 115 (1) of the Procedure for acquiring the scientific degree "doctor" at the Medical University – Plovdiv, the Rules of Organization and Activity of the Medical University – Plovdiv (2020), the Rules for Academic Development of the Medical University – Plovdiv (2021) and includes the following documents:

1.application to the Rector of MU – Plovdiv for disclosure of the dissertation procedure;

2.dissertation;

3. abstract of dissertation in Bulgarian and English;

4.curriculum vitae of the doctoral candidate (European format) with signature;

5.a certified copy of the diploma for medical doctor

6.a list of the author's publications related to the dissertation work;

7.copies of the author's publications;

8. Rector's order to enroll Hristina Ivanova in an individual form of study (Order R-3364/20.12.2022) and printed order for discharge with the right to defend (Order R-2039/14.07.2023);

8. protocol for completed doctoral exam in the specialty;

10.a declaration of originality and authenticity of the attached documents;

11.certificate for credits received from the group curriculum of Doctoral school

12.information card of NACID for a defended dissertation in Bulgarian;

13.information card of NACID for a defended dissertation in English.

Dr. Hristina Antonova Ivanova graduated in medicine from the Medical University – Plovdiv in 2012 and started her professional career as an intern at the Neonatology Department at USBALAG "Selena" – Plovdiv. In 2013 she started specializing in clinical hematology at the Clinic of Hematology, University Hospital "St. George", and since 2017 she has been an assistant in English-language training at the Department of Hematology, I-st Department of Internal Medicine, MU – Plovdiv. In 2019, she acquired a specialty in Clinical hematology and was employed at the Clinic of Hematology, University Hospital "Sv. Georgi" – Plovdiv, where she currently works.

Dr. Ivanova was enrolled as a PhD student in an individual form of study by Order No 3364/20.12.2022 with a dissertation on "Study of the impact of low level of FVIII on bone mineral density in adult patients with severe hemophilia A in correlation with markers of bone metabolism". It was deducted in time with the right to defend from FC and an order of the Rector of MU – Plovdiv No R-2039/14.07.2023.

2. Relevance of the topic

Therapeutic options to control hemorrhage episodes in patients with severe hemophilia have increased significantly in recent years and together with the decreasing incidence of hemophilic arthropathy, the focus of attention has shifted to other morbid factors relevant to their bone health.

After the first publications in 2007 and 2009, proving that about 70% of patients with hemophilia have osteopenia and osteoporosis, interest in this field is becoming the subject of various clinical studies. Despite their growing number, many questions related to the assessment of risk factors for their occurrence and prevention remain open and need further research. The results of available studies on the impact of low FVIII on bone mineral density (BMD) in patients with haemophilia A (HA) are ambiguous and an answer to the question whether low BMD is inherent in FVIII deficiency or a secondary phenomenon is still being sought.

On the other hand, in different countries and centers the therapeutic strategies for patients with severe hemophilia are different due to economic and other reasons. In this sense, the study of the problem of low BMD and the associated osteopenia and osteoporosis acquires great importance both for assessing their local frequency and severity, as well as to recommend algorithms/protocols adapted to the results from screening, prevention and therapeutic behavior.

In the context of the thus formulated reasons, I believe that the proposed topic is interesting and, with its unresolved problems, could serve as a basis for new scientific research.

3. Knowledge of the problem

The literature review is analytical, sufficient in volume and corresponds thematically to the purpose and tasks of the dissertation. The PhD student demonstrates a very good knowledge of the available information on the chosen topic and creative ability to structure and discuss literary data. At the end of the review, an analysis of the unsolved problems is made and the reasons for developing the dissertation are indicated. The review is written in literary Bulgarian and with a very good scientific style.

4. Methodology of the study

By nature, the study is diagnostic and controlled. The subjects of the study were patients with severe HA and healthy controls with clearly defined inclusion and exclusion criteria for participation. For its realization, modern methodological approaches and diagnostic methods were used, including the study of BMD by bone densitometry (DXA) and of plasma levels of some specific markers of bone metabolism. The tests are carried out using established technologies and with certified reagents. The statistical analysis is consistent with the type of data and the distribution of magnitudes.

