

Statement

from

Assoc. Prof. Emilia Stambolova Kostadinova, PhD, MD

Acting Head of the Department of Pediatrics, Faculty of Medicine, Trakia University – Stara Zagora

Address: 6000 Stara Zagora, 11 "Armeiska" St., Faculty of Medicine, Department of Pediatrics

Phone: +359 888 260 100

E-mail: kostadinovaemilia@abv.bg, emiliya.kostadinova@trakia-uni.bg

Regarding the dissertation for the award of the educational and scientific degree "Doctor"

Professional field: 7.1 "Medicine"

Doctoral program: 03.01.50 "Pediatrics"

Author: Dr. Petya Petkova Markova

Full-time PhD student at the Department of Pediatrics "Prof. Dr. Ivan Andreev", Faculty of Medicine, Medical University – Plovdiv

Topic: "Monitoring of Renal Function in Children Undergoing Chemotherapy"

Scientific Supervisors:

Prof. Maria Spasova, PhD, MD and Prof. Polina Miteva-Shumnalieva, PhD, MD

According to Order No. R-616/11.02.2025 issued by the Vice-Rector for Research and Innovation at the Medical University of Plovdiv, for participation in the Scientific Jury, approved by a decision of the Faculty Council of the Faculty of Medicine at MU Plovdiv, Protocol No. 1/22.01.2025.

1. General Presentation of the Procedure and the Doctoral Candidate

The submitted set of materials on an electronic medium complies with Article 70 (1) of Section I – Acquisition of the Educational and Scientific Degree "Doctor" at the Medical University of Plovdiv, according to the Regulations of MU-Plovdiv dated January 28, 2021, and includes the following documents:

-Application to the Rector of MU-Plovdiv for initiating the procedure for the defense of the dissertation.

-Curriculum vitae (CV) in European format, signed by the doctoral candidate.

-Notarized copy of the diploma for higher education.

-Orders for enrollment in the doctoral program, interruption of studies (due to maternity leave), continuation of studies, and completion with the right to defend.

-Order for conducting an examination from the individual study plan and the corresponding protocol for the successfully passed examination or doctoral minimum in the specialty.

-Protocol from the Departmental Council regarding the preliminary discussion of the dissertation and the decisions taken for initiating the procedure and forming the Scientific Jury.

-Dissertation thesis.

-Abstract of the dissertation.

-List of scientific publications related to the dissertation topic.

-Copies of the scientific publications.

-List of participations in scientific forums.

-Declaration of originality and authenticity of the submitted documents.

2. Biographical Data of the Doctoral Candidate

Dr. Petya Markova is an established pediatrician with 18 years of professional experience in pediatrics and a recognized pediatric nephrologist with 8 years of expertise in the diagnosis and treatment of pediatric kidney diseases.

She possesses certified skills in abdominal ultrasound, central venous catheter placement, and percutaneous renal biopsy.

Dr. Markova has successfully passed the exams of the international pediatric nephrology course "IPNA - Master for Junior Classes", completing modules over three consecutive years.

She actively participates in diagnostic and therapeutic activities, teaching, and scientific research at the Department of Pediatrics, Faculty of Medicine, MU-Plovdiv.

Since 2021, she has been a full-time doctoral student at the Department of Pediatrics, enrolled by Order No. R-2374/17.12.2021.

3. General Structure of the Dissertation

The dissertation submitted for review consists of 167 pages, structured as follows:

- 48 pages dedicated to a review of the relevant medical literature on the topic.
- 1 page outlining the aim and objectives of the study.

- 7 pages describing the materials and methods, including study design and methodology.
- 71 pages presenting the results of the author's clinical studies and discussion of the defined objectives.
- 1 page detailing a clinical-diagnostic algorithm for monitoring renal function in children undergoing chemotherapy.
- 2 pages covering the conclusion, key findings, and self-assessment of contributions.
- 3 pages listing publications, conference presentations, and a research project related to the dissertation.
- The bibliography includes 247 references, with more than half published in the last 10 years.
- The dissertation is illustrated with 29 tables, 63 figures, and 1 appendix.

4. Relevance of the Topic

Over the past decades, advancements in chemotherapeutic concepts and protocols, along with improved risk stratification management and supportive care, have significantly contributed to achieving remission and relapse-free survival in children with onco-hematological diseases.

