To
The Scientific jury
appointed by order No. RD-38-297/21.06.2022
of the Rector of Sofia University "St. Kliment Ohridski"

REGARDING:

Dissertation review on topic

Macrophage activation syndrome in childhood - analysis of clinical and laboratory changes, evaluation of the diagnostic approach and therapeutic effectiveness for the awarding of the educational and scientific degree "DOCTOR", to Dr. Kalin Yordanov Lisichki, a free doctoral student in the doctoral program 7.1. Medicine ((03.01.50) "Paediatrics" at the Department of "Internal Diseases, Pharmacology and Clinical Pharmacology, Paediatrics, Epidemiology" , infectious and skin diseases", Faculty of Medicine, SU "Kliment Ohridski".

Review

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Dissertation topic:
Macrophage activation syndrome in childhood - analysis of clinical and laboratory changes, evaluation of the diagnostic approach and therapeutic effectiveness

Doctoral student: **Dr. Kalin Yordanov Lisichki,**

Scientific supervisors: Prof. Dr. Stefan Nedev Stefanov, MD,
Ass. Professor Yordanka Georgieva Uzunova, Ph.D
Form of doctoral studies: open doctoral studies

1. Presentation of the doctoral student. Autobiographical history.

Dr. Kalin Yordanov Lisichki is born on 11.10.1958. " In 1976, he graduated from Frederic Joliot-Curie Secondary School, Sofia, and in 1984 - from the Faculty of Medicine, MU, Sofia with excellent results. From 1984 to 1987, he worked as a paediatrician in the Regional Hospital - Dupnitsa. From 1987-2007, he worked firstly as an assistant, following the senior assistant post and then chief assistant post, in the Paediatric Rheumatology Clinic at the University Children's Hospital (NIP, later named "SBALDB"). He is actively engaged in medical-diagnostics, teaching and research work. He acquired the paediatric specialty in 1991, and six years later, in 1997, the subspecialty of paediatric rheumatology, thus being one of the first specialists in the newly formed subspecialty.

From 2007 to 2013, he is a doctor at Tokuda General Hospital, Department of Paediatrics, and from 2013 to the present, he is the head of the department (Clinic of Paediatrics, Tokuda General Hospital).

He is a member of the Bulgarian Paediatric Association, Bulgarian Medical Union, PRINTO.

He has 43 scientific publications in scientific journals in Bulgaria and 4 abroad. He has participated with posters and reports in over 30 scientific forums in Bulgaria and 1 abroad. He speaks French and Russian very well.

2.Relevance of the chosen topic, justification of the goal and tasks

The topic of the dissertation is macrophage activation syndrome (SMA) - a difficult-to-diagnose disease, critical in its clinical course, with a mortality rate of up to 40% if left unrecognized and untreated. It lacks diagnostic clinical and laboratory markers, therapeutic protocols, its differential diagnostic clarification is carried out within the febrile-rash syndrome, which includes one of the most severe diseases in childhood. The detailed study of the syndrome and its nomenclature clarification has been the focus of medicine in the last 20 years, made possible due to confirmation through genetic and immunological studies. The descriptions and detailed presentation of cases with SMA enriches the experience of a large number of specialists due to the multisystem manifestations of the disease and the overlap with a number of infectious and hematological conditions. Macrophage activation syndrome (MACS) is a subset of hemophagocytic lymphohistiocytosis (HLH) particularly in autoimmune, malignant diseases and some infections. The disease is a hyper-inflammatory syndrome, reaching a cytokine storm, hemophagocytosis and multi-organ failure. In the scope of rheumatological diseases in children, the highest frequency is observed in the systemic form of juvenile idiopathic arthritis (cJIA), but it also occurs in other autoimmune and / or auto inflammatory conditions including systematic lupus, Kawasaki disease, periodic fever syndromes, etc. SMA in its unfolded manifestation is observed in 10-20% of patients with cJIA, sometimes in its subclinical form it occurs in 30-40%,