5. Characterization and evaluation of the dissertation and contributions

The dissertation includes 119 pages. It contains 22 figures and 12 tables, which are informative and illustrate the statistically significant results. It is structured correctly in sections, the volume of which corresponds to generally accepted principles.

The literature review is developed on 31 pages and includes 201 sources, of which 10 in Cyrillic and 191 in Latin. The majority of them (129) are from the last 10 years. The current knowledge on the biology and regulation of bone metabolism, the diagnosis of BMD, the main causes of osteoporosis and the specific factors contributing to its greater incidence in patients with hemophilia are presented in great detail: presence of haemophilic arthropathy, decreased physical activity, lower Vit. D levels, lower body mass index (BMI) and higher incidence of blood viral infections. Special attention is paid to the new concepts for the development of osteoporosis in severe FVIII deficient patients and the modern possibilities for prevention and treatment of this complication.

Summarizing the literature data on the frequency and risk factors for the development of osteoporosis in patients with hemophilia, the dissertant finds grounds for studying the problem in the adult patients treated in their center with severe HA and formulates his scientific hypothesis. In addition to the well known mechanisms responsible for bone loss in the general population and the additional risk factors identified so far in HA, the dissertant suggests that osteoporosis may be a direct

consequence of low plasma FVIII levels and some of its extrahemostatic functions as a key regulator of bone metabolism. The author assumes that by understanding the role of FVIII in reduced BMD in HA, it will be possible to answer the question whether innovative nonfactor replacement therapies would reduce the risk of developing osteoporosis in patients with severe and moderate FVIII deficiency.

The aim of the study is short and precisely defined.

The tasks for its implementation are clearly formulated, follow the logic of the literature review and their solutions are in line with the diagnostic methods used.

Section ***Material and methods*** is in a volume of 9 pages. The study included 28 men with severe HA and 33 healthy controls with similar age, demographic and anthropometric data. Appropriate inclusion and exclusion criteria were specified for their selection. All participants were assessed for physical activity and the FRAX index was calculated for all patients over 40 years of age. In addition, patients with haemophilia were assessed for the severity of hemophilic arthropathy by the *Gilbert* score.

The applied diagnostic methods are divided into two categories – instrumental and laboratory. The dual energy X-ray absorptiometry (DXA) method was used to evaluate the BMD, which is well justified as a choice and described in details - algorithm and requirements to perform. Laboratory methods have been used to study plasma levels of several markers of bone metabolism exploited in various studies in recent years: osteocalcin (OS), osteoprotegerin (OPG), nuclear factor receptor activator Kappa beta Ligand (sRANKL), C-terminal telopeptide of collagen type 1 (CTX-1) and Vit. D. They were evaluated by high-sensitivity ELISA-reagents with high precision and low variability in series and time.

The statistical methods, together with the software programs for data processing, are presented in details and competently.

The presentation ***of the results*** is spread over 27 pages .They follow the sequence of tasks and are also illustrated with 11 tables and 20 figures. After the detailed assessment of the general characteristics of the studied cohorts, the results are grouped and summarized according to the tasks.

On *task 1*, the doctoral student found that patients with severe HA were characterized by significantly lower BMD values, higher OC and OPG levels, and lower sRANKL levels compared to healthy controls.

On *task 2*, when analyzing the incidence of low BMD, osteopenia and osteoporosis, the author found that a total of 60.1% of patients have low BMD, with the relative share of cases in patients aged > 50 years being significantly higher than in the subgroup aged ≤ 50 years.

On *task 3* in the group of patients with HA, the dissertant proved a significant association between low BMD and lower body weight, complete lack of physical activity and more frequent presence of polyarticular involvement of joints.

Under *task 4,* the PhD student proved significant associations of BMD at the spinal level with two of the biochemical markers of bone metabolism – OC and sRANKL.

Under *task 5,* the author was able to identify OC with an acceptable degree of reliability as a biomarker for dysregulation of bone cycling.

The data analysis was carried out with the statistical program SPSS for Windows 22, and a full justification was made for the statistical methods used in each of the surveyed indicators.

The discussion of the results is drawn up in a separate section, which is developed on 10 pages and follows the logic of the conducted study and the tasks set. In it, the dissertant demonstrates in-depth discussion skills and conclusions about the results obtained. The comparison of own results with those of other foreign studies is in terms of all parameters studied, proportionally distributed in terms of volume of content.

