# PEER REVIEW

**From Assoc.Prof. Nikolay Conev, MD, PhD**
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**CONCERNING: Dissertation thesis of Jeliazko Iliev Arabadjiev, MD**

**Entitled "PREDICTIVE AND PROGNOSTIC VALUE OF TUMOR-INFILTRATING LYMPHOCYTES IN WOMEN WITH BREAST CANCER"**

**for acquisition of the educational and scientific degree "Doctor"**

**Scientific supervisor: Assoc. Prof. Eleonora Dimitrova-Gospodinova, MD, Ph.D.**

*The review is prepared according to the requirements of:*
- *The Law for Development of the Academic Staff in the Republic of Bulgaria (LDASRB);*
- *Regulations for implementation of the LDASRB*
- *Regulations on the terms and conditions for acquiring scientific degrees and holding academic positions at MU – Varna*

**Candidate's biography**

Dr. Jeliazko Iliev Arabadjiev graduated in medicine at the Medical University - Varna in 1997. From 2008 to 2014 he was a specialist in the Clinic of Medical Oncology of USHATO - Sofia. In 2012 he graduated from the University of National and World Economy with a degree in Health Management, and in 2014 he acquired a degree in Medical Oncology. From 2015 to 2017 he worked at the Acibadem City Clinic Oncology Center in the Clinic of Medical Oncology, and in June 2018 he moved to the Department of Medical Oncology at Acibadem City Clinic Tokuda Hospital, where he held the position of head of department. In March 2019 he was enrolled as a freelance doctoral student at the Medical University - Varna in the Department of Oncology. Since October 2019 he has been an assistant professor at Sofia University "St. Kliment Ohridski "- Sofia, Faculty of Medicine, in the Department of Propaedeutics of Internal Medicine.

For the period 2015 - 2019 he is a representative of Bulgaria in the European Society for Medical Oncology (ESMO).

During the period 2008 - 2019 he actively participated in a number of national and international congresses, conferences and others.

Dr. Arabadjiev speaks English, Russian and Italian.

He is a member of:

- European Society for Medical Oncology (ESMO);

- American Society for Clinical Oncology (ASCO);
- American Association for Clinical Research (AACR);
- General Medical Council (GMC) in the United Kingdom, Specialists Register;
- Educational Academy of Oncology (AAO) - co-founder, member of the Board;
- Young Oncologist Club Association, Deputy Chairman

He is a member of the editorial board of Pro Medic, European Medical Journal, Oncostar, Bulgarian Association for Clinical Trials.

***Breast cancer (BC) is the most common malignancy in women in the world;***
***According to the World Health Organization (WHO), the incidence of BC worldwide is about 2.1 million new cases diagnosed annually;***
***In Bulgaria about 4000 new cases are registered annually;***
***In Bulgaria, one in 16 women is likely to get BC.***

Breast cancer (BC) is the most common malignancy in women in Bulgaria as well as in states of EU and North America. According to the World Health Organization (WHO), the incidence of BC worldwide is about 2.1 million new cases diagnosed annually, which represents one forth of all malignancies in women.

Despite significant advances in diagnosis, a significant percentage of new BC cases are still detected at an advanced stage with the presence of distant metastasis, which is noted as an unfavorable fact. In these cases, the goal of treatment is to slow the progression of the disease, preserve the quality of life and delay the time to reach palliative and symptomatic treatment.

In order to optimize the clinical behavior in breast cancer, prognostic and predictive factors are sought to determine the course of the disease and the effect of treatment, respectively. Recently, BC has been increasingly viewed as an immunogenic tumor, and as such the immune system's response to cancer cells is of particular importance for prognosis and treatment effect. The immune system and the immune response play a key role in the onset and development of cancer. Recently, it has been investigated whether the degree of peri- and intratumoral lymphocyte infiltration, quantified by tumor-infiltrating lymphocytes (TILs), can serve as a prognostic and predictive factor in breast cancer. It is currently assumed that the degree of lymphocytic infiltration, assessed only on the basis of hematoxylin and eosin (H&E) stained tumor sites, provides a reliable and statistically confirmed prognostic and prognostic value in some subtypes of BC (eg TNBC and HER2 +). At this stage, the need for detailed information on the immune subpopulations of the infiltrate is not associated with additional information on the prognosis of the patient with BC. Regarding the degree of lymphocytic infiltration, as an expression of the immune response, the results of some clinical studies show that increasing the number of TILs in the tumor and stroma prolongs overall survival (OS), disease-free survival (SBP) and improves the clinical response to treatment. Despite significant advances in BC therapeutic approaches, there are still unresolved issues. One of these unresolved problems in the treatment of BC is finding reliable markers on which to base the personalized individualized therapeutic approach. This determines the need to study the importance of lymphocytic infiltration of the tumor and peritumoral stroma for OS and the

effect of treatment, if possible, to create an exemplary algorithm for assessing this factor and make recommendations for its use in clinical practice.