which is often interpreted as overactivity of the main disease, but it is also possible that the disease begins with the symptoms and diagnosis of SMA. Signs of SMA in other rheumatic diseases may remain unrecognized because they are similar to those of autoimmune symptoms during its active phase or be unrecognized and confused with septic complications. In clinical literature, as well as in present publications, cases with cJIA/SMA are analyzed using the PRES*EULAR criteria introduced in 2015. This analysis is necessary for the validation of similar criteria in other rheumatological diseases and their application in provocation of SMA by infectious triggers. The search for infectious triggers is required in the diagnostic process, as they could lead to SMA, and can be seen independently and in the presence of a systemic disease. In-depth knowledge of laboratory deviations that reflect in pathogenetic mechanisms and their dynamics determine the therapeutic approach and its order during clinical observations.The seriousness of the prognosis, reflecting the need for rapid diagnostic orientation which leads to the choice of timely targeted treatment according to the severity of the condition define the topic as current, modern and significant - which is necessary for the general paediatric practice and paediatric rheumatologists. This research material is the first covering SMA as a clinical problem in Bulgaria.

3. Literature review, recognition of the problem, choice of goal and tasks

The literature review of 28 standard typewritten pages is comprehensive, specific and clear. It shows precise knowledge of the problem by the author, his ability to select, cite and analyze objectively and critically the scientific, laboratory and clinical experience of others. The aetiology, genetic characteristics, pathogenesis, pathohistology, diagnostic criteria, clinical pictures, laboratory parameters and therapeutic strategies in SMA are reviewed in detail. Attention is drawn to the described variations of the lymphohistiocytic model, which also determine the differences in the established diagnostic criteria. Of essential pathogenetic and therapeutic importance is the consideration of the diverse cytokine influences against the background of genetic variants, which are the subject of future studies. Also included is the expanded future diagnosis of the syndrome through the study of new biomarkers, - the study of the low activity and reduced number of NK cells in the active phase of a systemic form of JIA, through the levels of sCD25 and sCD163 and the increase of - Follistatin 1 (FSTL- 1) to identify a subclinical syndrome of macrophage activation. The pathogenetic mechanism of the

treatment with biological agents as present and future is described in detail, which is used then to select the proper therapeutic approach in patients. The author rightly questions the extent to which biological treatments of the underlying diseases mask the currently used diagnostic criteria and whether they could be applied in its course.

The literature review is logical and represents a reasonable transition to the next chapter of the dissertation work.

The purpose of the current dissertation is specifically, precisely and clearly defined - to carry out a clinical-laboratory, diagnostic and therapeutic analysis of patients with SMA. This is the first study covering the SMA problem in Bulgaria.

Eight tasks are set with which to achieve the goal of the work, namely - determination of SMA triggers, analysis of clinical characteristics, laboratory changes, therapeutic influence in clinical and laboratory aspects. The last two tasks are summarized with suggestions for an up-to-date diagnostic approach and an effective therapeutic strategy. The goal, as well as the tasks, require serious and in-depth coverage of the studied groups of children and accurate analysis which require good knowledge of the features of the disease such as clinical pictures, course and evolution - tasks that the doctoral student coped with at the level of a doctor with long-term and rich clinical experience experience.

4. Material and research methodology

The "Materials and methods" section is reflected in 6 pages. Included is the data of 20 children with macrophage activation syndrome, diagnosed and treated in the period of time from 2013 to 2019. Evaluated was the time interval for follow-up of the most important diagnostic laboratory parameters, as well as the effect of treatment on the change in ferritin levels and on the time required for follow-up. A control group of 21 patients with other diseases occurring with a pronounced inflammatory clinical-laboratory syndrome, not meeting the criteria for SMA-19 with acute infectious-provoked diseases and 2 with rheumatological-Kawasaki and SLE, as well.

The study is retrospective, covers a 6-year period - a basis for in-depth knowledge of the problem and a long enough collection and follow-up of patients to obtain meaningful clinical results and objective conclusions.

