

REVIEW

from

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in his capacity as a member of the scientific jury based
by order of the Rector of MU - Varna № P-109-469

on a dissertation on a topic

"EXPRESSION OF TETRASPANIN MARKERS IN BENIGN HYPERPLASIA AND PROSTATE CANCER"

for awarding educational and scientific degree "Doctor"

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Materials on paper and electronic media have been submitted - dissertation, abstract, biographical and professional data, administrative documents, copies of publications by Dr. Lyuben Lyudmilov Stoev.

Dr. Lyuben Lyudmilov Stoev was born in 1991, graduated from the First Language School, Varna in 2010, and in 2016 - a master's degree in medicine at the Medical University - Varna. Since 2017 he has been working as an assistant in the Department of General and Clinical Pathology, Forensic Medicine and Deontology at the Medical University of Varna and as a resident in the University Hospital "St. Marina". Since 2018 he has been working as a tissue donation explantator at OSTEO-CENTER EAD Bulgaria. After successfully passing the state exam for acquiring a specialty in "General and Clinical Pathology" in May 2021, Dr. Stoev was appointed as an assistant physician at the University Hospital "St. Marina".

Dr. Stoev has 4 years of experience as a teacher of Bulgarian and English students in "Medicine" and "Dental Medicine".

He is a member of the Bulgarian and European Society of Pathology, the International Society of Urological Pathology (ISUP) and the BMA.

The dissertation of Dr. Lyuben Lyudmilov Stoev is dedicated to a current problem in the field of human pathology - prostate cancer. Prostate cancer is the second most common malignancy in men and is one of the most common causes of death due to neoplastic progression. In some patients, the disease is detected at an early stage and has a favorable clinical course, but in many cases at the time of diagnosis, the tumor is locally advanced and has even metastasized to regional and distant lymph nodes, bones and, less frequently, internal organs. It is the most commonly diagnosed cancer in men in more than half (112 out of 185) of the world. Mortality is a less variable (from 3.1 to 27.9 per 100,000), but it is present. And in Bulgaria there is the lowest survival rate among the European countries that participated in the study - 68%. These data prove the relevance of the work of Dr. Stoev, which is dedicated to the study of prostate cancer.

The presented dissertation work, as well as the abstract, are in compliance with the requirements for structure and volume, laid down in the regulations of MU-Varna. The dissertation is written on 115 standard pages and includes the following main chapters: abbreviations used (2 pages), introduction (2 pages), literature review (34 pages), purpose and tasks (1 page), materials and methods (6 pages), results (37 pages), discussion (12 pages), contributions (1 page), conclusions (1 page), publications (1 page) and bibliography - 18 pages including 194 titles, 4 of which in Cyrillic, and the rest in Latin, a significant part of which from the last 10 years. It is illustrated with 38 figures, incl. 14 microscopic photographs and 48 tables.

The literature review is well structured and comprehensive. It identifies the most important issues related to epidemiology, risk factors such as age, smoking, obesity, race, dietary habits and the importance of putative genetic factors in prostate cancer. Linking to mutations in BRCA1, BRCA2, the defective HPC1 gene, decreased expression of the GSTP1 gene, etc. is suggested. It has been found that in the case of gene damage, carcinoma develops at a younger age (about and over 40 years of age), with a frequency of about 10%. In these cases, it is a true hereditary prostate cancer. The review presents in detail the morphological characteristics and histological variants, duly describes the principles determining the differentiation by Gleason - Gleason score (GS), the new concepts of group grading (GG), the rules for staging from the point of view of the affected areas inside and outside the gland - seminal vesicles, lymph nodes, other organs; the prognostic histological factors are presented - morphological variant, lymphovascular invasion, perineural invasion, resection lines, presence of necrosis. An important place is given to the study of the serum marker PSA, which along with GS, incl. GG is also an important parameter in the Cambridge prognostic groups for non-metastatic prostate cancer from 2018. The immunohistochemical markers used in the diagnosis of prostate cancer, as well as the rules of treatment are not missed.

