

To
The chairman of the scientific jury,
Determined by Order № P - *49*... *10806* 2021 of the Rector of the Medical
University-Plovdiv

REVIEW

From **Professor Sevdalin Slavov Nachev, DMSc**
Laboratory of Clinical Pathology of St. Ivan Rilski University Hospital – Sofia

of dissertation study for acquisition of educational and scientific degree '**Doctor**'
professional field: **7.1. Medicine "Pathoanatomy and Cytopathology"**

Author: **Dr. Milena Gulina**

Type of doctoral studies: **free doctoral studies**

Department: **General and Clinical Pathology**

Topic: **IMMUNOMORPHOLOGICAL ASPECTS OF UROTELIAL
CARCINOMAS OF THE BLADDER**

Supervisor: **Assoc. Prof. Dr. Dorian Ivanchev Dikov, MD, PhD**

1. General presentation of the procedure and PhD candidate

The presented set of materials on paper / electronic media is in accordance with Art. 115 (1) of the Procedure for acquisition of ONS "Doctor" in MU - Plovdiv; Regulations of MU-Plovdiv from 06.11.2014 and includes the following documents:

- Application to the Rector of MU-Plovdiv for disclosure of the procedure for defense of the dissertation;
- CV in European format with the signature of the doctoral student;
- notarized copy of the diploma for higher education;
- orders for enrollment in doctoral studies, interruption of studies and expulsion with the right to defense;
- order for conducting an examination from the individual plan and a respective protocol for passing an exam or a doctoral minimum in the specialty;
- Minutes of the department council for preliminary discussion of the dissertation and the decisions taken for opening the procedure and for the composition of the scientific jury;
- dissertation study;
- abstract;
- list of scientific publications on the topic of the dissertation;
- copies of scientific publications;
- list of participations in scientific forums.

The doctoral student has attached 3 / three/ publications.

Notes and comments on the documents - none

2. Brief biographical data about the doctoral student

Dr. Milena Gulinac graduated 2005y. from High School with a medical profile in Kumanovo, North Macedonia. In 2011y. she graduated from the Medical University - Faculty of Medicine in Plovdiv. The same year she started specialization in the Department of General and Clinical Pathology of MU - Plovdiv, with a base of practical training at University Hospital "KASPELA" - Plovdiv. She received a degree in general and clinical pathology and cytopathology in 2016y. Since 2016, she has been an assistant professor at the Department of General and Clinical Pathology, Medical University of Plovdiv and started working as a pathologist at the Asenovgrad Hospital where she still works. In November 2019y. she is enrolled as a PhD candidate.

3. Relevance of the topic and expediency on the set aim and tasks

The relevance of the problem developed in the dissertation study in scientific and scientific-applied terms is related to the fact that **bladder cancer** is in the ten most common malignant processes in both genders and the most common cancer of the urinary system. This determines the aim and main tasks for a resolution in the present study, namely: *To study the epidemiological, morphological and immunohistochemical / IHC / features of urothelial carcinoma with special attention to the expression of PD-L1 and the immunomorphology of giant cells in tumor stroma.*

In pursuit of the set aim, Dr. Gulinac solves some tasks: *to study the indicators frequency, age, gender, histological variant of the tumor and its stages in two populations of patients - French and Bulgarian; to correlate between the expression of PD-L1 and the above-mentioned indicators; to derive a differential diagnostic algorithm from the study of giant cells in the stroma of urothelial carcinomas.*

4. Knowledge of the problem

The doctoral student knows the state of the problem and the literature related to it. In this direction she is facilitated by the fact that her research supervisor has been working in the same field for many years, thus a continuity and further development of the creative achievements of a proven specialist such as Assoc. Prof. D. Dikov is obtained.

5. Research methodology

The chosen research methodology: classical histological techniques, histochemical, immunohistochemical methods and statistical methods with applied software, allows to achieve the set goal and obtain an adequate answer to the tasks solved in the dissertation. The material includes a huge number of studied patients, from which 395 cases with neoplastic pathology were selected / **263 from the Bulgarian** population and **132 from the French** /. In **105** of them, the expression of **PD-L1** was studied.

6. Characteristics and evaluation of the dissertation

The dissertation study is written on 153 pages, including 10 tables, 25 figures and 37 microscopic photographs. The bibliographic reference contains 157 literature sources. It is presented in 8 sections: Introduction and literature review; Purpose and tasks; Material and methods; Results; Discussion; Conclusions; Contributions; Publications related to the dissertation.

