REVIEW

by

Assoc. Prof. Dr. Elena Galabova Poryazova – Markova Ph.D.

Medical Faculty, Department of General and Clinical pathology

Medical University – Plovdiv

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under the procedure for obtaining the educational and scientific degree "Doctor"

professional field: medicine, doctoral program "Pathoanatomy and Cytopathology", code 03.01.03.

Author: **Dr. Lilyana Nikolova Petkova**

Form of doctoral studies: **full-time doctoral studies**

Department of General and Clinical Pathology, Forensic Medicine and Deontology

Medical University -Varna "Prof. Dr. Paraskev Stoyanov"

Topic: **"Expression of Cyclin D1, BCL2, p53 and other melanocyte markers in malignant melanoma of the skin and melanocyte nevi - a comparative analysis of immunohistochemical expression, morphological profile and their importance for diagnosis and tumor progression"**

Scientific supervisor: **Prof. Dr. Bogomila G. Manevska, MD**

Brief biographical data and professional development of the doctoral student

Dr. Lilyana Nikolova Petkova was born on September 23, 1961. She graduates from the Mathematical High School in Dobrich in 1979. She has a master's degree in medicine from the Medical University - Varna in 1988. Acquired a degree in general and clinical pathology in 1995. Dr. Petkova has a total work experience of over 30 years. Immediately after graduation and currently working as a pathologist at the University Hospital "St. Marina" - Varna. She has worked as a resident doctor in UMBAL - Dobrich and Ruse and as head of the Department of Clinical Pathology at UMBAL - Dobrich. Since 2014 she has been an intern in the Clinic of General and Clinical Pathology at the University Hospital "St. Marina" Varna and since 2019 she is an assistant at the Medical University - Varna, Department of General and Clinical Pathology, Forensic Medicine and Deontology and a full doctoral student. Dr. Petkova is fluent in written and spoken English and Russian.

Assessment of the personal participation of the doctoral student in the dissertation

The topic of the dissertation is relevant and does not repeat other studies in connection with malignant melanoma in Bulgaria and abroad. Malignant melanoma (MM) is a malignant tumor originating from epidermal melanocytes and is the most serious oncological problem in dermatology. Diagnosis of MM is difficult for the experienced pathologist. The correct diagnostic approach requires knowledge and evaluation of a large number of histological parameters and often additional immunohistochemical analysis, both to prove the origin of the tumor and to differentiate malignant melanoma from mimicking borderline lesions. Long experience and excellent morphological knowledge are required in such cases. The incidence and mortality from malignant melanoma in the world and in our country is increasing in recent decades. The prognosis remains unfavorable. Early diagnosis, followed by adequate surgical excision, leads to recovery in over 90% of low-risk melanomas. Timely and accurate diagnosis correlates with treatment success and patient survival by improving prognosis.

The dissertation is written in clear and precise Bulgarian language in a good scientific style. The dissertation contains 154 standard typewritten pages and is illustrated with 41 figures and 30 tables. The literature reference includes a total of 126 literary sources, of which 10 in Cyrillic and 116 in Latin. The bibliography is structured according to the requirements. The construction of the dissertation is in the usual sequence (introduction, literature review, purpose and tasks, material and methods, results of own research, discussion, conclusions, contributions, bibliography).

Two groups of biomarkers (immunohistochemical antibodies) were selected: **melanocytes** (S100 protein, Melanosome clone HMB45) and **non-melanocytes** (Cyclin D1, BCL2, p53 protein), which significantly increase the possibility of more reliable confirmation of the malignant

potential of pigmented skin lesions and more accurate predictors of tumor progression. The comparative analysis of the immunoreactivity of such biomarkers in benign nevi, atypical pigmented lesions and malignant melanoma improves the diagnostic capabilities of the pathologist and protects him from the possibility of diagnostic inaccuracies. These biomarkers can be used for both diagnostics and research. Morphological features in combination with the expression of **melanocyte and non-melanocyte biomarkers** for each type of lesion - melanoma, benign and atypical nevi, can help in early diagnosis and subsequent therapy.

The introduction is short, precise and points to the purpose of the development and the topic of the dissertation. The literary review is well structured and shows the doctoral student's ability to handle literary sources. It is presented on 41 standard pages. Describes current trends in the English literature regarding the classification and etiology of pigmented skin lesions. The importance of risk factors and genetic mutations for the progression of malignant melanoma is presented. Literary sources are from the last 10 years, and authors from recent years are also cited. The epidemiology, etiology and pathogenesis of pigmented skin lesions are discussed in 9 chapters. The modern classification and histological characteristics of benign pigmented neoplasms and malignant melanoma are described in detail, a brief description of the main histological subtypes of malignant pigmented skin neoplasms, as well as the features of atypical pigmented nevi and methods for their diagnosis.