At the end of the review there is a place for the characteristics of tetraspanins. Tetraspanins are a family of membrane proteins containing 4 transmembrane fragments, representing 12% of the protein composition of exosomes. Exosomes are membrane vesicles 30-200 nm in size secreted into the intercellular space of various cells, including tumor cells. They are involved in many physiological and pathological processes such as intercellular signaling, stromal remodeling, induction of angiogenesis, participation in metastasis processes and resistance to chemotherapy. Tetraspanins form tetraspanin microdomains that interact with large amounts of transmembrane and cytosolic signaling proteins. Dr. Stoev has chosen to study in his work the expression of two tetraspanin markers - CD9 and CD151. The main functions of CD9 are that it is a factor associated with cell motility, and CD151 is associated with maintaining the integrity of the cell membranes of epithelial cells and has an effect on carcinogenesis

and metastasis. Interest in proteins from the group of tetraspanins is increasing and they are increasingly established as molecular biomarkers with pronounced prognostic and diagnostic potential. Their key role as coordinators of cellular motility and intercellular interactions in the extracellular matrix under physiological and pathological conditions puts them in the focus of a number of scientific studies. Therefore, in order to elucidate the molecular mechanisms of CD9 and CD151 and their role in the neoplastic process in prostate cancer, further studies are needed. Their inclusion in the dissertation of Dr. Stoev gives an innovative character to the work.

In conclusion, the review logically points to the aim of the dissertation: to analyze the clinical and morphological parameters of patients with non-advanced and advanced prostate cancer in relation to the immunohistochemical expression of tetraspanins CD9 and CD151 and to clarify their role in tumor progression.

In connection with it, 4 main tasks are set, formulated precisely and specifically. They are to study the clinical and morphological characteristics of patients with non-advanced and advanced cancer, to perform a comparative analysis between the individual clinical and morphological parameters in these two groups of patients, to study the immunohistochemical expression of CD9 and CD151 in prostate hyperplasia and prostate cancer (non-advanced and advanced).

Prostate biopsy materials of a total of 101 patients were used to solve the tasks, of which the target group was 91 patients diagnosed with prostate cancer, 10 patients had no tumor process, they had BPH and formed the control group; 50 of the patients with carcinomas were without distant metastases (M0) (non-advanced carcinomas) and 41 were with carcinomas with distant metastases. The intensity of cytoplasmic expression of CD9 and CD151 in the glandular epithelium was determined in all patients.

The methods used are routine biopsy technique and determination of the following clinical and morphological parameters: histological type of tumor, TNM stage, degree of differentiation according to Gleason score and ISUP Grade groups, perineural invasion, tumor necrosis, extraprostatic extension, presence of cribriform histology, IHC, which is enough to give adequate answers to the tasks.

Along with the morphological ones, statistical methods are also applied: 1. the chi-square test was used for the testing of hypotheses for connection between two qualitative variables. 2. Spearman's rank correlation was used to study the relationship between two quantities measured on the ordinal scale. 3. The correlation between two quantitative variables was tested by Pearson correlation analysis. 4. The comparisons between two quantitative variables are made with t Student's test, and in comparisons between more than two groups - with one-way analysis of variance ANOVA.

Each indicator is presented with a 95% confidence interval. Statistical analyzes were performed with the statistical package IBM SPSS ver. 21, and the graphs are built in Microsoft Excel for Windows. In this way they confirm the objectivity of the established data.

The results show that the mean age of patients with prostate cancer is 69.39 ± 7.98 years, with a minimum age of 45 years and a maximum age of 90 years. Of these, 50 (54, 95%) had non-advanced cancer and 41 (45, 05%) had advanced cancer.

