

## STANDPOINT

From **Assoc. Prof. Dr. Veselin Todorov Belovezhov, MD, PhD**

Department of General and Clinical Pathology,  
Medical University of Plovdiv

as a chairman of the scientific jury  
based by order of the Rector of MU - Plovdiv № R- 19 / 08.01.2021

*On the topic:*

**"IMMUNOMORPHOLOGICAL ASPECTS OF UROTELIAL CARCINOMAS OF THE URINARY BLADDER"**

*for acquiring the educational and scientific degree 'DOCTOR' in direction 7. Healthcare and sports, professional direction 7.1. Medicine and scientific specialty "Pathology and cytopathology" with code 03.01.03*

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

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*Materials on paper and electronic device have been submitted – the dissertation study, abstract, biographical and professional data, administrative documents, copies of publications by Dr. Milena Gulinac.*

Dr. Milena Gulinac was born on April 30, 1986. After graduating from high school with a medical profile, she completed a master's degree in medicine at the Medical University - Plovdiv in 2011. Dr. Gulinac began her professional career as a pathology resident at the University Hospital "KASPELA", where the basis for practical training is and for theoretical - the Department of Pathology of MU - Plovdiv. From 2013 to 2020 she worked as pathologist at Department of pathology in the University Hospital "St. George", and since 2016 at the same time in the hospital in Asenovgrad and as an assistant professor in the Department of General and Clinical Pathology at Medical University of Plovdiv, leading lessons for students of medicine, dental medicine and pharmacy at Bulgarian and English.

In 2016, Dr. Gulinac acquired a degree in general and clinical pathology. She speaks Serbo-Croatian and English and is a member of the Bulgarian Society of Pathology, the European Society of Pathology, the Union of Scientists in Plovdiv and the Bulgarian Medical Association (BMA).

Dr. Gulinac's dissertation study is dedicated to urothelial carcinoma (UC) of the bladder. This tumor ranks 7th among the most common malignancies in the world and at the same time is the most common cancer of the urinary system and the second most common neoplasm of the urogenital system characterized by a tendency to recurrence and a specific course of development. The complications

caused by UC worsen the quality of life of patients with this tumor. These data determine the relevance of the dissertation work on the study of immunomorphological aspects of UC.

An important place in the dissertation is occupied by the study of PD-L1 in the tumor and the giant multinucleated cells found in it. These are essential innovative researches, dedicated to a new methodology, essential for the treatment and to a classical morphological phenomenon, which emphasizes the diverse possibilities of pathology as a medical specialty and the ability of the dissertation student to apply them.

The presented dissertation study is in accordance with the requirements for structure and volume of such works comparable to the requirements of MU - Plovdiv. The dissertation is written on 153 pages and includes the following main chapters: title and content (2 pages), abbreviations used (2 pages), introduction (1 page), literature review (28 pages), purpose and tasks (1 page), materials and methods (13 pages), results (52 pages), discussion (25 pages), conclusions (2 pages), contributions (5 pages) and references - 18 pages including 157 titles, 8 of which in Bulgarian and the rest in English, a significant part of which from the last 10 years. It is illustrated with 37 microscopic photographs, 25 figures and 10 tables.

The literature review is well structured and comprehensive. It identifies the most important issues related to the epidemiology, frequency, etiology, pathogenesis and clinical symptoms of UC. The types of carcinomas, the morphological variants and the modern rules for staging and grading of the tumor are described.

Particular attention is paid to the role and place of the PD-L1 receptor, which allows tumor cells to inhibit cytotoxic T lymphocytes and block the effective antitumor immune response. In order to restore the effective antitumor immune response, we are working on the synthesis of checkpoint inhibitors - drugs that block and "break" the interaction between PD-1 and PD-L1, leading to the restoration of a complete antitumor immune response.

Attention is also paid to a poorly understood phenomenon such as the presence of mono or multinucleated giant mesenchymal stromal cells in normal and pathology.

Based on the review, conclusions were formed, which emphasize that there is a lack of information in the national literature on the expression of PD-L1 in bladder UC; in the world literature these studies are also not numerous and the correlation statistical studies of this expression of PDL -1 in bladder UC would be interesting. It has also been correctly assessed that the presence of giant multinucleated cells in the bladder mucosa and bladder stroma in UC, as well as their IHC phenotype, has been very poorly studied to date.