The increasing number of childhood cancer survivors necessitates the identification of biomarkers for early and long-term side effects of treatment on renal function, aiming to preserve their quality of life.

Nephrotoxicity associated with chemotherapy in these patients can lead to acute kidney injury (AKI) with a multifactorial etiology, as well as to chronic kidney disease (CKD).

The assessment of renal function in children with onco-hematological diseases during chemotherapy is a modern, significant, and highly relevant issue in pediatric medicine.

5. Knowledge of the Problem

The literature review is well-structured, focused, and balanced, providing comprehensive and accurate information that introduces and supports the chosen scientific problem.

A contemporary perspective on acute kidney injury (AKI) in childhood is presented, including a detailed analysis of causes, risk factors, classification, clinical course, differentiation of major AKI types in children, and treatment principles.

Current studies on novel biomarkers for early acute kidney injury are highlighted, including:

- Inflammatory biomarkers: Neutrophil gelatinase-associated lipocalin (NGAL) and pro-inflammatory cytokines (Interleukins IL-6, IL-18).
- Cell injury biomarkers: Kidney injury molecule-1 (KIM-1), Liver fatty acid binding protein (L-FABP), and Sodium/hydrogen exchanger-3 (NHE-3).
- Cell cycle markers: Urinary tissue inhibitor of metalloproteinases-2 (TIMP-2) and Insulin-like growth factor-binding protein 7 (IGFBP-7).

The dissertation provides a well-organized and synthetic overview of acute kidney injury (AKI) in pediatric onco-hematology, considering both etiological and pathophysiological aspects.

The study describes conventional chemotherapy and its associated nephrotoxicity, emphasizing key risk factors such as tumor infiltration, tumor lysis syndrome, treatment with nephrotoxic agents, and comorbidities.

A detailed review of nephrotoxic drugs used in pediatric oncology is presented, along with strategies to mitigate the nephrotoxicity of Cisplatin, Carboplatin, Ifosfamide, and Methotrexate.

The dissertation provides an in-depth assessment of chemotherapy-related nephrotoxicity in children, highlighting the low sensitivity and limited applicability of conventional markers such as elevated serum creatinine and oliguria.

The phenomenon of pseudonormal serum creatinine levels is discussed, linked to: reduced muscle mass due to cachexia. hemodilution caused by chemotherapy-associated hyperhydration. non-oliguric tubular nephrotoxic injury. 0Additionally, the study presents a thorough analysis of the mechanisms and types of drug-induced nephrotoxicity at both glomerular and tubular levels.

The literature review is systematically structured in accordance with the topic, clearly indicating that the doctoral candidate possesses an exceptional understanding of the global advancements in the research area. The candidate demonstrates the ability to critically analyze the literature in a comprehensive and innovative manner.

At the conclusion of the literature review, a synthetic analysis is provided of the well-established and still unresolved issues related to chemotherapeutic nephrotoxicity in pediatric oncology, forming the foundation for the scientifically grounded hypothesis that underpins the objective of the dissertation.

6. Aims and Objectives of the Dissertation

The aims and objectives of the dissertation are logically presented, based on the problems outlined in the scientific review, and are focused on the monitoring of renal function in children undergoing chemotherapy. The objectives are precisely formulated and are sufficiently comprehensive to achieve the stated aim of the dissertation.

7. Research Methodology

In the Materials and Methods section, the doctoral candidate justifiably selects the clinical groups and defines the parameters for clinical, laboratory, and instrumental monitoring based on established criteria. The study covers a total of 281 children with a wide range of onco-hematological diseases. The retrospective part of the study includes 213 children hospitalized between 2016 and 2020 at the Department of Pediatric Onco-Hematology, Pediatrics Clinic, University Hospital "St. George." The cross-sectional study involves 40 children undergoing

chemotherapy with nephrotoxic medications at the same department. Additionally, a prospective study is conducted on a group of 28 children who completed their treatment with nephrotoxic drugs at least one year prior, to analyze and assess the development of chronic kidney disease (CKD).

The clinical selection of pediatric patients provides a significant clinical sample necessary to achieve the aims and objectives of the dissertation. The methods employed include clinical, imaging (instrumental), and laboratory techniques, including functional studies for the determination of acute kidney injury (AKI) according to the KDIGO 2022 classification and chronic kidney disease (CKD) based on the KDIGO classification, as well as Fe F% (fractional excretion of phosphate) and renal threshold for phosphate (T_{mp}/GFR).

