

OPINION

from



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"Doctor"

Subject: Dissertation project for awarding the educational and scientific degree "Doctor"

Field of higher education: 7. "Health and sports"

Professional direction: 7.1. "Medicine"

Doctoral program: "Hematology and blood transfusion" 03.01.39

Author: Dr. Hristina Antonova Ivanova

Form of doctoral study: full-time doctoral study

Department: First Department of Internal Medicine, Section of Clinical Hematology

Topic: INVESTIGATING THE EFFECT OF LOW FVIII LEVELS ON BONE MINERAL DENSITY IN ADULT PATIENTS WITH SEVERE HEMOPHILIA A IN COMPARISON TO MARKERS OF BONE METABOLISM

Research supervisor: Prof. Janet Grudeva-Popova, MD, PhD

General presentation of the procedure

The presented set of materials on the dissertation work on the topic "Investigation of the influence of the low level of FVIII on bone mineral density in adult patients with a severe form of hemophilia A in comparison with markers of bone metabolism" of Dr. Hristina Antonova Ivanova is available in electronic and paper formats carrier. It is in accordance with the procedure for acquiring the educational and scientific degree "Doctor" and the Rules of the

MU-Plovdiv for the development of the academic staff and includes all the necessary documents for the procedure: dissertation, author's abstract, European format of the doctoral student's resume, as well as all other required administrative documents from the various stages of the doctoral student's education, including those necessary for the procedural advancement of the presentation of the dissertation. In total, the presented publications are 3. Two of the publications are in the journal "Hematology", one in "Folia Medica", referenced and indexed in the international database "Scopus". Overall, 4 scientific reports from participation in national and international scientific forums are also presented.

Presentation of the PhD student

Dr. Hristina Antonova Ivanova graduated from the Medical University of Plovdiv in 2012. She obtained a specialty in clinical hematology in 2018, and since 2017 is an assistant professor at the "Hematology" section of the First Department of Internal Medicine of the Medical University - Plovdiv. Dr. Ivanova participated in a number of conferences and training courses in the field of hematology. Dr. Ivanova's scientific interests are in the field of haemorrhagic disorders and thrombophilia. She is a part of the teams in several international clinical trials. She speaks English and German. She has 10 years of experience in the field of clinical hematology.

Actuality of the topic and knowledge of the problem

The dissertation work is devoted to a modern, socially significant problem concerning the impact of low levels of FVIII on bone metabolism in patients with Hemophilia A. Hemophilia A is a rare disease, but it is the most severe congenital coagulation disorder. With advances in therapeutic intervention such as the introduction of purified and recombinant factors, as well as factor products with extended half-lives and non-factor products, the quality of life and survival of patients with hemophilia A have improved significantly. Comorbidities and complications such as low bone mineral density (BMD) and osteoporosis are increasingly becoming a priority problem for patients with Hemophilia A. Studies have demonstrated that 70% of patients have significantly reduced BMD, which is associated with an increased risk of fracture following minimal trauma. The pathogenesis of the condition is not yet fully understood. In addition to the known mechanisms responsible for bone loss in the general population and the additional risk factors proposed so far in patients with hemophilia A, data on a direct effect of low FVIII on bone metabolism through inhibition of RANKL and the associated osteoclast response are of interest. Until now, the literature lacks data on the

frequency and characteristics of reduced BMD in Bulgarian patients with a severe form of hemophilia A, as well as data on bone metabolism markers and their role in prevention, diagnosis and treatment algorithms. This gives reason to the dissertation student to develop the current dissertation work.

Knowledge of the scientific problem

The well-formulated scientific hypothesis shows that the author understands the scientific problem in depth and conducts an analysis, the results of which would lead to an improvement in the treatment and follow-up of patients with a severe form of Hemophilia A and an increase in their quality of life.

Research methodology

The research methodology was correctly chosen. The dissertation work was carried out as a single-center prospective study, conducted in the First Department of Internal Medicine, Hematology Section at the Medical University of Plovdiv and Clinical Hematology Clinic UMBAL "St. Georgi", the city of Plovdiv between 2019 and 2021. Patients were selected through strict inclusion and exclusion criteria to eliminate the impact of known risk factors for reduced BMD and were divided into two subgroups according to age: ≤ 50 years and >50 years.

Characterization and evaluation of the dissertation work

The presented work is structured according to the accepted standards of a dissertation for obtaining the educational and scientific degree "Doctor" in medicine. The dissertation is written in 119 standard pages and includes: table of contents (1 page), abbreviations (1 page), literature review (33 pages), aim and objectives (1 page), materials and methods (7 pages), results (31 pages), discussion (19 pages), conclusions (1 page), main contributions of the dissertation work (1 page), bibliography (19 pages). The bibliographic list contains a total of 201 literary sources, 10 of which are in Cyrillic and 191 in Latin. The work is illustrated with 12 tables and 22 figures.

Literature review

The literature review is presented in 33 standard pages in depth and focused on the scientific problem. In separate paragraphs, the biology of bone metabolism, the cellular elements of bone and the extracellular matrix, bone formation and bone turnover, the regulation of bone metabolism are discussed, and the studied bone biomarkers - OPG, RANKL, osteocalcin, CTX-I, as well as the levels of Vit.D. The known risk factors for the general population and for patients with Hemophilia A and the various pathogenetic mechanisms of bone turnover

dysregulation resulting from FVIII deficiency are reviewed in detail. Modern treatment options for coagulation deficiency and osteoporosis are also discussed.