**Characteristics of the presented for peer review dissertation thesis.**

The dissertation thesis consists of 118 pages including the bibliography and contains 21 tables and 31 figures. The dissertation thesis has a classical structure: “Introduction”- 3 pages, “Literature review” 52 pages, “Methods of research” – 7 pages (with sub-chapters “ Purpose and tasks“, “Patients selection”, “Specific methods of research”), “Results” – 22 pages, “Discussion” – 3 pages, “Conclusions” and “Contributions of the dissertation thesis”- 2 pages, “Apendices” – 5 pages, “List of scientific publications and releases in connection to the dissertation thesis” – 6 publications, “Participation in scientific forums with published abstracts” – 1. Bibliography contains 246 literature sources.

The study examined the data of 118 women with BC for the period from January 2019 to January 2020, which were analyzed retrospectively by medical records of patients diagnosed with BC from 1997 to 2020. The in-depth study included 58 women with histologically confirmed lobular or ductal breast cancer in stages I to IV. Bases of the study - Acibadem City Clinic Tokuda Hospital EAD: Department of Medical Oncology and Department of General and Clinical Pathology.
The main focus of the **literature review** are tumor-infiltrating lymphocytes (TILs), as well as the fundamental role of the immune system in the processes of tumorigenesis, the influence on the behavior of cancer cells through the so-called cross-talk - between cancer cells and their immune microenvironment, the presence or absence of certain immune elements that determine the immune "landscape" around the tumor and their prognostic significance. The author presents evidence that dispels the "myth" that breast cancer is not an immunogenic tumor and provides a scientific overview of the role of immune infiltration in breast cancer. Techniques for assessment and recommendations for analysis of TILs are also discussed. Last but not least, the literature review analyzes the factors related to the etiology, pathogenesis and development of breast cancer. Examines in detail the possibilities of modern drug treatment, focusing on registration studies that have established one or another treatment regimen in clinical practice. The author also discusses the place of hormone therapy and biological therapy for breast cancer.
The **Purpose** of the author is to study the degree of TILs in patients with BC in order to establish their prognostic and predictive value and the possibility of specific application in practice.
To achieve the set goal the author sets the following tasks:
Selection of patients with BC in adjuvant and metastatic stages and determination of the degree of TILs in the tumor material. Analysis of the correlations between the degree of TILs with the clinical and pathological characteristics of patients with BC in the study - age, location, IHC, stage of the disease, disease development, drug treatment, adjuvant

radiotherapy. Determination of the prognostic potential of the degree of TILs depending on the development of the disease and IHC and analysis of the predictive value of the degree of TILs for response to drug antitumor therapy and adjuvant radiotherapy (ART).
Inclusion and exclusion criteria were clearly formulated to select the appropriate patients for the study, and the parameters for examination and follow-up were pre-defined and retrospectively collected within the study.

**Results**

The study included data from 118 women with BC for the period from January 2019 to January 2020, and the in-depth study included 58 women with histologically confirmed lobular or ductal breast cancer in stages I to IV. All patients were stratified by demographic and clinical pathology characteristics and received adjuvant treatment and / or palliative treatment for metastatic disease - drug treatment (chemotherapy and / or hormone therapy) and / or radiation therapy. Regarding the stage of the disease, they are divided into 3 groups: adjuvant without progression (adjuvant + SD), adjuvant with progression (adjuvant + PD) and metastatic at diagnosis.