The literature review includes: On 17 pages / 1/3 of the text / the issues for distribution of tumor pathology according to **gender and** age as well as the histological variant, differentiation and invasion in the context of TNM - classification are considered; The issues of possible geographical peculiarity of the **pathology** as well as correlations related to the histological grading are also considered. The remaining 33 pages are devoted to the **immunohistochemical** features (PD-L1) of urothelial carcinoma and giant cells.

The detailed elaboration of the material studied by Dr. Gulinac allows us to draw some important conclusions:

1. Urothelial carcinoma of the bladder is more common in men around the age of 70 years / 70-80 years. / as the degree of invasion is not related to the gender and age of the patient. Histological grading, according to the two systems for its reporting, statistically more indicates the **presence** of a correlation with a tendency to increase the number of male patients.
2. Expression of PD-L1 is observed in more than ¼ of the cases (28.8%) with carcinoma in men and it is more common in invasive high-grade carcinoma with squamous cell differentiation. The histological variant and the TNM stage do not change these data.
3. Giant cells in the stroma of urothelial carcinoma are found in more than 1/3 / 35.6% / of the cases again more often in carcinoma with a high degree of malignancy and their number increases with increasing degree of tumor invasion in the bladder wall.
4. The morphology of giant cells located in the tumor stroma and their immunophenotype distinguishes them from giant **tumor** cells. They are cytokeratin (-) and PD-L1 (-). The detailed immunomorphological panel used allows the author to claim that these cells reflect the phenomenon of cell fusion (shown electron microscopically by other authors) under conditions of "tissue stress".

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

The contributions of the present work are presented by the author by sections / tasks. I must note that it is incompatible to put original and confirmatory data in one section. Furthermore, priority research in the native literature does not mean that it should be considered original! As I had the opportunity to point out in another dissertation, I must emphasize here that the extravagance in listing the merits of the work does not make it more significant.

I must especially emphasize the methodological contributions of the work:

1. A method for testing PD-L1 expression in urothelial carcinoma has been introduced.
2. Giant cells in the stroma of urothelial carcinoma were examined.
3. Statistical analysis was used to study various parameters of both urothelial carcinoma and the accompanying giant stromal cells.
4. The above-mentioned introductions **in the study of urothelial carcinoma allow to make the right decision in its subsequent treatment!** A diagnostic-therapeutic algorithm is created.

I will especially emphasize the magnificent illustrations presented in the work. Their quality is in itself a contribution for those studying this problem in the future.

8. Evaluation of the publications on the dissertation

The 3 /three/ publications of the author are presented. Two of them are in a foreign magazine and one - in a Bulgarian one. All three articles are in English. Two posters are also presented.

9. Abstract

Very well formed, according to the requirements, and fully reflecting what is presented in the dissertation.

Immunomorphological studies were performed on only 105 cases in 2 main groups: 28/105 with non-invasive carcinoma and 77/105 with invasive bladder carcinoma.

10. Critical remarks and recommendations

I made critical remarks on the individual sections.

I will point out that the title of the paper is too general. The goal is probably to be short. In this case, in my opinion, it would be more appropriate to use: Immunomorphological aspects of urothelial carcinoma. Because not all immunomorphological aspects have been studied.

It is promising to further study the cell composition in the vicinity of the tumor, as well as the tumor itself, in order to reveal the presence of stem cells and their importance in tumor behavior and the undertaking of adequate treatment (CPS).

CONCLUSION

The dissertation study *contains scientific and applied results, which represent a contribution to science* and **meet all** the requirements of the Law for the

Development of the Academic Staff in the Republic of Bulgaria. The presented materials and dissertation results fully comply with the specific requirements of MU - Plovdiv.

The dissertation shows that PhD candidate Dr. Milena Gulinač has in-depth theoretical **knowledge and professional skills** in the scientific specialty of pathology and cytopathology by demonstrating qualities and skills for independent research.

Due to the above, I confidently give my positive assessment of the research presented by the above-reviewed dissertation, abstract, results and contributions and ***I invite the esteemed scientific jury to award the educational and scientific degree 'Doctor' to Dr. Milena Gulinač in doctoral program in general and clinical pathology.***

January 15, 2021.

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DMSc)

Reviewer:



(Prof. Sevdalin Nachev,