Thus, the review logically points to the aim and tasks of this dissertation study.

The aim is to study the epidemiological, morphological and some immunohistochemical features of UC - with special focus on the expression of PD-L1 and the immunophenotype of giant cells in the tumor stroma, by determining the diagnostic and prognostic value of the above indicators in comparison with histological, histological differentiation and tumor stage.

In connection with the aim, which I find correct, 5 tasks are set, formulated precisely and specifically, and briefly they require to study the epidemiological features of the UC of the two groups of patients, histological variant, tumor stage, histological differentiation, PD-L1 expression in UC, correlation of the

obtained results with the gender and age of the patients, with stage, with the histological type and grading, and study of the morphology of the giant cells in the stroma of the UC.

Materials from the 3021 cases of patients: Bulgarian samples of patients - 2758 (91.3%) have non-tumor pathology and 263 (8.7%) - with neoplastic diseases, and France sample of patients - 518 cases of patients, of which 386 were (74.5%) have non-tumor pathology and 132 (25.5%) have neoplastic diseases. They are retrospective and current urological materials from the bladder (biopsies and TUR) from the Department of Clinical Pathology of the University Hospital "St. George " - Plovdiv, and the Department of pathology of Grand Hôpital de l'Est Francilien, Jossigny, France, for a period of 4 years (2016 - 2020). The number is sufficient and I believe that it gives grounds for objectivity of the obtained data.

The methods used - conventional histological examination, histochemistry, immunohistochemistry, including for PD-L1 expression testing and statistical methods - Software, package for statistical analysis (SPSS®), IBM 2009, version 19 (2010) - I consider reliable enough to obtain correct results.

The results section states that the incidence of UC in the Bulgarian samples of patients is 8.7% and in the French 74.5%. The most commonly affected age decades are the 6th and 7th decades. Over 70% of patients are men. Classical urothelial carcinoma is the predominant histological type - 86.7%; 26.7% are non-invasive carcinomas (pTa), 44.8% infiltrate the mucosal chorion, the rest infiltrate the muscle wall. Regarding the differentiation - 29.5% are well differentiated UC, 35.2% - moderately differentiated and 35.2% - with poorly differentiation. The data from the point of the WHO 2016 classification show that 40% are low-grade and 60% high-grade. PD-L1 positive are 28.8% of cases, more often they are observed in men, 30% of positive cases are in classic urothelial carcinoma, histologically, followed by that with squamous differentiation; the expertise increases synchronously with stage and invasiveness and is observed mainly in infiltrative tumors. Presence of giant stromal cells combined with UC is found in 35.6% of cases (for both nationality), only in the Bulgarian sample of patients (67.6%) are more often high-grade UC (moderately differentiated - 45.9%), mainly in stage pT1 (43.2%). Multinucleated giant cells are negative for Perl's staining and IHC demonstrates mesenchymal, myofibroblast differentiation, expressing both a macrophage marker (CD 68) and a marker of cell degeneration (p16).

The "Discussion" chapter comments on the results in parallel with other similar studies. Bladder cancer has been found to be a multifactorial disease characterized by an aggressive course and frequent recurrences. In the last 2-3 years, however, there is a qualitatively new stage in the treatment approach, covering in addition to the established standard methods such as chemotherapy and surgery, and the use of immunotherapy. That is based on an IHC examination of PDL-1 expression. Regarding the epidemiological data, it is stated that there are no significant deviations from those of other authors published in the native and foreign literature. However, the drastic difference between the percentage of diagnosed UC in the Bulgarian contingent of patients (8.7%) and the French (74.5%) is impressive. According to the authors, this can be explained by the different diagnostic and therapeutic strategy. I would also add the level of preventive measures and the results achieved in pre-hospital care.