The author pays special attention to the limitations in the study, which shows its criticality in developing the problem. Emphasis is placed on the possibilities of increasing the statistical reliability of the results by increasing the number of studies at new time points or by the number of participants studied.

In the discussion, the doctoral student brings out and formulates recommendations for further studies in order to more precisely identify the mechanism of low BMD in HA and to develop strategies for the prevention and treatment of osteoporosis and fracture events in high-risk patients.

The proposed *Algorithm for prevention, diagnosis and treatment and follow-up of low BMD, osteopenia and osteoporosis in patients with severe Haemophilia A* includes recommendations based mainly on existing contemporary literature data and are educational and wishful in nature.

The conclusions of the dissertation are 10 and extract the results of the six tasks. It is noteworthy that some of them have correlations with results from other studies, which is most likely related to a technical error (item 1, item 10). The conclusions defined in points 8 and 9 are based on results obtained at untested plasma levels of FVIII and are not consistent with the individual clearance of prophylactic applications FVIII and with the complex interaction of the biochemical markers studied with other known risk factors for reduced BMD. All these details and parameters are not included in the analysis, suggesting a significant heterogeneity of the results and a tentative nature of the conclusions drawn.

6. Evaluation of the contributions of the dissertation

The dissertation work ends with the presentation of contributions which are divided into four categories. I firmly accept the first two of the category "Of an original character", the first and the third of the category "Confirmatory" and the two proposed by the category "Scientifically applied".

7. Evaluation of publications and personal contribution of the doctoral candidate

The PhD student presents four publications in connection with the dissertation, 3 of which are in a referenced database. There are also two scientific reports that are presented at international events and have published abstracts in prestigious journals with a high impact factor.

In all publications and scientific reports Dr. Ivanova is the first author, which is an indirect proof of her independent merit in the development of the scientific concept, the collection of data, the design of the study and the writing of the dissertation itself.

8, Abstract

The abstract of dissertation is spread over 46 pages and structured in the same way as the dissertation. Gives a very good idea of the set goals and tasks, achieved results, conclusions and self-assessment of the contributions. The exhibition is laconic, concrete and qualitatively illustrated with 10 tables and 20 figures.

9. Critical remarks and recommendations

With the exception of the remarks, in the Discussion and Conclusions sections, I have no other critical remarks. The inconsistencies allowed, as well as the small number of technical and grammatical inaccuracies, do not diminish the value of the dissertation. As a recommendation, I would insist that the PhD student continue to apply the acquired knowledge in his scientific and professional path and to contribute to the development of scientific quests and clinical implementations. The current state of the problem and the contributions of the dissertation motivate future research to identify the mechanism of bone damage and to develop strategies for prevention and treatment of groups at high risk for osteoporosis and fracture events. The introduction of new treatment strategies in patients with HA would contribute to more definite conclusions about the impact of FVIII on bone health in the Bulgarian patient population and to provide guidelines for effective and safe preventive strategies in patients with hemophilia.

Conclusion

The dissertation work of Hristina Ivanova,MD contains scientific results, some of which have been studied for the first time in patients with HA in Bulgaria and have an original character. They meet all the requirements of the Law on the Development of Academic Staff in the Republic of Bulgaria , the Regulations for the Implementation of the Law on the Development of the Academic Staff in the Republic of Bulgaria and the relevant Rules of the Medical University – Plovdiv. The topic of the presented work is original, unexplored in Bulgaria, and the presented materials and dissertation results fully comply with the specific requirements of MU – Plovdiv.

The dissertation shows that the PhD candidate Hristina Ivanova,MD has in-depth theoretical knowledge and professional skills in the scientific specialty "Hematology and blood transfusion", demonstrating qualities and skills for synthesis and analysis of scientific information and independent conduct of scientific research.

In the context of everything stated so far, I give ***a positive assessment*** of the conducted research, presented by the peer-reviewed dissertation, abstract of dissertation, results and contributions, and I propose to the honorable scientific jury ***to award the educational and scientific degree "Doctor"*** to Hristina Antonova Ivanova, MD.

3 February 2024

Prepared the review. .....

Prof. Dr. Valeria Kaleva, MD, PhD