Of these 50 cases with non-advanced cancer, 46 cases were tested for PSA levels, where 22 (47.83%) were found to be below 10.00 ng / mL, 11 (23.91%) with PSA between 10, 00 and 20.00 ng / mL

, 9 (19.57%) between 20.01 and 50.00 ng / mL and 4 (8.70%) with a PSA above 50.00 ng / mL. Of all the 50 non-advanced cancers studied, a cribriform growth pattern was found in 27 (54%) cases, while in 23 (46%) cases it was absent. In 12 (24%) of non-advanced cancers Gleason score is 6, in 22 (44%) is 7, in 9 (18%) is 8, in 6 (12%) is 9 and only in 1 (2%) is 10. When distributed by prognostic groups it is found that respectively 12 (24%) are in Grade Group 1, 17 (34%) - in Grade Group 2 and 7 (14%) respectively in Grade group 3, 4 and 5. Perineural invasion is found in 35 (70%) of all non-advanced cancers. In non-advanced carcinomas, 29 (58%) were restricted to the prostate gland, 20 (40%) showed extraprostatic extension in the capsule or seminal vesicles, and only 1 (2%) involved adjacent prostate structures.

For advanced carcinomas, 2 (5.71%) cases were found to have PSA below 10.00 ng / mL, 2 (5.71%) with PSA between 10.00 and 20.00 ng / mL, 4 (11.43%) between 20.01 and 50.00 ng / mL and 27 (77.14%) with PSA above 50.00 ng / mL. In 30 cases (73.2%) there is a cribriform growth model, while in 11 (26.8%) - no. In 1 (2.4%) of advanced cancers Gleason score is 6, in 4 (9.8%) is 7, in 15 (36.6%) is 8, in 16 (39%) is 9 and in 5 (12.2%) is 10. Distribution by prognostic groups is respectively 1 (2.4%) in Grade Group 1, 4 (9.8%) in Grade Group 2, none in Grade group 3, 15 (36.6%) in Grade group 4 and 21 (51.2%) in Grade group 5. There is perineural invasion in 28 (68.3%) of the advanced cases, and in 13 (31.7%) they are absent. In 41 cases of advanced cancer there are tumor necrosis, and in 37 (90.2%) cases they are absent. In 35 (85.4%) cases, the tumor was confined to the prostate gland, 3 (7.3%) showed extraprostatic extension in the capsule or seminal vesicles, and 3 (7.3%) involved structures adjacent to the prostate gland.

The comparative analysis of the variables up to and over 70 years of age and PSA did not reveal a statistically significant relationship between them, both in the group of non-advanced tumors and in the group of advanced carcinomas. The comparative analysis between the age variables and the Gleason score showed that in the older age groups the Gleason score is higher, excluding the age over 80 years.

PSA values in the group of non-advanced carcinomas and cribriform histological growth indicate that at high PSA values, tumors are more likely to have cribriform structures. In non-advanced PSA values in the groups with different Gleason score shows a tendency to increase PSA with increasing Gleason score. The level of PSA in tumors with perineural invasion is higher than in carcinomas without evidence of perineural invasion, while there is no relationship between PSA value and T-stage in patients without metastases. In advanced tumors, the mean PSA values in the cribriform growth model are higher than in the absence of such a model. There was no statistically significant relationship between PSA meanings in the advanced tumor group between PSA levels and the Gleason score, nor between mean PSA values in the metastatic tumor group, PSA levels, and the presence of perineural invasion. Mean PSA values in prostate cancer patients tended to increase in the group of advanced tumors compared to non-advanced ones. In only one case there is histological evidence of cribriform growth. In non-advanced carcinomas without perineural invasion, most are those with Gleason score 6 - 7. Again, in the majority of tumors - 66.7%, which are in pathological stage T2, there is no cribriform growth model. There are statistically significant differences in non-advanced cancers between Gleason score with stage as the sum of 6 is more common in T1 and T2, and a higher sum in T3 and T4. Also in the more advanced T-stage the frequency of perineural invasion is 87% and it is significantly higher in the M0 tumors.

