To
Chairman of the Scientific Jury,
According the Order No P-255/22.01.2024
of the Rector of the MU- Plovdiv
Prof. Angel Uchikov, MD, PhD, DMSc

OPINION
by Prof. Dr. Maurizio Nordio, MD, PhD, DMSc
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1. Procedural requirements

Presented for opinion is a dissertation entitled "CHRONIC AUTOIMMUNE HASHIMOTO THYROIDITIS AND CO-MORBIDITIES" by Dr. Dimitar Manolov Troev, doctoral student of independent training at the Department of Endocrinology, Medical Faculty of the Medical University - Plovdiv, with scientific supervisors: prof. Dr. Maria Orbetzova, Department of Endocrinology and Prof. Dr. Mariela Geneva-Popova, Department of Propaedeutics of Internal Diseases "Anton Mitov", Faculty of Medicine, Medical University - Plovdiv.

The provided set of materials on paper and electronic media is in accordance with the requirements for acquiring the educational and scientific degree "doctor" and the Regulations of MU-Plovdiv from 2021 and includes all the required documents.

2. Brief biographical data of the candidate

Dr. Dimitar Manolov Troev was born on 06.12.1964. He graduated from "Dimitar Blagoev" secondary school in Plovdiv in 1982. He completed his higher education in medicine at the High Medical Institute - Plovdiv in 1990. Since 1991, he has been a doctor at the Clinic for Endocrinology and Metabolic Diseases at "Sv. Georgy" University Hospital - Plovdiv. He worked as an assistant, later senior and chief assistant at the Endocrinology Section of the Second Department of Internal Medicine, MF, MU - Plovdiv. After the separation of the Endocrinology Section into an independent department in 2020 and in connection with the legal requirements, Dr. Troev became an assistant, then a teacher, being enrolled for a doctoral thesis of independent training in 2022. From 2021, he is an administrative assistant at the Clinic of Endocrinology and metabolic diseases.

Dr. Dimitar Troev has acquired clinical specialties - internal medicine (1995), endocrinology and metabolic diseases (1998). He has completed training courses and acquired highly specialized qualifications related to his medical activity.

3. Actuality of the topic

Globally, Hashimoto's thyroiditis is the most common autoimmune thyroid disease of any age, and its incidence has been increasing in recent decades. Basically, autoimmune thyroid diseases, which mainly include Hashimoto's thyroiditis and Graves' disease, affect approximately 5% of the general population, making them one of the most common organ-specific autoimmune diseases. Hashimoto's thyroiditis is known to be associated with other autoimmune diseases, which are a wide spectrum according to studies, ranging from involvement of endocrine or non-endocrine organs, specific or systemic diseases. Hashimoto's thyroiditis is part of the Autoimmune Polyglandular Syndromes (APS) and is a main element of the type 3. In this view, the most common is the combination with diabetes mellitus type 1, defined as APS 3A. In addition, non-autoimmune diseases, including malignancies, occur more frequently when an autoimmune thyroiditis is present, than in the general population. Most studies reported in the scientific literature that have evaluated different constellations of autoimmune diseases, usually examined the prevalence of Hashimoto's thyroiditis in nonthyroidal autoimmune diseases, rather than the opposite.

In Bulgaria, there are no large-scale studies characterizing the co-morbidities in Hashimoto's thyroiditis. In this sense, the dissertation, which is dedicated to studying the type and frequency of

autoimmune and non-autoimmune diseases, associated with autoimmune Hashimoto's thyroiditis in a Bulgarian population of hospitalized patients, is modern and relevant.

4. Structure of the dissertation; aim and objectives; material and methods

The dissertation is properly structured in terms of the main components. The volume is 180 pages, contains 29 tables and 33 figures, which help to present in a synthesized form the information from the extensive statistical analyses and enrich the presentation of the material.

A targeted *literature review* was made on the problem under consideration, with some general conditions regarding the development of polyautoimmunity and the predisposing factors, the characteristics of Hashimoto's thyroiditis, its presence within the autoimmune polyglandular syndromes. Combinations with autoimmune arthropathies and connective tissue diseases, particularly rheumatoid arthritis, are discussed in detail. Emphasis is placed on the relationship with gastrointestinal diseases and the role of zonulin as a biomarker of increased intestinal permeability and a possible mediator of autoimmune processes.

As formulated, the *aim* of the dissertation was to determine the type and frequency of associated autoimmune and non-autoimmune diseases in a randomized representative sample from the Bulgarian population of hospitalized patients with proven autoimmune Hashimoto's thyroiditis; to study the prevalence of Hashimoto's thyroiditis, resp. autoimmune polyglandular syndrome type 3A in patients with diabetes mellitus type 1; to study the prevalence and analyze the impact of Hashimoto's thyroiditis on the clinical course of one of the most common non-thyroid autoimmune co-morbidities - rheumatoid arthritis; to shed light on the role of zonulin in Hashimoto's thyroiditis as a biomarker of increased intestinal permeability and a possible predictor of thyroid autoimmunity progression.