The results are presented in hierarchical categories and are very well visualized, divided into the following sections:

1. Correlations with respect to overall survival and event-free survival time;
1.1. Correlations regarding overall survival and time of event-free survival to disease stage;
1.2. Correlations regarding overall survival and event-free survival time to disease development;
1.3. Correlations regarding the time of PBZ compared to the achieved 3-year survival (<36 and> 36 months);
2. Evaluation of the degree of tumor-infiltrating lymphocytes in relation to some characteristics of the disease
2.1. Evaluation of the degree of tumor-infiltrating lymphocytes in relation to the development of the disease;
2.2. Evaluation of the degree of tumor-infiltrating lymphocytes according to the stage of diagnosis;
2.3. Evaluation of the degree of tumor-infiltrating lymphocytes relative to tumor location;
2.4. Evaluation of the degree of tumor-infiltrating lymphocytes with age;
2.5. Evaluation of the degree of tumor-infiltrating lymphocytes according to the type of surgical intervention;
2.6. Evaluation of the degree of tumor-infiltrating lymphocytes in relation to performed adjuvant radiotherapy (ART);
2.7. Evaluation of the degree of tumor-infiltrating lymphocytes in relation to the immunohistochemical characteristics (IHC) of the tumor;

3. Correlation of overall survival and event-free survival time to the degree of tumor-infiltrating lymphocytes and disease development;
4. Correlation of overall survival and event-free survival time to the degree of tumor-infiltrating lymphocytes and the type of drug treatment received;
5. Correlation of overall survival and event-free survival time to the degree of tumor-infiltrating lymphocytes and ART;
6. Correlation of overall survival and event-free survival time to the degree of tumor-infiltrating lymphocytes and the immunohistochemical characteristics of the tumor;
7. Correlation of disease-free survival and tumor-infiltrating lymphocytes and disease development.

**Some emphasis on results:**
An important result is that in low TILs, patients treated with endocrine therapy or a sequence of chemotherapy and endocrine therapy have a greater benefit in terms of OS than those with high lymphocyte infiltration, whereas in the group of patients treated with chemotherapy alone, OS has been found to be prolonged with higher lymphocytic infiltration.
There was a statistical dependence for low OS with increasing degree of TILs in the group of HR-positive / HER2-negative variants (as TILs are a prognostic factor for shorter survival) and lack of statistical significance or observed statistical trend in the groups of HR-positive / HER2-negative variants. positive / HER2-positive and HR-negative / HER2-negative IXX variants.
An interesting and provocative statistical trend has been identified, which may define the need for ART to lead to prolonged OS in patients with low TILs, regardless of the IHC type.

The **discussion** on the obtained results emphasizes their importance, giving guidelines for interesting future developments.

**Conclusions**: 9 clear conclusions are formulated, which lead to logical contributions of **scientific-theoretical nature** and **scientific-practical nature**.

The **abstract** is 44 pages long and presents in a concise form the main problems and conclusions presented in the dissertation: formulated goals and objectives, methods used, results and discussion, conclusions and contributions of the dissertation. A list of the main publications of the candidate related to the topic of the dissertation is also presented.

**Critical remarks and recommendations:**
I am familiar with the many tasks, functions and responsibilities that Dr. Arabadjiev has in recent years, which naturally and logically does not allow him to develop the full potential he has and to carry out this full-scale research. The main weakness of the dissertation is the relatively small number of patients who are studied and on the other hand their overstratification, which leads to even smaller groups and hence to the difficulty of statistical analysis. I am sure that this project will be expanded and optimized in the near future.

**The scientific production** of the candidate, related to the topic of the dissertation, includes 4 publications and one participation and presentation of the results of a scientific forum.

**Conclusion**

The dissertation contains scientific and scientific - applied results with original contribution to science and meets all the requirements of the Law for development of the academic staff in the Republic of Bulgaria (LDASRB), the Regulations for application of LDASRB, the Regulations of MU - Varna. The presented materials (dissertation, publications and scientific communications) correspond to the specific requirements of LDASRB.

The dissertation shows that the doctoral student Dr. Jeliazko Iliev Arabadjiev has in-depth theoretical knowledge and professional skills in the scientific specialty of medical oncology, demonstrating qualities and skills for independent research.

Due to the above, I give my POSITIVE assessment of the research presented by the above-reviewed dissertation, abstract, results and contributions by proposing to the esteemed scientific jury to award the educational and scientific degree "Doctor" to Dr. Jeliazko Iliev Arabadjiev in a doctoral program “Oncology”.

August 06, 2020

Peer Reviewer:

**Assoc. Prof. Nikolay Conev, MD, PhD**