REVIEW

From: Assoc. Prof. Dr. Veselina Goranova-Marinova, MD
Head of the Hematology Section, Medical Faculty, MU-Plovdiv
External member of the scientific jury by order of the Rector of MU - Varna № P-109-377 06.10.2020

Subject: Procedure for obtaining the scientific and educational degree of doctor
Field of higher education: 7. "Health and sport"
Professional field: 7.1. "Medicine
Doctoral program: "Hematology and blood transfusion", code 03.01.39
Author: Dr. Vanya Slavcheva Popova, PhD student in independent form of education, at the II Department of Internal Medicine, Faculty of Medicine, Medical University – Varna
Form of doctoral studies: Independent preparation
Topic: "Clinical application of prognostic factors and their integration into a scale for risk assessment and time to treatment in untreated patients with B-chronic lymphocytic leukemia"
Scientific adviser: Prof. Dr. Liana Gercheva-Kyuchukova, MD

1. **General presentation of the procedure**. The presented set of materials on paper and flash memory is in accordance with the procedure for acquisition of scientific and educational degree "PhD" in MU-Varna, the Rules of MU-Varna for the development of the academic staff and includes all necessary documents. The orders for enrollment and deduction, the protocols of extended department councils and the passed doctoral minimum exam are presented, which reflect all stages of the doctoral student's development, including the Dissertation and the Abstract. The stages of the doctoral program have been observed. There is no change in the initial topic and the supervisor. The research was approved by the Commission for Scientific Ethics. A list and copies of the publications related to the dissertation are attached to the set of materials. The total number of publications is 4, in which the Dr Slavcheva is a leading author. Two of the publications are in JIMAB magazine - referenced and indexed in the international Web-of-Science database. The doctoral procedure has been followed.
2. **Presentation of the doctoral student.** Dr. Vanya Slavcheva Popova is graduate in the field of medicine in 1990 - Pleven Medical Institute, and since 1996 she has been working as an assistant at the Clinic of Hematology at the University Hospital "Dr. Stranski". Over the years she acquired BC/ BE degree in Internal medicine and Clinical hematology. The doctoral student's scientific interests are in the field of malignant lymphoproliferative diseases, including CLL. Dr. Slavcheva is a leading author and co-author of over 20 articles in the field of hematology, immunology and genetics, published in Bulgarian and foreign journals. She has been involved in the development of research projects related to CLL disease. Dr. V. Slavcheva is a member of the working group "Lymphomas" at BMUH. She speaks Russian and English. *The doctoral student has theoretical knowledge, practical experience, additional qualifications and focused scientific and practical interests in the field of doctoral studies.*
3. **Actuality on the subject**. In the dissertation Dr. Vanya Slavcheva develops an important, current and of great practical importance problem in modern clinical hematology. Chronic lymphocytic leukemia (CLL) is one of the most common leukemias among adults, as the risk of disease progressively increases with age. It is a fact that about 80-85% of newly diagnosed patients remain under observation, but the clinical course of the disease is extremely heterogeneous - from sluggish indolent

forms that do not require treatment for years, to aggressive, evolutionary course, difficult to treat and with a quick fatal end. At the moment there are many clinical studies for measurable, reliable and stable indicators that can reproductively predict the evolution of the disease, the time to start treatment and the appropriate therapeutic choice. The dissertation develops the clinical application of prognostic factors and their integration into a scale for risk assessment and time to treatment in untreated patients with B-CLL. After year 2000, thanks to the development and improvement of immunology, immunogenetics and molecular biology, a number of author teams intensively analyzed and offered prognostic models, indices and scales for risk stratification in patients with newly diagnosed B-CLL. However, at present for our country there is no consensus and reported studies based on evidence related to a comprehensive assessment of available molecular genetic and classical prognostic factors. The need for a more accurate assessment of the risk of progression is becoming increasingly important as new B-CLL drugs emerge. This gives the dissertation a reason to develop the present dissertation. ***The topic of the dissertation is relevant, of great social and clinical importance.***

