To the Chairman of the Scientific Jury
determined by Order of the Rector of
University of Sofia "St. Kliment Ohridski"

Opinion for ONS "Doctor"

by Prof. Ivan Olegovich Litvinenko, MD, Department of Paediatrics at Medical University - Sofia, Head of Children's Neurology Clinic at SBALDB "prof. Ivan Mitev" - Sofia

Contact address: 1309 Sofia, St. Troitsa bl. 150 entrance And app. 1,
GSM 0888592015, e-mail: i.o.litvinenko@gmil.com

Appointed as a member of the Scientific Jury by Order No. RD 38-279/21.06.2022, according to Art. 4, paragraph 2 of the Law on the Development of the Academic Staff in the Republic of Bulgaria, decision of the Faculty of Medicine of the Faculty of Medicine dated 11.05.2022, protocol No. 104, in connection with the defense of Dr. Kalin Yordanov Lisichki - a free doctoral student in a professional direction /doctoral program 7.1. medicine (paediatrics), for awarding the educational and scientific degree "DOCTOR". Professional direction: 7.1 Medicine (03.01.50) "Paediatrics".

Author of the doctorate: Dr. Kalin Yordanov Lisichki
Form of doctoral studies: free

Department: "Internal diseases, pharmacology and clinical pharmacology, paediatrics, epidemiology, infectious and skin diseases" at Sofia University "St. Clement of Ohrid", Faculty of Medicine.

Topic: "MACROPHAGEAL ACTIVATION SYNDROME IN CHILDHOOD - ANALYSIS OF CLINICAL AND LABORATORY CHANGES, ASSESSMENT OF DIAGNOSTIC APPROACH AND THERAPEUTIC EFFECTIVENESS"

Scientific supervisors: Prof. Dr. Stefan Nedev Stefanov, MD, Assoc. Professor Yordanka Georgieva Uzunova, MD.

1. General presentation of the procedure and the doctoral student

The presented set of paper materials electronic media is in accordance with the Regulations of Sofia University "St. Clement of Ohrid". It includes the following documents: 1. curriculum vitae; 2. diploma of higher education and its application; 3. dissertation work; 4. abstract; 5. printed scientific works related to the dissertation work; 6. declaration of authorship; 7. certificate of compliance with the national minimum requirements for the ONS "doctor" for the relevant scientific field;

2. Brief biographical data for the doctoral student

Dr. Kalin Lisichki is born on October 11, 1958. In 1984, he graduated from the University of Sofia. After successfully passing the exam in 1991, he acquired the specialty of Paediatrics, and in 1997 - Paediatric Rheumatology. He began his career as a paediatrician 1984-

1987 in the Regional Hospital of Dupnitsa. From 1987-2007, after a competition, he was appointed assistant, senior assistant, then chief assistant at the University Children's Hospital, Clinic of Paediatric Rheumatology. In the period from 2007-2013, he was a doctor at the Tokuda General Hospital, Clinic of Paediatrics. From 2013 to the present, he is the head of the Paediatric Clinic, Tokuda UMBAL. In 2002, he acquired a certificate in abdominal ultrasound, and in 2001, a certificate in health management. In 1997, he completed a two-month course in France under the TEMPUS program. His special professional skills also include joint ultrasound and joint punctures. He speaks English and Russian.

3. Actuality of the topic and appropriateness of the set goals and tasks

Macrophage activation syndrome (SMA) is a serious condition with possible severe consequences (mortality ranging from 8% to 40% according to different authors), which is why it is of interest to a number of researchers. Work is being done in the direction both of clarifying the detailed mechanisms in the pathogenesis of the disease, and in the direction of determining accurate diagnostic criteria, based on the clinical manifestations of SMA and analysis of certain laboratory markers. Placing the diagnosis of SMA is a reason for starting timely treatment aimed at suppressing the hyperinflammatory state and bringing it under permanent control. Treatment regimens include high-dose regimens with corticosteroids and cyclosporine. In addition to these, attempts have been made with intravenous immunoglobulins, antithymocyte globulin, etoposide, rituximab, with variable success. Modern therapeutic strategies, including biological agents, developed in recent years find their place in the treatment of SMA. The data from the studies conducted so far on the application of biological therapy show encouraging results. SMA occurs in both adults and children. The main characteristic of this pathological condition is that it is severe and often ends in death. The lack of definitive clinical features and characteristic laboratory changes make it even more challenging. There is no safe and generally accepted therapeutic protocol that guarantees a good outcome of the disease. This gives reason to study the problem in more and more detail, to analyze a maximum number of patients in order to develop the most accurate diagnostic criteria and apply the most effective therapeutic strategies. The earlier the diagnosis is made and the faster and more timely the treatment is started, the greater the chance of a good outcome of the macrophage activation syndrome.