The purpose is clearly and precisely stated. A well-selected biopsy array of tumor and non-tumor skin lesions is tested with melanocyte (S100 protein, Melanosome clone HMB45) and non-melanocyte (Cyclin D1, BCL2, p53 protein) biomarkers in order to look for significant differences and to develop criteria for help of the morphological diagnosis, especially for the differentiation of borderline lesions from malignant melanoma with possible prediction of biological behavior in these tumors.

The tasks are properly structured. There are 5 tasks, the first of which is related to the selection of the three groups of research material - benign, borderline lesions and malignant melanomas. The task is to study the immunohistochemical expression of biomarkers in parallel, looking for significant differences in this expression, to develop a diagnostic algorithm that supports and provides morphological diagnosis in diagnostically difficult cases that may imitate malignant melanoma. After analysis of the obtained results to look for possible prognostic signs for the behavior of borderline lesions and malignant melanoma.

The material used in the study is sufficient to obtain statistically reliable results. A total of 91 pigmented neoplastic lesions were examined, including 57 (fifty-seven) benign melanocyte nevi, 10 (ten) atypical nevi, and 24 (twenty-four) malignant melanomas. The entire material for the present study was diagnosed in DKC 2 EOOD Dobrich, for a 5-year period - from 2014 to 2019.

The research methods are modern and the dissertation student shows that she knows how to read and describe the results. The groups are methodologically correct. Routine histological

methods are presented concisely, accurately and clearly. Emphasis is placed on morphological and immunohistochemical studies, which are described in detail. The criteria for assessing the immunohistochemical expression of all antibodies used are clearly formulated and justified. The statistical research methods used are also described.

The results of the research obtained during the tasks are well explained and correctly described, accompanied by well-structured tables. The correct choice of the used statistical methods is a prerequisite for the autheticity of the obtained results. Each result is accompanied by a logical scientific discussion, which helps to make it easier to perceive and make sense of the whole material.

Own results are presented in 57 standard pages, well illustrated with tables, figures, and graphs, as well as high-quality microscopic photographs and appropriate magnifications in the order of the tasks. In the presented discussion of the results, a critical review was made, which is based on the comparison of the literature data with the results of our own research. Literary sources have been skillfully used in order to concretize the data from other studies according to the needs for comparison with own results.

After summarizing the results obtained and the discussion, 12 **conclusions** were made, which summarize the criteria for accurate diagnosis and prediction of the biological behavior of pigmented skin neoplasms. **The formulated conclusions and contributions** are in accordance with the set tasks and the obtained results. With regard to the contributions, they are divided into original scientific and applied contributions and confirmatory ones. In my opinion, it is of the greatest importance to prove the relationship between the high prognostic value of the proposed triple combination of markers - Cyclin D1, p53 protein expression and HMB45 and the malignant potential in pigment lesions. The higher proliferative index in malignant melanomas reported with Ki 67 was associated with more aggressive tumor growth and metastasis, although these results showed lower values compared to other similar studies in other groups.

In connection with the dissertation, **Dr. Lilyana Petkova** has presented four publications, all of which she is the first author.

The abstract meets the requirements, is written on 108 pages and includes all the main parts of the dissertation, as well as tables, figures and photographs. The content of the abstract gives a detailed idea of the overall dissertation and reflects the main results and conclusions and contributions.

Assessment of the professional and personal qualities of the doctoral student

Dr. Petkova is an established specialist in general and clinical pathology, always with a clear position, both on discussed diagnostic cases and on organizational issues of the colleges she attended. From our joint work I know Dr. Petkova as an educated and intelligent colleague, a

very good diagnostician who constantly strives to improve both in her diagnostic work as a pathologist and in research, despite her heavy workload.

Conclusion

The dissertation developed by **Dr. Lilyana Petkova on** the topic: "Expression of Cyclin D1, BCL2, p53 and other melanocyte markers in malignant melanoma of the skin and melanocyte nevi - a comparative analysis of immunohistochemical expression, morphological profile and their importance for diagnosis and diagnosis progression" contains sufficient scientific and applied results, which are a contribution to the biopsy diagnosis of pigmented skin lesions and malignant melanoma. Based on the above facts, I believe that the presented dissertation contains all scientific and applied results, which are a contribution and meet all the requirements of the Academic Staff Development Act (ACASR) and the Regulations for the implementation of ACASR in the Republic of Bulgaria.

The doctoral student has the necessary theoretical knowledge on the topic of the dissertation and has professional skills for conducting independent research. The dissertation of Dr. Petkova contains significant and original results, which are skillfully presented as conclusions and contributions of original-applied and confirmatory character.

In conclusion, I confidently give a positive assessment and recommend the other members of the Scientific Jury to vote positively for the award of the Educational and Scientific Degree "Doctor" of Dr. Lilyana Petkova in the Doctoral Program in Pathology and Cytopathology.

14.02.2022

Prepared the opinion:

Assoc. Prof. Dr. Elena Poryazova-Markova, Ph.D.