The *tasks* derive from the objective and are 8 in total. They are aimed at realizing the main idea and objectives of the thesis and outline the nature of the clinical studies conducted. The tasks are precisely and clearly formulated.

The approaches used in the construction of the *design* of the clinical studies, the *material and the methods* are adequate to the purpose of the doctoral thesis and the set tasks. The methods (clinical, clinical-laboratory, instrumental and statistical) are presented in detail. The description of the methodology for determining the main hormonal, immunological and biochemical indicators is very precise, which speaks well for a clinician.

The 4 studies are presented as follows:

1. Randomized cross-sectional study on a sample of hospitalized patients with proven Hashimoto's thyroiditis (n=447) in the Clinic of Endocrinology and Metabolic Diseases, with analysis of anamnestic, clinical-anthropometric, clinical-laboratory data, including hormonal and immunological markers and imaging studies of the thyroid gland with an emphasis on co-morbidities.
2. Randomized cross-sectional study on a sample of patients with type 1 diabetes mellitus (n=67), admitted to the Clinic for Endocrinology and Metabolic Diseases in order to assess the prevalence of Hashimoto's thyroiditis, resp. APS type 3A, compared to a control group of age- and sex-matched clinically healthy individuals (n=124)
3. Retrospective analysis of data in a randomized representative sample of patients with early rheumatoid arthritis (n=180) hospitalized in the Clinic of Rheumatology, regarding the association with Hashimoto's thyroiditis and the follow-up of the latter's influence on the clinical course of the disease.
4. Study of zonulin levels in patients with newly diagnosed Hashimoto's thyroiditis (n=106), comparative analysis of clinical and laboratory markers in different functional states of the thyroid gland – hypothyroidism, euthyroidism and hyperthyroidism.

5. Results, discussion and conclusions

The *results* are presented according to the specific studies and their respective tasks, in a way that shows that the objectives of the doctoral thesis have been completely fulfilled and are personal work of Dr Troev. The *discussion* is logically structured and follows the description of the results of the various studies and ends with detailed conclusions. The doctoral student extensively comments

on the obtained data and compares them with those in the literature, and in addition to those mentioned in the review, analyzes many others, specifically related to the concrete aspects of the dissertation. This confirms a very deep knowledge of the thesis topic by the author, and sheds light onto the underlying ideas and his ability to perform a targeted analysis.

The *final conclusions* are 16 in number and represent a short summary analysis of the results regarding the main aspects of the doctoral thesis.

The *bibliography* is comprehensive, meets the requirements - it covers 460 basic and modern literary sources (8 in Cyrillic, 452 in Latin).

6. Contributions

The description of the characteristics of a large sample of 447 hospitalized patients with Hashimoto's thyroiditis is of important and informative value for practice, as such purposeful studies concerning this most widespread autoimmune thyroid disease are scarce. Hashimoto's thyroiditis was found more common in females (female:male ratio - 4.96:1), predominated in the age group of 29-61 years with the highest prevalence in the fourth decade (24.61%), with no significant differences in the percentage distribution in separate age groups by gender in this sample of hospitalized patients with proven disease.

The dissertation provides very valuable results regarding the spectrum of co-morbidities in Hashimoto's thyroiditis, the prevalence of which is very high. Thus, 84.8% of patients had co-morbidities without significant sex-related differences in their frequency - 83.6% in women, 90.7% in men, with the exception of hematological diseases - 20% in men and 11% in women.

The search for predisposing and risk factors for the appearance of associated diseases in Hashimoto's thyroiditis is also of practical importance. With increasing age, the probability of developing co-morbidities increased, with the average age of patients without concomitant diseases being significantly lower (36.80±10.63 years), compared to those with non-autoimmune (46.22±16.29) and autoimmune (45.54± 14.77) co-morbidities, which is important in terms of follow-up the course of the autoimmune process. The general co-morbidity was not directly related to the function of the thyroid gland - it was observed in 85.9% of patients with euthyroid, in 80.2% with hypothyroid and in 86.7% with hyperthyroid state, with the exception of rheumatological diseases - most common in hyperthyroidism (33.3%), in 16.7% with hypothyroidism and least often with euthyroidism (9.9%).