4. Recognizing the problem

On the basis of the above, reflected in the overview part of the dissertation, being aware of the emerging unclear points and contradictory data in the literature, the doctoral student logically derives the goal of the present work - **to describe in detail the childhood patients with macrophage activation syndrome in Bulgaria; to analyze established clinical and laboratory changes; to evaluate the applied diagnostic approach and the effect of the treatment carried out.** For the fulfillment of the stated goal, the following tasks were solved: 1. Classification by gender and age was carried out; 2. An attempt was made to establish the triggers of the underlying disease; 3. The clinical characteristics of the syndrome are presented; 4. The changes in the laboratory indicators in SMA are examined in detail and evaluated; 5. The effect of the administered medications to control the clinical changes in SMA is analyzed; 6. The effect of the administered medications on the changes in the laboratory indicators is analyzed; 7. A proposal for an up-to-date diagnostic approach is given; 8. An effective therapeutic strategy is offered.

5. Material and research methodology

The chosen research methodology allows the realization of the set goal and obtaining an adequate answer to the tasks of the dissertation work. In the present work, is included the data of 20 children with SMA that is analyzed, diagnosed, treated and followed up in the children's clinic of "Acibadem City Clinic Tokuda Hospital" - Sofia, Children's Rheumatology Clinic at SBALDB "Prof. Ivan Mitev" - Sofia, children's clinic at the university multispecialty hospital for active treatment "St. Georgi" - the city of Plovdiv and the children's clinic at the university multidisciplinary hospital for active treatment - the city of Varna. Thus, in practice, the experience on this problem from the whole country is summarized, something that does not happen often and deserves admiration. A significant group is formed, with this otherwise rare disease, and thus the results acquire greater value and make the dissertation work significant. Children diagnosed and treated for SMA between 2013 and 2019. An analysis of the clinical manifestations, laboratory indicators, imaging diagnostics, and bone marrow puncture was performed in all of them. All the indicated clinical and laboratory parameters were evaluated repeatedly in the patients - both at the time of diagnosis and in the subsequent period of treatment. After the diagnosis of macrophage activation syndrome, according to the criteria

mentioned above, treatment was started in all patients. The impact of the treatment on the change in serum ferritin levels and on the days that were needed to follow up the clinical condition of the children and the changes in their laboratory parameters was evaluated. Special attention is paid to the diagnostic value of serum ferritin. The diagnostic value of the ferritin/ESR ratio was evaluated in both groups of patients - those with a primary diagnosis of SMA and those from a control group of children with other main non-SMA diseases; investigated the sensitivity and specificity of the ferritin/ESR ratio in the context of CMA.

All results are presented in the form of tables and/or graphs, accompanied by corresponding detailed explanations regarding the studied parameters.

6. Characterization and evaluation of the dissertation work

The dissertation finalizes at a volume of 123 standard typewritten pages and is illustrated with 37 graphic figures and 8 graphic tables. The bibliographic reference contains 203 literary sources, of which 2 are by Bulgarian authors and 201 are in Latin, the majority of which are from the last 10 years. A huge amount of work has been done. A total of 20 patients with SMA were diagnosed and monitored in the leading clinics from all over Bulgaria.

With this dissertation, for the first time in Bulgaria, patients with SMA from the entire country have been summarized - the clinical and laboratory characteristics and the effect of the treatment are described and categorized. The diagnosis was made using the currently valid PRINTO/EULAR diagnostic criteria from 2016. As it is a rare disease, a statistical processing package was used for processing data from a small group of patients and the analyzes performed were performed by a professional specialist in statistics and medical data processing.

