

## REVIEW

By Prof. Iskra P. Altankova, MD

Department of Internal Diseases, Pharmacology and Clinical Pharmacology, Paediatrics, Epidemiology, Infectious and Skin Diseases, Faculty of Medicine, SU "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, "Paediatrics".

This review is written in accordance with the requirements of the Law on the Development of Academic Personnel and the Regulations of SU "St. Kliment Ohridski" for its application.

### **Relevance of the chosen topic**

In recent decades, an increase in the frequency of immune-mediated diseases, as well as the appearance of new ones, has been reported. Hemophagocytic lymphohistiocytosis is a complex pathological condition characterized by a hyperinflammatory response to various stimuli, which in most cases leads to severe and often fatal consequences. This syndrome can be determined by gene mutations of the perforin-dependent pathway for cytolysis of NK and CD8+ T-lymphocytes, but also as idiopathic and/or secondary within other diseases – most often autoimmune and malignant, as well as in a number of infections. The modern term that describes this process is Macrophage Activation Syndrome (SMA). It occurs in both adults and children. At the heart of SMA is a disturbed relationship between macrophages and T-lymphocytes, which leads to an uncontrolled proliferation of hyperactivated macrophages, cytotoxic T- and NK lymphocytes, which release an excessive amount of pro-inflammatory cytokines. And subsequent generalized organ dysfunction, often ending in a fatal outcome. This condition is also known as a "cytokine storm".

Macrophage activation syndrome is an extremely rare disease that is difficult to diagnose. Common features in the development and course of SMA such as genetic predisposition, immune dysregulation and autodestruction have been established, but they cannot explain some differences in the manifestations of the disease, as well as the insufficient effectiveness of therapeutic regimens. This supports the need for new and in-depth scientific research and clinical trials.

The present dissertation work deals with the characterization and refinement of the diagnosis and therapy of SMA in the pediatric population and among Bulgarian children. Therefore, I believe that the dissertation work is relevant, current and very useful for clinical practice.

### **Evaluation of the dissertation work**

The dissertation is written on 123 standard pages and is illustrated with 37 figures and 8 tables. The bibliographic reference contains 203 literary sources, of which 2 are in Bulgarian and 201 are from foreign medical teams.

The dissertation is structured according to the generally accepted model: literature review, aim and tasks, material and methods, own results and discussion, in an optimal ratio. Sixteen conclusions and 11 scientific contributions were formulated. The work is well illustrated with 8 tables and 37 figures. It is written in a very good, competent and understandable scientific Bulgarian language. In connection with the dissertation, 6 scientific articles were published (2 in international and 4 in Bulgarian journals) and the work was popularized among the scientific community with 4 reports at scientific forums in our country.

The dissertation begins with a comprehensive but focused **literature review** illuminating multiple aspects of aberrant macrophage activation and the clinical diseases associated with it. The main centers of the review are: pathogenesis of SMA, genetic features, other diseases, bacteria and viruses as possible triggers of SMA, ferritin as a key participant in SMA, diagnostic criteria, etc. In addition to their objective presentation, the author critically interprets them. The currently unsolved

problems are highlighted and the purpose and tasks of the dissertation are scientifically substantiated. This speaks of the indisputable personal competence of the doctoral student, but also of the experience of his scientific supervisors. I believe that the above facts prove the in-depth educational competence of the doctoral student not only in the field of pediatrics, but also in clinical immunology and, in particular, pediatric rheumatology.

**The goal** of the dissertation is to describe in detail the pediatric 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 provided. To fulfill the goal, the following **tasks** have been formulated:

1. To determine the gender and age distribution.
2. To try to establish the triggers of the underlying disease.
3. To present the clinical characteristics of the syndrome.
4. To examine in detail and evaluate changes in laboratory parameters in the syndrome of macrophage activation.
5. To analyze the effect of the administered medications to control clinical changes in macrophage activation syndrome.
6. To analyze the effect of the administered medications on the changes in laboratory indicators.
7. To offer an up-to-date diagnostic approach.
8. To give a proposal for an effective therapeutic strategy.

### **Methods and materials**

Twenty children with SMA, diagnosed, treated and followed up in the country's leading pediatric clinics for the period 2013-2019, were included as clinic material. The currently valid PRINTO/EULAR diagnostic criteria from 2016 were used for the diagnosis. All patients had anamnesis taken and physical status examined, in accordance with the generally accepted rules in pediatric practice. This assessment was made for each child multiple times - upon admission to the hospital, during the hospital stay, as well as in the post-hospitalization period. A group of children with other, non-SMA diseases was also analyzed as a control

group for the sensitivity and specificity of the ferritin / ESR ratio indicator in the context of SMA.

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. A very wide range of hematological, biochemical and other clinical and laboratory indicators was used. All laboratory and imaging studies were conducted in certified laboratories, using automatic hematological and biochemical analyzers and age-appropriate reference values, without the possibility of manual correction of the obtained data.

After the diagnosis of macrophage activation syndrome, according to the accepted criteria, treatment was started in all patients. The initial choice of medication is a corticosteroid - methylprednisolone in pulse doses - 30 mg/kg/day on three consecutive days. Depending on the effect on disease control, some patients were also treated with other medications: cyclosporine, intravenous immunoglobulins, biological agent - anti-interleukin 1 receptor antagonist - Kineret, Etoposide.