4. **Knowledge of the scientific problem.** The different and variable clinical course of chronic lymphocytic leukemia in patients classified in the same clinical stage is probably related to the different molecular genetic profile of each of them. The fact is that some of the unfavorable prognostic markers are present at the time of diagnosis of the disease. They are synonymous with aggression and determine a shorter time to start antileukemic therapy. In this regard, the need for their early and timely identification in each patient with B-CLL is justified. ***The author knows the scientific problem in depth, can formulate a scientific hypothesis and conduct an analysis, the results of which would lead to improved risk - starting, predicting the time to start treatment, choosing the most appropriate therapeutic strategy to prolong survival and improving the quality of life of CLL patients.***
5. ***Characteristics and evaluation of the dissertation.*** The dissertation is developed on 123 pages. It contains 24 tables and 49 figures. 199 literature sources were cited, purposely selected. The obligatory sections of the scientific work are professionally developed, and an acceptable ratio between them is observed. A clear and accurate, grammatically correct Bulgarian language is used.

Literature review. The literature review is presented on 37 standard pages, in-depth and focused on the scientific problem. Objectively, data from numerous studies that consider the prognostic factors in CLL as a variable that is assessed before starting treatment and on the basis of which a good or poor clinical outcome can be expected, regardless of the choice of therapy. The doctoral student pointed out that such indicators have been analyzed in many scientific studies in order to identify patients with the need for early treatment, to determine the time to initiate treatment, to choose risk-adapted therapy (drug toxicity), and to determine follow-up strategy for CLL patients. Such a stable indicator that does not change in the course of the disease is the mutational status of the genes for the variable region of the immunoglobulin heavy chain (IGVH), defined by sequencing methods. Considered in a complex with the so-called "classical prognostic factors", the latter serve as a basis for forecasting and selection of targeted therapy and are part of the presented in 2016 international prognostic index - CLL-IPI. Although originally developed to predict overall survival, several clinical trials involving multiple groups of patients have validated its use in estimating the time to initiation of initial treatment (Parikh AS 2018). The mutational status of IGVH, as well as the factors influencing it, are extremely important and require good knowledge, as taken together they indirectly reflect the aggressiveness of the disease and are related to the survival and outcome of the disease. ***The literature review concludes with a***

motivated basis for studying the impact of available clinical-laboratory and molecular-genetic factors related to the specific characteristics of both the patient and his disease, with an assessment of their impact on time to the need to start treatment in untreated patients with B-CLL and allows the formulation of a clear goal and scientific hypothesis.

Purpose and tasks. The goal is precisely and clearly formulated and logically set 6 main tasks.

Material and methods section. The Material and Methods section is presented on 9 standard pages. It was carried out both as a retrospective and prospective study, conducted at the University Hospital "Dr. Georgi Stranski" EAD - Pleven, Clinic of Hematology, Central Clinical Laboratory, Medical Laboratory of Immunodiagnostics, Clinic of Imaging, genetic and molecular research were conducted in Laboratory of molecular biology and cytogenetics at SBATHD - Sofia, within three years 2016-2019. - CLL patients are divided into three groups: Group I - 97 patients with standard laboratory tests, flow cytometric analysis of peripheral mononuclear cells, beta 2-microglobulin level and staging procedures .; Group II, consisting of randomly selected 61 patients, from the first group of patients who underwent FISH for del17p-, del13q-, del11q- for and group III, comprising 48 patients (randomly selected from group II), in whom an additional PCR analysis was performed for ADAM29 / LPL as a surrogate marker for IGHV mutation status. The statistical processing of the obtained results is done competently with correctly selected analyzes and the data are visually presented. The SPSS 19.0 statistical package was used. for Windows (SPSS Inc, Chicago USA) and the methods of classical statistics, including graphical analysis to illustrate the results. The methodology is chosen correctly. It calls for the recruitment of a sufficient number of patients to conduct statistical analysis and obtain reliable results.

5.4.The "Results" section is presented on 30 standard pages. The presentation of the section is concise and is presented in an overview of tables, figures, graphs and diagrams. The sequence of the set tasks is logically followed. Time to initiation of first treatment (TTFT), defined by default - the interval from diagnosis of the disease to the start of treatment, or the date of the last follow-up, or death (censored). The Kaplan Meier log-rank test method was used. The indications for treatment are in accordance with the recommendations of IWCLL - 2008. (Hallek M et al, 2008). The results show that the short time of leukocyte milking, the high absolute number of lymphocytes at the time of diagnosis, the high levels of serum β-2 MG, the presence of del11q and del17p, including the combination of more than a chromosomal aberration and unmutated IGVH status determined by the surrogate markers LPL and ADAM29 predetermine a shorter time to progression and need for therapy.