Aim and tasks

The aim of the study was clearly and precisely formulated, namely, to investigate the frequency of low BMD and the influence of low FVIII level in adult patients with severe form of hemophilia A and to look for a relationship with some of the biochemical markers of bone remodeling. The synchronously set 5 main tasks are clearly formulated and follow the aim of the study.

Material and Methods Section

The Material and Methods section is presented in 9 standard pages. Twenty-eight men with a severe form of Hemophilia A over 18 years of age and a control group of 33 clinically healthy men, similar in terms of demographic and anthropometric data to the patients, participated in the study. Inclusion and exclusion criteria are precisely defined. Imaging and clinical-laboratory methods for the study of BMD are appropriately selected in accordance with the recommendations for the evaluation and follow-up of osteoporosis. Physical activity was assessed with a questionnaire, a physical method for clinical assessment of the severity of homophilic arthropathy, and a FRAX index was calculated for all patients over 40 years of age. Levels of bone turnover markers were assessed using original ELISA Kits Immunidagonstic for sRANKL, OPG, osteocalcin, CTX-1 as well as competitive heterogeneous immunoenzymatic method for determination of 25(OH)D levels. The statistical methods used and the software product for the purpose of data analysis and visual presentation are described in detail.

The Results section is presented on 32 standard pages. The results of the set five tasks are analyzed, systematized, presented concisely and consistently and illustrated with the help of tables, figures, graphs and box plots. Hemophilia A men had statistically significant lower BMD g/cm² and Z/T-score and higher levels of Osteocalcin and OPG and lower levels of RANKL and RANKL/OPG and did not differ by marker levels of bone resorption CTX-I and the mean level of Vit.D. A total of 60.71% of patients were found to have low BMD at the spinal level, proximal femur and/or femoral neck. Regardless of age group, patients had a significantly higher relative proportion of cases with low BMD compared to healthy controls (12.12%). Of patients ≤50 years, 43.75% had low BMD (<-2) by Z-score. Of the patients >50 years, 83.33% have low BMD by T-score. The cases of osteopenia and osteoporosis are evenly distributed at 41.67%. A significant association was found between low bone density and the following factors: lower

weight, complete lack of physical activity and more frequent presence of polyarticular involvement, and no association was found with BMI and Vit D level. Both were equally affected locations, regardless of the severity of arthropathy in most of the patients. The extent of hemophilic arthropathy and the associated chronic inflammatory response and loss of joint function is a risk factor for low BMD at the femoral neck (FN) level.

Higher levels of osteocalcin and lower levels of sRANKL were observed in patients with lower BMD measured in g/cm² and Z/T-score at the spinal level (LS). Osteocalcin was determined as a biomarker with significant diagnostic and prognostic function at a criterion value > 0.86 ng/ml to identify patients with low bone density with an accuracy of 78.10%. The proposed hypothesis of an extra-hemostatic role of coagulation factor in bone metabolism is supported by the results of statistically significant low level of RANKL and high level of OPG and osteocalcin after FVIII administration.

The Discussion section is spread over 19 pages. The doctoral student analyzes and discusses the obtained own results in the context of the data published so far from the scientific literature, looking for reasons for the similarities and differences in the obtained results. The presented algorithm for the prevention, diagnosis, treatment and follow-up of low BMD, osteopenia and osteoporosis in patients with severe Hemophilia A makes practical sense for improving patient care.

There are 10 **conclusions** and they logically follow the goal and the set tasks.

The bibliography contains 201 literary sources, of which 10 are by Bulgarian authors. The scientific publications after 2015 are 30%. The sources are selected purposefully, which shows the ability of the doctoral student to select important scientific information with high research awareness in the chosen field.

Evaluation of the contributions of the dissertation work

The work ends with the presentation of scientific and scientific-applied contributions. Three of the contributions are of an original nature and three of a confirmatory nature. The results of the study and the proposed recommendations can be used in the development of an algorithm for screening, prevention, diagnosis and treatment of low BMD, osteopenia and osteoporosis in Bulgarian patients with a severe form of Hemophilia A.

Personal participation of the doctoral student

The doctoral candidate has personal involvement in the formulation of the scientific idea, the design of the study and the analysis of the obtained results. Conclusions and contributions are also independently derived.

Publications related to the dissertation work

In total, the presented publications are 3, in journals referenced and indexed in the international database "Scopus". In all three, the dissertation student is the lead author. Two of the publications are in the journal "Hematology" and one is in the journal "Folia Medica". Additionally, 4 scientific reports from participation in national and international scientific forums, in which the dissertation student is the lead author, are presented.

Abstract

The abstract contains 46 pages, makes a summary of the dissertation work. The most important studies, results and discussions on the scientific problem are summarized. Figures and tables have been selected to present the selected data.

Conclusion

The dissertation work of assistant professor Dr. Hristina Ivanova meets the requirements of the Law on the Development of Academic Degrees in the Republic of Bulgaria, the Regulations for its implementation and the Regulations of MU-Plovdiv for awarding the scientific and educational degree "Doctor". The topic of the dissertation work is up to date, not developed in Bulgaria. The dissertation contains important scientific and scientific-applied results, including those that represent an original contribution to science.

Based on the topic of the dissertation work and the scientific and practical value of the presented results, I confidently give my positive assessment for the dissertation work on the topic "Investigation of the influence of the low level of FVIII on bone mineral density in adult patients with a severe form of Hemophilia A in comparison with markers of bone metabolism" and I propose to the respected scientific jury to vote "for" the award of a scientific and educational degree "doctor" to Dr. Hristina Antonova Ivanova.

Date: 12.02.2024

Reviewer:


/Prof. Ilina Dimitrova Micheva, MD, PhD/

Заличено на основание
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