Special attention is paid to the diagnostic value of serum ferritin as a differentiating marker between SMA and other severe inflammatory diseases. For this purpose, a control group with non-SMA diagnoses was also used. All primary data have been adequately processed statistically and are presented in graphic and textual formats.

### **Evaluation of the obtained results**

The obtained own clinical and laboratory data, which are subjected to analysis, are grouped in accordance with the tasks set and are presented in this order. All results are presented in the form of tables and/or graphics, as per the corresponding detailed explanations. The adopted structure of the exhibition is clear and informative. However, repetitions are also made. In the presentation of the therapeutic results, this is somewhat changed and their perception is difficult. All obtained results and analyzes are discussed in detail and summarized in the chapter "**Discussion**". The results of the dissertation work are presented in 16 conclusions, which are quite detailed and largely meet the tasks of the work. It can be seen that most often SMA in children develops as a complication of systemic form of juvenile arthritis, but it turns out that SMA can also appear in the course of some infections (mycoplasma, Epstein-Barr virus and Parvovirus B19) or other autoimmune diseases. Clinical symptoms usually include fever combined with a rash syndrome, lymphadenomegaly and hepatosplenomegaly, presented to varying degrees. Of the changes in 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. Serum ferritin has been shown to be extremely important in SMA in children. It has a special weight both in establishing the diagnosis of SMA and in monitoring the course of the disease and the effect of the treatment being carried out. The proposed ferritin/ESR ratio is a useful, efficient and rapid method for differentiating SMA from other inflammatory and autoimmune diseases.

Making the diagnosis of SMA requires prompt initiation of high-dose corticosteroid treatment, in combination or not with cyclosporine and last-generation biological therapy.

### **Evaluation of contributions**

In the dissertation, 3 groups of scientific contributions are self-assessed, which I accept, but I would summarize as follows.

#### **Contributions with original character**

1. For the first time in Bulgaria, the data of Bulgarian children diagnosed with macrophage activation syndrome are described and summarized. The provoking factors responsible for the occurrence of SMA in the pediatric population have been established.
2. The age, gender distribution and clinical manifestations of SMA among Bulgarian children are characterized.
3. The diagnostic significance of changes in laboratory indicators in children diagnosed with SMA, as well as their spontaneous or therapeutic dynamics with different therapeutic regimens, is defined.
4. The role of serum ferritin in childhood and especially in children with SMA has been fully and thoroughly studied. An additional indicator ESR / ferritin ratio with maximum sensitivity and specificity for the diagnosis of SMA in children was proposed, which successfully complements the model of ferritin significance in SMA in childhood.
5. The effectiveness of the therapeutic regimens used for the treatment of SMA in childhood is analyzed.

#### **Contributions of a confirmatory nature**

1. It is confirmed that the changes in laboratory parameters currently accepted by PRINTO/EULAR as diagnostic criteria are valid as such for SMA in childhood.

2. It is confirmed that changes in the values of LDH, D-dimers, total protein and albumin also have a high diagnostic value in children with SMA.
3. It is confirmed that the therapeutic scheme, including a high-dose corticosteroid (methylprednisolone 30 mg/kg/day or another corticosteroid medication in an equivalent dose) in combination or not with cyclosporine at this stage has no significant alternative. The importance and perspective of using biological agents in the treatment of SMA has been confirmed.

#### **Contributions of an applied nature**

1. The use of the ferritin / ESR ratio is proposed as a rapid and effective method for differentiating SMA from non-SMA patients.
2. The personal experience and the algorithm of application of biological therapy with anti-IL-1-receptor antagonists are described, as well as its effectiveness in Bulgarian children with SMA.

#### **Critical notes and recommendations to the dissertation**

I have no significant notes related to the design of the dissertation work, the methodology, the obtained results and scientific contributions. I have critical remarks about the technical layout of some figures – inscriptions and units on the axes, the legends, lack of explanations under the figures, etc. In some places, information in tables, figures and text overlaps.

#### **Brief biographical details of the candidate**

Dr. Kalin Lisichki graduated in Medicine in 1984 at the Medical Academy, Sofia with excellent results. He immediately started working as a pediatrician, and from 1987 he became an assistant in the Pediatric Rheumatology Clinic at the University Children's Hospital (NIP, later SBALDB), where he developed successfully as a teacher and pediatrician. He has 2 medical specialties - pediatrics and pediatric rheumatology and several additional qualifications. Since 2013, he has been working at the Pediatric Clinic, Tokuda UMBAL, and is currently its head of department. He had written 43 scientific publications and reports in over 30 scientific forums in Bulgaria and 1 abroad. He is member of the Bulgarian Pediatric Association, Bulgarian Medical Union, PRINTO.

## **Conclusion**

The dissertation reflects the broad medical competence of Dr. Kalin Lisichki as a long-term pediatrician and teacher, but also his taste for scientific and applied research and medical practice in the border area between pediatrics, clinical immunology and new therapeutic approaches aimed at deciphering key diagnostic and therapeutic aspects of SMA. I believe that both the educational and the scientific part of this PhD program in "Pediatrics" have been successfully completed. I highly value the dissertation work - its relevance and concept, the modern methods used, the results obtained, the contributions and the publications related to the work. I believe that they fully meet the requirements of the Law on the Development of Academic Personnel and the Rules for its Application. Therefore, I strongly recommend to the respected Scientific Jury to vote positively for awarding the educational and scientific degree "Doctor" to Dr. Kalin Lisichki, and I will gladly vote YES.

15. 08. 2022 г.

Reviewer:

Prof. Iskra P. Altankova, MD