Regarding the histo-epidemiological study, the results do not differ from other scientific studies - the highest frequency of the classic variant of UC, the predominant part of the primary diagnosed UC of the bladder are tumors with invasion of the lamina propria (pT1) - 44.8%, followed by non-invasive UC (pTa) -26.7%, the majority of patients for both genders (45.2%) were diagnosed for the first time in stage T1, cases of moderately and poorly differentiated carcinomas (G2 and G3) predominate equally over well

differentiated (G1). High-grade (HG) bladder UCs were more common than low-grade (LG) UCs in both nationalities, and the expression of PD-L1 in tumor cells did not differ significantly from other studies (28.8%), suggesting that the methodology and evaluation are applied adequately. Stromal cell changes are nonspecific. Giant cells in the stroma of the UC of the bladder and in the surrounding lamina propria are more common in the French samples of patients; in the predominant part of the cases they are established in a criminal case with a high degree of malignancy; there is no statistically significant difference between the bladder stage of the bladder and the presence of giant multinucleated cells. PD-L1 is expressed in 28.8% of cases of bladder cancer more often in men between the ages of 50 and 80 years. PD-L1 expression is more common in invasive high-grade (HG) bladder UC.

At the end of the work are formed 7 conclusions corresponding to and based on the results obtained. The most significant of them is: UC of the bladder is a relatively common malignant tumor disease, most often affecting men around the age of 70, most often of the classic (conventional) type; the other histological variants have a more aggressive course of development. Histological grading of bladder UC at the time of its initial diagnosis by the pathologist is more often high (G2-G3 according to WHO 1973; high degree of malignancy according to WHO, 2016). Giant cells in the bladder stroma are found in 35.6% of cases, more often in high malignancy (HG). They show simultaneously mesenchymal, myofibroblast and macrophage immunophenotype. Their morphogenesis reflects the phenomenon of cell fusion under conditions of chronic tissue stress.

The presented scientific work contains contributions of original and confirmatory value, and I would like to emphasize those that I consider to be particularly important:

For the first time: systematic quantitative study with statistical analysis of the main epidemiological indicators of bladder management with elements of geographical pathology, systematic quantitative histo-epidemiological study with statistical analysis of the main morphological indicators of bladder management; histological variant and tumor stages comparable in the context of the latest revisions of the histological classification and the TNM system; of the histological grading of the bladder UC in a comparative aspect (according to the two systems used - WHO 1973 and WHO 2016).

For the first time in the native literature, a systematic IHC study is performed with statistical analysis of PD-L1 expression in bladder UC in correlation with histological grading and histological variant of bladder UC, as well as statistical analysis of PD-L1 expression in correlation with the TNM stage.

For the first time in the literature a systematic histo-epidemiological study is performed with statistical analysis of giant cells in the stroma of the UC, IHC examination, detailed and complex diagnostic, differential diagnostic algorithm for giant cells in tumor and non-tumor context and patho- from the morphogenesis of these cells in the stroma of the UC.

The data from the dissertation study are presented in three scientific publications, both in foreign language journals and in two scientific communications. In all of them, Dr. Gulnac is the first author, and in two she is the only one, which shows the commitment in the preparation of the work.

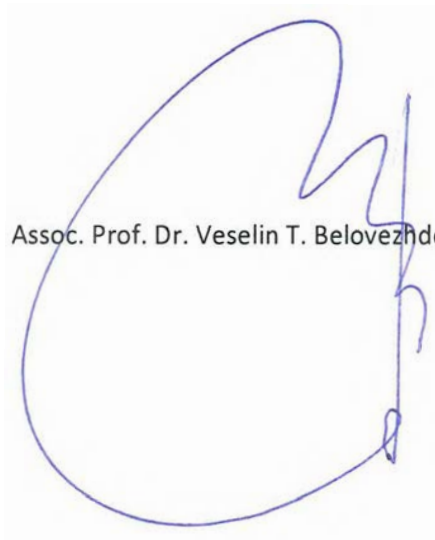
The dissertation study presented to me for an opinion is a very well-formed work written in a good and understandable style. It is a diligently prepared scientific work, which testifies to a good knowledge of the subject and to the hard work of the dissertation. It contains sufficient quality information, which gives grounds for positive assessment. I believe that Dr. Gulnac's work is completely finished, contains

original results and those of practical significance. It meets the requirements of the Law for the development of the academic staff in Bulgaria and the terms and conditions for obtaining scientific degrees at MU - Plovdiv.

**That is why I give my positive assessment, I will vote for, and I would like to recommend to the members of the respected Scientific Jury to support the acquiring of the educational and scientific degree "Doctor" to Dr. Milena Gulina.**

15.01. 2021

Assoc. Prof. Dr. Veselin T. Belovezhkov, MD, PhD

A handwritten signature in blue ink, consisting of a large, stylized 'V' followed by a vertical line and a small flourish at the bottom.