Overt metabolic syndrome was found in 26.85%, type 2 diabetes mellitus in 20.13%, separate components of metabolic syndrome - dysglycemia in 0.89%, dyslipidemia in 2.01%, which leads to the conclusion that about half of patients with Hashimoto's thyroiditis is with high cardiovascular risk. In addition, a large percentage of patients had arterial hypertension (38.26%) and ischemic heart disease (11.86%). These facts speak eloquently that accurate assessment of carbohydrate and lipid parameters and cardiovascular function is necessary, regardless of the functional status of the thyroid gland.

Relatively more often, anemia and gastroenterological diseases were detected, which requires active screening for iron and B12-deficiency already at the diagnosis of Hashimoto's thyroiditis, regardless of functional and immunological status, as well as during follow-up with a view to early diagnosis and timely treatment.

The percentage of autoimmune co-morbidities was high - 13.65%, and Hashimoto's thyroiditis was a component of APS in 7.16% of cases. Of the other accompanying autoimmune diseases, the most common were diabetes mellitus type 1, skin and rheumatological diseases, TAO. In samples of hospitalized patients, APS type 3A occurred in 3.58% of Hashimoto's thyroiditis patients, while in diabetes mellitus type 1 - 20% in men and 32.4% in women, indicating that the latter is the leading cause of hospitalization, during which the diagnosis of autoimmune thyroid diseases is made more accurately and in time due to targeted search. No significant difference was found in relation to the family anamnesis of diabetes mellitus in both male and female diabetics, while in relation to the family anamnesis of autoimmune thyroid disease was found in women only.

The study on the combination of rheumatoid arthritis and Hashimoto's thyroiditis in a representative sample of 180 individuals also has a great practical contribution, regarding the follow-up of thyroid autoantibodies and thyroid function in patients with newly diagnosed rheumatoid

arthritis. As an expression of polyautoimmunity, Hashimoto's thyroiditis can appear later in patients with rheumatoid arthritis, which is why initial screening for autoimmune thyroid disease is necessary, regardless of the presence of clinical symptoms, and also during the evolution of the disease, functional and immunological follow-up of the thyroid gland is important. The role of thyroiditis on joint destruction at the beginning and at different stages of development of rheumatoid arthritis was also evaluated. There was no significant association of Hashimoto's thyroiditis with the disease activity at the beginning and in the evolution of rheumatoid arthritis, but more pronounced radiographic changes of the joints were observed in the first 3 years.

Very interesting are the data on zonulin, studied in a sample of 106 patients with Hashimoto's thyroiditis, which are pioneering for Bulgaria. The levels of this biomarker were found to be significantly higher in carriers of an autoimmune thyroid disease compared to normal subjects, which indicates an increased intestinal permeability. An association of zonulin with body weight and insulin resistance was found - its levels positively correlated with weight, BMI and HOMA-index, being significantly higher in overweight and obesity, which supports its role in the pathogenesis of obesity and related disorders. This fact can be compared with the established significant relationship between weight and general co-morbidity in patients with Hashimoto's thyroiditis - higher in the presence of combined pathology. Data from the present study will shed light on the involvement of the gut microbiota in modulating autoimmunity and co-morbidity. In fact, it is well known that microbiota is considered a modulator of our immune system and its alteration may enhance the possibility to develop autoimmune diseases and/or insulin resistance.

I accept the distribution of the dissertation's contributions as original and confirmatory, with a predominantly scientific-theoretical and scientific-applied nature. The study is a pioneer for the country in a number of aspects examining Hashimoto's thyroiditis, and especially with regard to zonulin - also on a global scale.

7. Publication activity

In connection with the dissertation, the following materials have been presented: 1 participation in a monograph, 8 full-text publications, of which 2 in international journals, 1 with an Impact Factor and 2 publications in Folia Medica - journal of an international character; 7 participations in scientific conferences (3 international) and participation in 2 research projects.

8. Summary of the dissertation

The presented summary is properly structured, contains all the required details and reflects the most important aspects of the doctoral thesis, which fully meets the requirements of the Regulations of the MU-Plovdiv, in terms of content and layout.

9. Conclusion

Based on the above, I consider that the developed topic "CHRONIC AUTOIMMUNE THYROIDITIS OF HASHIMOTO AND CO-MORBIDITIES" is up-to-date and dissertable, meets all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for the Implementation of the latter and the Regulations for the organization and activities of the MU-Plovdiv for the acquisition of the educational and scientific degree "Doctor" in the doctoral program "Endocrinology".

I give a very positive opinion and strongly recommend to the members of the respected Scientific Jury to give a positive vote for the implementation of the procedure for awarding a doctorate degree to Dr. Dimitar Manolov Troev, on the basis of the dissertation work presented by him.

25.01.2024
Rome

Signature:
/Prof. Dr. M. Nordio/