The statistical methods chosen for data analysis align with current standards for validating clinical-laboratory methods and processing scientific data.

8. Evaluation of the Dissertation and Contributions

Dr. Petya Markova, as a pediatrician, pediatric nephrologist, and diligent researcher, conducts a comprehensive study on the frequency, characteristics, and types of acute kidney injury (AKI) in patients with onco-hematological diseases over a 5-year period. She investigates the role of classical AKI markers—creatinine and GFR—along with early markers of proximal tubular nephrotoxicity, such as Fe F%, T_{mp}/GFR, and NGAL in urine, as potential early biomarkers for AKI in children undergoing chemotherapy.

Dr. Markova also analyzes the frequency of chronic kidney disease (CKD) in children who underwent chemotherapy more than one year ago, as well as the role of NGAL as a marker for chronic kidney disease.

The significant clinical material utilized in this study makes the research valuable from both a scientific and practical perspective. The proposed tasks are innovative, and the algorithm for monitoring renal function in children treated with nephrotoxic chemotherapy offers a timely and relevant approach to their care.

Own results. The monitoring of renal function in patients with solid tumors or malignant hematology has been conducted thoroughly, providing strong evidence that Dr. Markova possesses a deep understanding and effectively applies modern diagnostic and therapeutic algorithms in pediatric nephrology and pediatric oncology-hematology.

In the retrospective part of the study, a distribution was made according to the degree of AKI (acute kidney injury), identified in 94 children (44.1%), with 72.3% of cases being classified as stage I. The frequency of causes for AKI was also noted. Among the identified etiological causes, drug-induced nephrotoxicity related to chemotherapy administration was the leading cause, accounting for 58.51% of all AKI episodes, followed by infections, including sepsis (22.24%), renal infiltration at disease onset (14.89%), and tumor lysis syndrome (2.12%). No statistically significant correlation was found between tumor type, gender, and the presence of AKI. A tendency for a higher frequency of AKI was observed in school-aged children (49%) and at disease onset. AKI stage III was observed in children with solid tumors (9.30% of children with solid tumors/2.5% of children with malignant hematopathies).

For the first time in Bulgarian children with oncological and hematological diseases undergoing chemotherapy, a study was conducted to identify a suitable, early, highly sensitive biomarker for subclinical renal injury and the early diagnosis of AKI through the monitoring of uNGAL. The results from the cross-sectional study involving 40 children on chemotherapy with high and moderate nephrotoxicity indicate that uNGAL is not a reliable early marker for diagnosing AKI resulting from chemotherapy-induced nephrotoxicity. The absence of uNGAL dynamics is most likely due to the hyperhydration protocol applied to all children during each chemotherapy cycle. In this context, an investigation of the uNGAL/creatinine ratio in a single urine sample collected 12 hours after a chemotherapy cycle revealed a statistically significant increase in the marker only in the "4-10" cycle group, indicating that uNGAL levels rise as a result of the cumulative nephrotoxic effect. The findings of other studies on the role of uNGAL in similar clinical contexts are also contradictory, suggesting cumulative tubular nephrotoxic injury.

The existing challenge of asymptomatic AKI in children undergoing chemotherapy necessitates the search for and confirmation of nephron dysfunction at various levels, which is of critical diagnostic importance. Of practical application is the use by the author of the adapted Schwartz formula based on body surface area, as well as the Brandt formula for assessing glomerular filtration rate (GFR). The high percentage of hyperfiltration, i.e., $eGFR > 160 \text{ mL/min/1.73 m}^2$, observed among pediatric oncology patients undergoing chemotherapy is most likely associated with the hypermetabolic state in which children with oncological diseases find themselves, particularly during chemotherapy.