The analyzed indicators were divided into groups by gender, age and according to the triggers of SMA (15-cJIA, 1-dermatomyositis, 4-infectiously provoked SMA). A contribution is the analysis of the patient with SMA provoked by Mycoplasma pneumoniae, which showed a severe course of the disease, necessitating treatment with an anti-IL 1-receptor antagonist. Similar cases described in the literature are single.

The clinical and laboratory indicators by which the patients were diagnosed and followed up are described thoroughly. The EULAR/ACR criteria from 2016 were used, taking into account also the criteria generally valid for HLH (HLH 2004 Diagnostic criteria developed by the Study Group of the Histiocyte Society).

The statistical methods used to process the results use a package for statistical processing of data for small groups of patients. Descriptive and diagnostic analysis, Fisher exact test, ROC analysis to assess the specificity and sensitivity of the ferritin/ESR ratio, analysis of the difference in mean values ANOVA t-test, assessment of the level of significance of certain empirical characteristics were performed. Statistical methods enable full processing of the results and reaching objective conclusions.

5. Characterization and evaluation of the dissertation work, results and discussion

Dr. Lisichki's dissertation is written on 123 typewritten pages and illustrated with 8 graphic tables and 37 graphic figures. The bibliographic reference contains 203 literary sources, of which 2 are in Cyrillic and 201 are in Latin. The abstract contains 50 pages. The design of the dissertation is according to the accepted requirements, namely - literature review, aim and objectives, method and materials, results and discussion, conclusions and contributions. 20 children with SMA diagnosed and treated at Tokuda UMBAL and the university clinics of Sofia, Varna and Plovdiv over a period of 6 years were studied. This is a sufficient number of cases collected from several centres, given the fact that it concerns a rare disease. However, the small number of patients does not due justice to the varied clinical palette. A control group of 21 children with diseases occurring with pronounced inflammatory laboratory syndrome, not meeting the criteria for SMA, was also included. In them, the sensitivity and specificity of the

ferritin/ESR ratio as a new important marker in the diagnosis of SMA, also helping to differentiate SMA from other acute inflammatory conditions, has been scientifically investigated. The obtained results represent a serious contribution to the dissertation.

The results are presented on 43 pages. They are logically systematized and illustrated in well-designed colors and black-and-white tables and figures. The clinical and demographic characteristics of the patients are included, the results of the laboratory indicators and the therapy are analyzed.

In analyzing the data, the personal experience of Dr. Lisichki on the researched topic and his personal attitude towards determining the importance of the monitored parameters is evident. A special place is rightfully allocated to ferritin, as the most important marker for diagnosis and correlating with disease activity. The interpretation of its significance and its dynamics follows the author's extensive pathogenetic studies indicated in the literature review. Its comparative follow-up in patients with SMA and the control group of patients with diseases with a high inflammatory background provides valuable differential diagnostic information. For the first time in his patients, Dr. Lisichki researched and applied in the diagnosis of SMA /introduced/ the ferritin/ESR ratio, the expediency of which has been reported in the literature in recent years.The doctoral student conducted his own study examining the sensitivity and specificity of the ferritin/ESR ratio in a control group of non-SMA patients and those with SMA and reached his own result of 100% sensitivity and 100% specificity. (I quote - "this is a fast, relatively cheap and high-quality way that facilitates the diagnosis of macrophage activation syndrome.")

The results of the follow-up of the laboratory parameters under the treatment effects are reflected. The follow up for the treatment effect was carried out in stages, individually, according to the clinical course and laboratory deviations - from classically applied therapy to the inclusion of biological agents. In Bulgaria, for the first time, an anti-IL-1 receptor antagonist was administered to two of the children included in the present study. Especially valuable are the correlations - SMA trigger, therapy and ferritin level decreases, as the main biological marker. Of interest are the results that give reason to think that in children with infectiously provoked SMA, the control of the disease and serum ferritin is slower and their stabilization is twice as long.

The results are interesting from a scientific and practical point of view and enable objective and interesting conclusions from a clinical and theoretical point of view.