What are the results of the advanced cancers: no statistically significant relationship was found between the cribriform growth model and the "perineural invasion"; also between Gleason score and perineural invasion; the incidence of the cribriform growth pattern is higher in advanced tumors.

Data from the immunohistochemical study showed the highest mean levels of cytoplasmic expression of CD9 in the BPH group. In carcinomas, the values are lower in both non-advanced carcinoma and advanced tumors, but there is no statistically significant difference. The mean cytoplasmic expression of CD151 in non-advanced and advanced carcinoma and BPH showed the highest levels, but the difference was not statistically significant, with the lowest mean level in the BPH group.

The results were compared in discussion with other similar studies. The publications whose data coincide with the results and those in which there are differences are marked.

At the end of the work, the following 11 conclusions were made, which I consider relevant and which can be briefly summarized as follows: at high PSA values, the incidence of cribriform structures and perineural invasion is also high, in non-advanced prostate cancer there is no relationship between PSA and age, as well as between PSA and T-stage of prostate cancer, perineural invasion is common in the cribriform growth pattern and is associated with T-stage, the cribriform growth pattern does not show a T-stage dependence; in advanced prostate cancer there are high PSA values, regardless of the cribriform growth pattern and perineural invasion, the cribriform growth pattern is 3 times more common in advanced cancer; in non-advanced carcinomas, cytoplasmic CD9 expression decreases after age 70; in PNI, patients with prostatic carcinoma without distant metastases have a higher expression of CD9 than in patients with metastases, in advanced carcinomas the expression of CD9 does not show a dependence on morphological parameters cribriform growth pattern, GS and PNI; high CD151 expression in stage M0 prostate cancer occurs in cribriform growth pattern, high Gleason score and perineural invasion, in advanced carcinomas the expression of CD151 in prostate cancer tumor tissue does not depend on the age of patients, cribriform pattern, Gleason score and PNI.

The dissertation work of Dr. Stoev has the following contributions, indicated by him, which I fully accept:

1. Immunohistochemical expression of CD9 and CD151 was analyzed in order to clarify their role in the prognosis in patients with prostate cancer - an original contribution.
2. There was analysis of the significance of the different values of PSA in relation to the clinical and morphological indicators, the prognostic value of the cribriform model and the expression of CD9 and CD151 in relation to the clinical and morphological indicators - practical contributions was assessed.

The data from the dissertation are presented in two scientific publications and one scientific report.

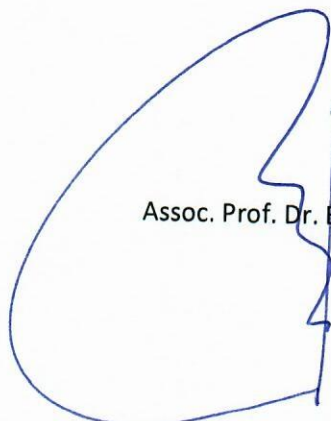
Dr. Stoev's dissertation is written in good language and is adequately illustrated. It presents many in-depth and practically useful results related to the morphology of prostate cancer, examining in detail the relationships between GS, PNI, the presence of cribriform structures, which will contribute to a more detailed assessment of their significance for the prognosis of this common tumor. IHC studies of little-studied markers, tetraspanins CD9 and CD151, have shed light on new unexplored factors associated with the pathogenesis of prostate cancer. This is evidence of the innovative nature of the dissertation.

Based on the above, I believe that the dissertation of Dr. Stoev is fully completed, containing 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 acquiring scientific degrees at MU - Varna.

This is why I give a positive assessment of the work, I will vote for, and I allow myself to recommend to the members of the respected Scientific Jury to support the awarding of the educational and scientific degree "Doctor" to Dr. Lyuben Lyudmilov Stoev.

20.11.2021



Assoc. Prof. Dr. Belovezhdov