SMA is most often a complication of other underlying diseases and deserves to be studied, as it begins suddenly, its onset is severe, it is difficult to respond to treatment and greatly endangers the lives of patients. Through the analysis of the presented data, it becomes clear that it most often develops against the background of the systemic form of juvenile arthritis, but it turns out that SMA can also appear in the course of some infections or other autoimmune diseases. Clinical symptoms usually include fever combined with a rash syndrome, lymphadenomegaly and hepatosplenomegaly, presented to varying degrees. From the changes in

the laboratory indicators, hyperferritinemia is of the greatest diagnostic importance. In addition to the laboratory parameters included as diagnostic criteria, special attention should be paid to changes in the values of LDH, total protein and serum albumin, as well as D-dimers. The diagnosis of SMA requires prompt initiation of treatment. Usually, initial therapy is with a high-dose corticosteroid, with or without cyclosporine. In the absence of sufficient effect, additional treatment regimens with second-line drugs should be considered; in cases that are difficult and incomplete to respond to standard treatment, it is appropriate to apply treatment with biological agents (anti IL-1-antagonists). Although the experience with the application of biological therapy is small in the world, and even more so in Bulgaria, tendency shows that successful treatment of this serious disease will most likely develop in this direction.

On the basis of the obtained results, after a detailed discussion of each one of them, comparison with the literature data and subjecting to a thorough analysis, 16 conclusions were formed. They are in response and consonance with the set goal and tasks and are well reasoned. I accept the conclusions drawn. Of particular importance are the following conclusions: **number 3** - That children with infectiously provoked SMA are followed-up for twice as long until they stabilize their condition, which implies slower recovery and the need for longer treatment; **number 7** - Waiting to meet all diagnostic criteria for SMA can lead to a fatal delay in diagnosis; **number 8** - Serum ferritin is a laboratory marker of extreme importance. It has a special weight both in making the diagnosis of SMA and in monitoring the course of the disease and the effect of the treatment. Values above 600 ng/ml are in favour of macrophage activation syndrome; **number 9** - The ferritin/ESR ratio is an extremely useful, effective and rapid 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 than 11.3, the test has 100% sensitivity and 100% specificity for SMA; **number 15** - The independent application of more than 3 pulse therapies with methylprednisolone has no statistically significant effect regarding the course of SMA; and **number 16** - The inclusion of a biological agent - an anti-IL-1-receptor antagonist leads to the achievement of full clinical-laboratory control of the disease and allows for gradual discontinuation of the ongoing treatment.

7. Contributions and significance of the development for science and practice

I fully accept the original and confirmatory scientific contributions outlined by the dissertation. Among them of those of an original character I would distinguish that:

- *For the first time in Bulgaria, the data of patients diagnosed with macrophage activation syndrome in childhood are described and summarized.*

- *The data on changes in laboratory indicators in children diagnosed with SMA are analyzed and provided, and their diagnostic significance is determines.*

- *Changes in ferritin values in SMA and non-SMA patients are compared and analyzed.*

8. Evaluation of publications on the dissertation work

The doctoral candidate has submitted 6 publications, two of which are in a scientific publication referenced and indexed in a world-renowned database of scientific information - the journal of Paediatrics and 2 in international publications with an impact factor - Journal of Biogeneric Science and research with an impact factor of 0.071 and Journal of Rheumatology with an impact factor 4.666, as well as 4 report at national medical forums. The publications reflect the results of the dissertation work.

9. Personal participation of the doctoral student

I believe that it is undoubtedly the personal participation of the doctoral student in the conducted dissertation research, and the formulated contributions and obtained results, that should and are mainly his personal merit. In favour of the undoubted personal contribution of the PhD student is that he is either the first or maybe the last author on these publications.

10. The abstract is made according to the requirements and reflects the main results achieved in the dissertation.

11. Critical remarks and recommendations

I have no critical remarks on the essence of the work.

CONCLUSION

The dissertation contains scientific, scientific-applied results, which represent an original contribution to science and meet all the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB and the relevant Regulations of Sofia University "St. Clement of Ohrid".

The dissertation work shows that the doctoral student has in-depth theoretical knowledge and professional skills in the scientific specialty of paediatrics, demonstrating qualities and skills for independent conduct of scientific research.

Due to the above, I confidently give my **positive assessment** of the conducted research, presented within the framework of the dissertation work, its abstract, achieved results and contributions, and **I propose to the honourable Scientific Jury to award the educational and scientific degree of "Doctor"** to Dr. Kalin Yordanov Lisichki.

01.08.2022 год. Reviewer: .....

Prof. I. Litvinenko, d.m.