The conclusions of the dissertation work, 16 in number, are clearly and precisely defined. They derive from the results and correspond to the set goals and tasks of the work. From a clinical point of view, I find the most important **conclusion number 9** (The ferritin/ESR ratio is an extremely useful, effective and quick method for differentiating SMA from other diseases with similar clinical symptoms and laboratory changes. According to the data obtained, at a value equal to or greater greater than 11.3 the test has 100% sensitivity and 100% specificity for SMA) and **conclusion number 10** (Ferritin/ESR ratio is essential for early diagnosis of subclinical SMA, in which the ESR may be normal.), as well as c**onclusion number 16** (The inclusion of a biological agent - anti-IL-1-receptor antagonist leads to the achievement of complete clinical - laboratory control of the disease and allows a gradual cessation of the ongoing treatment. The personal attitude and the accumulated clinical experience of the dissertation student is clearly visible in conclusions 6,7,15.

I share the contributions of the work - 6 with an original character, 4 with a practical meaning and 3 with a **validating character.** The most important of all of them is that for t**he first time in Bulgaria the data of patients diagnosed with macrophage activation syndrome in childhood are described and summarized and the therapeutic approach is analyzed.**

The literature reference includes 203 literary sources - 2 of which are in Cyrillic and 201 in Latin. Most of the cited works have been published in the last 15 years. They are selected thematically according to the purpose of this dissertation. During their selection, the dissertation student's ability to select, evaluate and cite accumulated foreign scientific experience is evident. The number of Bulgarian authors cited is small, but this is logical in view of the fact that no similar studies have been conducted in Bulgaria so far. The number of literary sources is sufficient for the development of a dissertation work for awarding the scientific and educational degree “doctor".

The abstract meets the requirements. It reflects in a synthesized form the most essential moments of the dissertation work in all its sections. It is appropriate to indicate on a separate page the publications in connection with the dissertation work.

The dissertation work represents an extremely personal activity of the doctoral student in the treatment of patients, conducting the necessary examinations in the necessary time, follow-up, creating a control group of healthy children, monitoring the results.

In conclusion - I have no significant recommendations and remarks to the author and the doctoral student. The chosen topic of the dissertation ***"Macrophage activation syndrome in childhood - analysis of clinical and laboratory changes, evaluation of the diagnostic approach and therapeutic effectiveness"*** is significant for two reasons. SMA is rare and any systematic analysis of the disease is of interest. The presented work is the first systematic analysis in our country of patients with this disease.

The dissertation work is thoroughly, precisely and in detail developed with very in-depth knowledge of the problem, with a thorough and critical interpretation of the results acquired by Dr.Lisichki and their objective comparison with the results of other authors with similar studies. The results and conclusions are real, with an indisputable scientific and practical contribution.The quality of the dissertation work is completed with good illustration along with clear and precise Bulgarian language. In the selection of the topic and the entire work, the maturity of the extremely good clinician is evident, who researches and gives his experience and contribution in the clarification of a difficult-to-differentiate critical condition with manifestations of multisystemicity and the need for a rapid therapeutic response.

This gives me reason to recommend to the respected members of the jury appointed by order No. RD-38-297/21.06.2022 of the Rector of Sofia University "St. Kliment Ohridski" to vote a positive evaluation of a dissertation work for the acquisition of the scientific and educational degree "doctor" for Dr. Kalin Yordanov Lisichki, free doctoral student in the doctoral program 7.1. Medicine ((03.01.50) "Paediatrics" at the Department of "Internal Diseases, Pharmacology and Clinical Pharmacology, Paediatrics, Epidemiology, Infectious and Skin Diseases", Faculty of

Medicine, SU "Kliment Ohridski" on the topic: "Syndrome of macrophage activation in childhood" with scientific supervisors Prof. Dr. Stefan Nedev Stefanov, PhD, and Associate Professor Yordanka Georgieva Uzunova, PhD.

25.07.2022 г. Reviwer:

Assoc. Dr. Albena Telcharova-Mihailovska d.m.