The identification of early proximal tubular damage as a manifestation of nephrotoxicity was achieved through the use of accessible formulas for the investigation of fractional phosphate excretion (FeP%) and renal threshold for phosphates (Tmp/GFR). A statistically significant difference in FeP% levels in urine before and after each chemotherapy cycle was observed, indicating that as the number of chemotherapy cycles progresses, the risk for tubular damage increases. The examination of the renal threshold for phosphates (Tmp/GFR), a more accurate marker as it is compared to GFR, also revealed a statistically significant difference in the renal threshold for phosphates at zero and 12 hours across all chemotherapy cycles. With the increasing number of chemotherapy cycles, the percentage of patients with a low renal threshold for phosphates also increased, confirming proximal tubular damage. The clinical significance of tubular damage is reflected by the establishment of hypophosphatemia, statistically significant at the third and "4-10" cycles, as a risk factor for skeletal demineralization with osteomalacia, bone pain, spontaneous fractures, proximal muscle weakness, and hypophosphatemic rickets. Hypomagnesemia was observed in the second, third, and "4-10" cycles. Other urinary markers confirming proximal tubular damage include low-molecular-weight proteins such as beta-2-microglobulin (B2-M), retinol-binding protein, and bicarbonaturia with serum bicarbonate levels $< 20 \text{ mmol/l}$. The study of urinary osmolality and pH provides an accessible method for determining distal tubular function.

The conducted study reveals a 37.9% prevalence of CKD in children who underwent chemotherapy with nephrotoxic chemotherapeutic agents more than a year ago. The author has excellently presented the results of the tasks carried out, professionally and competently outlining

the findings from the long-term monitoring of renal function in children with oncological and hematological diseases.

The in-depth and analytical discussion of the clinical results deserves high recognition. The results from the author's own clinical studies are presented accurately and analytically, with statistical processing enabling the establishment of statistical significance, serving as the foundation for the comparative and correlational conclusions drawn. The author compares her results with data from international and national databases, and the discussions presented in the individual chapters highlight the significance of the dissertation work.

The conclusions drawn are consistent with the obtained results and are accurately formulated. They are directly linked to the set objectives and tasks, reflecting the author's ability to summarize and synthesize the findings of the scientific research. The conclusions have both theoretical and clinically applicable significance, relating to the potential for implementation in clinical practice.

9. Evaluation of Publications and Personal Contribution of the Doctoral Candidate

The doctoral candidate has included three full-text publications in Bulgarian peer-reviewed scientific journals related to the dissertation work, one of which is indexed in the Scopus database. Additionally, three participations in national scientific forums are presented, along with one participation in an international scientific forum on pediatric nephrology - The 56th Annual Meeting of the European Society for Paediatric Nephrology (ESPN), Valencia, 2024, with an abstract published in *Pediatric Nephrology*, 2024. In all full-text publications and scientific forum presentations, Dr. Markova is the first author, which confirms her personal involvement in the conducted dissertation research, her individual contribution to the obtained results, and in the formulation of the contributions.

I have no critical remarks regarding the conducted research and presented materials. I recommend the publication of the results in international scientific journals.

10. Abstract

The presented abstract, in terms of content and quality, complies with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria and reflects the main results achieved in the dissertation.

11. CONCLUSION

The conducted monocentric retrospective and prospective study on renal function in children undergoing chemotherapy provides data on the frequency and characteristics of AKI during nephrotoxic chemotherapy treatment and the prevalence of CKD as a long-term complication in these children.

The doctoral dissertation of Dr. Petya Petrova Markova contains scientific, applied-scientific, and practical results, which constitute an original contribution to pediatric science and, specifically, to pediatric oncological nephrology. The work meets all the requirements set forth by the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB), the Regulations for the Implementation of ZRASRB, and the Regulations of the Medical University of Plovdiv. The

presented materials and dissertation results fully comply with the specific requirements adopted by the Regulations of the Medical University of Plovdiv for the application of ZRASRB.

The doctoral dissertation demonstrates that the doctoral candidate Dr. Petya Petrova Markova possesses profound theoretical knowledge and professional skills in the scientific field of Pediatrics, while showcasing qualities and abilities for conducting independent scientific research.

Based on the aforementioned qualities of the presented doctoral dissertation by Dr. Petya Petrova Markova, I confidently give my positive evaluation of the conducted study, including the dissertation, abstract, published results, and contributions. I recommend to the esteemed scientific jury to award the educational and scientific degree of 'Doctor' to Dr. Petya Petrova Markova in the doctoral program in Pediatrics.

17.03.2025

Stara Zagora

Prepared the statement:

Assoc. Prof. Dr. Emilia Kostadinova, DM

Заличено на основание
Чл.5 §1, 6. "В" Регламент (ЕС)2016/679