Notes:

1. The distribution by sex, age and clinical stage, as well as other standard characteristics of the studied contingent to be considered and presented in the section "Material and methods"

2. Statistically significant predictive factors determining the time to start treatment to be assessed by multivariate analysis with Cox - the regression model, which would allow the derivation of independent predictive factors

3. To be unified in the whole dissertation: the time until the beginning of the treatment should be presented as "median" (recommended) or as "average value".

I accept the results obtained. Critical remarks do not underestimate their value. As a clinically important result, I note that PCR analysis allows the identification of high-risk patients with UM status, in whom the median time to initiation of treatment is significantly shorter compared to TTFT in individuals with MT status.

5.5 **Discussion section.** In 16 standard pages the doctoral student analyzes the obtained own results, discusses their significance and compares them with those of other author

teams. The established differences with the data from other scientific developments, the author explains with arguments and concrete facts. Such is the example with the independent analysis of the age factor, for which the study of Dr. Slavcheva did not confirm a prognostic value relative to the time to treatment. This author explains this with the influence of the proliferative activity of neoplastic cells) and the extent of the disease (clinical stage). The results of the multiplex PCR, which show twice the number of patients with UM status compared to those in whom the status was determined as MT, were discussed correctly and in confirmation of the generally accepted data. the mean time to initiation of treatment calculated by the authors in the UM status group was significantly shorter compared to the MT status group. The connection between the unfavorable chromosomal aberrations in the group studied by Dr. Slavcheva and the mutational status of IGVH is also confirmed. I accept the "Discussion" section without remarks.

5.6. The conclusions are 10 in number and clearly follow the set goals and objectives and are logically formulated according to the results obtained. Most important from a clinical point of view is the conclusion that the complex assessment based on molecular genetic markers available for study in patients with untreated B-CLL provides a better opportunity to stratify the risk of progression, determine the time to treatment, the period for follow-up of patients and, accordingly, the selection of the most adequate therapy**. *I agree with the conclusions formulated in this way.***

5.7 Literature sources. The bibliography includes 199 literary sources, 6 of which are by Bulgarian authors. The analyzed scientific publications after 2015 are> 30% of the total number. These data testify to the relevance of the problem and the great research interest on the topic in recent years. Note: The general standard is not observed in the writing of the bibliography

5.8 Evaluation of the contributions of the dissertation The paper ends with the presentation of contributions that have original (2 pieces), confirmatory (3 pieces) and applied character (2 pieces). I accept the contributions presented.

6. Personal participation of the doctoral student. The doctoral student is personally involved in the formulation of the scientific idea, the collection of the material and the design of the research. His participation in the statistical processing of the data and the analysis of the obtained results is personal. The conclusions and contributions were also presented with the participation of Dr. Slavcheva. The doctoral student has mainly personal participation in the development of the dissertation.

7. Abstract. The abstract contains 64 pages, gives a complete idea of the dissertation and fully reflects the individual sections. The figures and tables are selected specifically and present the necessary data.

8.Conclusion. The dissertation work of Dr. Vanya Slavcheva Popova meets the requirements of LDASRB, the Regulations for its implementation and the Regulations of MU-Varna for awarding the scientific and educational degree "Doctor". The topic of the dissertation has not been developed in Bulgaria, and some of the problems in it are still the subject of discussion internationally. The contributions of the dissertation have an original, confirmatory and applied character, which is a good basis for optimal risk stratification and forecasting the time to start treatment through available clinical-laboratory and molecular genetic factors of untreated patients with B-CLL. The dissertation of Dr. Vanya S. Popova shows developed qualities for analysis and synthesis of scientific information, ability to formulate conclusions and build scientific hypotheses. Extensive scientific knowledge and practical skills in the specialty "Hematology and blood transfusion" are presented. I give my positive assessment of the dissertation on "Clinical application of prognostic factors and their integration into a scale for risk and

time to treatment in untreated patients with B-chronic lymphocytic leukemia" and invite the esteemed scientific jury to vote "Yes" for awarding a scientific and educational degree "Doctor" to Dr. Vanya Slavcheva Popova

01.11.2020.

REVIEWER: .....
/ Assoc. Prof. Veselina Goranova-Marinova, MD /