Statement

By: assoc. prof. Ivanka Nenova - Chilova, MD, PhD

Regarding: a dissertation awarding the educational and scientific degree "Doctor"

Field of higher education: 7. " Healthcare and sport"

Professional field: 7.1. Medicine

Doctoral program: Hematology and Blood transfusions

Author: Hristina Ivanova, MD, doctor at UMHAT "Sveti Georgi" EAD, Medical university Plovdiv

Form of doctoral study: Independent preparation

Topic: STUDY OF THE IMPACT OF LOW FVIII LEVEL ON BONE MINERAL DENSITY IN ADULT PATIENTS WITH SEVERE HEMOPHILIA IN CORRELATION WITH MARKERS OF BONE METABOLISM

Scientific supervisor: Prof. Dr. Zhanet Grudeva-Popova, MD, PhD

General presentation of the procedure: The presented set of materials is in accordance with the regulations of MU Plovdiv regarding the procedure for acquirement of the educational and scientific degree "Doctor" namely the orders for enrolling and dismissing doctoral students, the protocols from the expanded departamental councils reflecting all stages of the doctoral student's development, dissertation, and abstract. The set of materials includes a list and copies of the publications related to the dissertation work. There are three publications in which the doctoral candidate is a leading author. Two of the publications are in the journal Haematology - the official journal of BMSH. One publication is in the journal Folia medica - the edition of MU Plovdiv. Both journals are referenced and indexed in the international database Scopus. Four scientific articles are presented in national and international forums. The procedure for the doctoral program has been followed.

1. Brief biographical data of the doctoral candidate

Doctor Hristina Antonova Ivanova graduated from MU Plovdiv in 2012. She acquired a specialty of Clinical Hematology in 2018. She has been an assistant at section Hematology at First department of Internal Medicine since 2017. The scientific interests of doctor Hr. Ivanova include benign hematological diseases - hemorrhagic diathesis and thrombophilia. She is a member of international scientific studies. She is fluent in English and German.
The doctoral candidate has theoretical knowledge, practical experience, additional qualifications and focused scientific - practical interests in the field of doctoral studies as well as demonstrated high moral and ethical values.

2. Relevance of the topic and understanding of the issue

Doctor Hristina Ivanova works on a relevant and socially significant issue of modern clinical hematology. Hemophilia A (HA) is the most severe congenital coagulopathy. With the increased life expectancy and the introduction of prophylaxis regimens an increasing attention has been paid to comorbidities such as low bone mineral density and osteoporosis. The pathogenesis of the condition is not clear. No studies on bone mineral density in Bulgarian patients with a severe Hemophilia A have been conducted. The mechanisms for osteoporosis in patients with Hemophilia A include the well - known mechanisms for bone mass loss in the general population, the additional risk factors in Hemophilia A as well as the FVIII deficiency which is a regulator of the bone cycle. The understanding of FVIII' role in low bone density in Hemophilia A is important for the choice of therapy including non - factor replacement therapies and the consequences for bone health. This is the rationale for this dissertation. **The topic of the dissertation is relevant and has a great social and clinical impact.**

3. Familiarity with the scientific issue

The presented scientific hypothesis suggests that low bone density resulting directly from FVIII deficiency (decreased thrombin synthesis and impaired proliferation of osteoblasts with thrombin receptors as well as desinhibition of RANKL - induced osteoclastogenesis) has a key role alongside the well - known mechanisms for bone mass loss in the general population and the additional risk factors for patients with Hemophilia A (severity of hemophilic arthropathy, lack of physical activity, low weight, BMI and vit. D deficiency). **The author has a deep understanding of the scientific issue, can formulate a scientific hypothesis and conduct an analysis the results of which can lead to improvements in treatment and monitoring of patients with a severe Hemophilia A and enhance their quality of life.**

4. Research methodology

The dissertation work is conducted as a single - center prospective study in First department of Internal medicine, Section of Clinical Hematology in Medical University Plovdiv and Clinic of Clinical hematology in UMHAT "Sv. Georgi" Plovdiv within a two - year period (2019 - 2021). Patients are selected based on strict inclusion and exclusion criteria to eliminate the influence of the well-known risk factors for low bone density and are divided in two groups based on their age: ≤ 50 and >50 years. **The methodology is appropriately chosen, the design includes bone density measurement and monitoring of the impact of prophylactically used Factor VIII product on the level of the tested bone biomarkers in patients with severe Hemophilia A and comparison with healthy control groups.**

5. Characterisation and evaluation of the dissertation work

The dissertation work is 119 pages, including 12 tables and 22 figures. 201 literature sources are cited. The mandatory chapters of the scientific work are professionally developed with an acceptable balance between them. Clear, precise and grammatically correct Bulgarian language is used.

6.1. Literature overview

Literature overview is presented in 33 standart pages. It describes bone marrow metabolism and its regulation by hormones and signal pathways. The tested bone biomarkers such as OPG, RANKL, osteocalcin, STN - 1 as well as vit. D levels are presented in a justified manner. The terms low bone density, osteopenia and osteoporosis along with their diagnostic methods are defined. The well - known risk factors for the general population and patients with Hemophilia A as well as pathological mechanisms of dysregulation of bone cycle, resulting from FVIII deficiency (investigated only in experimental models with animals and cell cultures until now) are taken into account. **Literature overview concludes with a motivated basis of a research on bone density in patients with FVIII and enables formulation of an objective and a scientific hypothesis.**

6.2. Objective and tasks

The objective of this dissertation is to research the frequency of low bone density and its influence on the low level of FVIII in adults with severe Hemophilia A and to look for correlations with some of the biochemical markers for bone remodeling. It is logically formulated and with logically put 6 major tasks.

6.3 Section Material and methods

It is presented in 9 standard pages. 28 men with severe Hemophilia A selected via precise inclusion and exclusion criteria participate in the research. The control group includes 33 healthy men. The imaging and laboratory methods for bone density evaluation are described in detail and are appropriately selected in accordance with the recommendations for diagnosing and monitoring of osteoporosis. The utilized methods are documentary method, physical activity assessment with a questionnaire, physical exam for clinical evaluation of the severity of hemophilic arthropathy. FRAX index has been calculated for all patients above 40 years. Clinical laboratory methods include bone markers measurement with ELISA Kits Immunodiagnostic.A gold standard for evaluation of bone density - DXA is used. The statistical result processing is conducted with appropriately chosen analyses and data is visually presented. **I completely accept the section Material and methods without any critique.**

6.4. Section Results

Section Results is presented in 32 standard pages. The exposition of the section is concise and is visually presented through tables, figures, graphics and diagrams. Men with Hemophilia A have statistically significantly lower levels of bone density and Z/T - score and higher levels osteocalcin and OPG and lower RANKL and RANKL/OPG and similar CTX-I levels and average vit. D levels. 60.71% of the patients have low bone density on the spinal level, proximal femur and / or femoral neck. Regardless of the age group, a significantly higher relative ratio of low bone density is observed in patients than in the control group (12.12%). 43.75% of the patients below 50 years are with low bone density (<-2) measured with the Z - score. 83.33% of the patients above 50 years are with low bone density measured with the T - score. The cases of osteopenia and osteoporosis are evenly distributed - 41.67%. A significant association is found between low bone density and the following factors: lower weight, lack of physical activity and

the presence of polyarticular involvement. No connection to BMI and vit. D levels is found. The severity of hemophilic arthropathy along with the related chronic inflammatory response and the loss of joint function are risk factors for low bone density at the level of bone neck.
Higher levels of osteocalcin and lower levels of sRANKL are observed in patients with lower bone density measured in g/cm2 and Z/T - score at lumbar spinal level. Osteocalcin is defined as a biomarker with a significant diagnostic and prognostic function with critical value > 0.86 ng/ml for the identification of patients with low density with an accuracy 78.10%.
In support of the chosen objective and tasks, the results show statistically significantly lower level of RANKL and higher level of OPG and osteocalcin after FVIII application, which supports the hypothesis for non - hemostatic role of the coagulation factor in bone metabolism. **I have no critique of the obtained results.**

6.5 Section Discussion
In 19 standard pages the doctoral candidate analyzes her own obtained results, discusses their importance and compares them to the ones by other authorial collectives. The discovered differences with other scientific discoveries are explained with sound arguments. The presented algorithm for prophylaxis, diagnostics, treatment and monitoring low bone density, osteopenia and osteoporosis in patients with severe Hemophilia A holds practical significance. **I accept Section Discussion.**

6.6. There are 10 **conclusions** that are logically derived in accordance with the obtained results. The most important clinical aspect conclusion is that low bone density in patients with severe Hemophilia A is a complication from a young age. Understanding the issue and the implementation of screening and prophylaxis could contribute to the patients. **I agree with the conclusions formulated in this way.**

6.7. Bibliography
The bibliography includes 201 literature sources, 10 of which are by Bulgarian authors. The analyzed scientific publications after 2015 are 30 % of the total number. I have no critique.

7. Evaluation of the contribution of the dissertation work
The dissertation work concludes with a presentation of contributions that have scientific and applicable character. Three of the contributions are original in nature and three are confirmatory. **I accept the presented contributions.**

8. Personal involvement of the doctoral candidate
The doctoral candidate has a personal involvement in formulation of the scientific idea, material collection and research design. She also has a personal involvement in statistical data processing and analysis of the results. The conclusions and contributions are independently derived. The doctoral candidate has a key personal engagement in the dissertation development.

9. Author's abstract
The author's abstract has 46 pages and gives a full overview of the dissertation work. The figures and tables are appropriately selected for data presentation.

10. Conclusion:The dissertation work of doctor Hristina Ivanova meets the requirements of LDASRB, the Regulations for its implementation, and the Regulations of Medical University - Plovdiv. The dissertation's topic is original for the Bulgarian medical science community. Its preparation is precise. The contributions of the dissertation have scientific and scientific - applicable character. They establish a solid foundation for optimal monitoring, early discovery of complications and right choice for therapy of patients with severe Hemophilia A. The dissertation work of doctor Hristina Ivanova shows deep scientific knowledge, analytical skills, synthesis of scientific data, ability to create scientific hypotheses and to obtain sound - proof scientific contributions.

I give a positive feedback for the dissertation work on topic STUDY OF THE IMPACT OF LOW FVIII LEVEL ON BONE MINERAL DENSITY IN ADULT PATIENTS WITH SEVERE HEMOPHILIA IN CORRELATION WITH MARKERS OF BONE METABOLISM. I recommend the honorable scientific jury to vote for awarding the educational and scientific degree "Doctor" to Hristina Antonova Ivanova.

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