МЕДИЦИНСКИ УНИВЕРСИТЕТ - ВАРНА
„Проф. д-р Параскев Стоянов"
Ул."Марин Дринов" 55, Варна 9002, България
Тел. : 052/ 65 00 57, Факс: 052/ 65 00 19
e-mail: uni@mu-varna.bg, www.mu-varna.bg

MEDICAL UNIVERSITY - VARNA
"Prof. Dr. Paraskev Stoyanov"
55, Marin Drinov Str., 9002 Varna, Bulgaria
Tel.: +359 52/ 65 00 57, Fax: + 359 52/ 65 00 19
e-mail: uni@mu-varna.bg, www.mu-varna.bg

R E V I E W
from
Prof. Dr. Lyudmila Boncheva Angelova, MD, PhD
Head of the "Medical Genetics" department

Specialty / scientific degree – Professor of Medical Genetics, Doctor of medicine
Institution – MU "Paraskev Stoyanov" Varna
Address and contact: "Hristo Smirnenski" Blvd. No. 1, UMBAL " St. Marina ",
E-mail address: liudmila.angelova@mu-varna.bg, phone 0898684701, ext. tel . 052/978225

REGARDING:

Dissertation on a topic *" Study of molecular genetic markers in patients with acute myelogenous leukemia"*

Author: Dr Dinnar Ali Yahya

Form of education **- full-** time, Department of " Medical Genetics " MU Varna, enrolled by doctoral student program **"Genetics"** for acquisition of **the ONS "Doctor"**, Code of Professional Field 4.3 Biological Sciences, Doctoral program: „Genetics."

Scientific supervisors: Prof. Dr. Ilina Dimitrova Micheva, MD, PhD
Assoc. Prof. Dr. Trifon Georgiev Chervenkov, MD, PhD

Procedure:

Pursuant to Order No. R- 109-82 / 21.03.2024 of the Rector of the MU - Varna, I have been appointed as a member of the Scientific Jury; based on Protocol No. 1 / 22.03.2024, from the first (on-line) meeting, I have been appointed as the chairman of the Scientific Committee Jury and Reviewer of Dr. Dinnar Dr. Dinnar Ali Yahya's Dissertation for Awarding the Educational and Scientific Degree "Doctor" in Science specialty "Genetics" .

Dr. Yahya is enrolled as a full-time doctoral student in " Genetics" with a study period of 3 years. (Order R-109-475 / 04.11.2020 of the Rector of MU-Varna). The stages of the doctoral studies for admission to the public defense have been met and the doctoral student has been dismissed on the proposal of a meeting of the Departmental Council of the Department of "MEDICAL GENETICS" according to Art. 61, para. 1 and para. 2 of the Regulations for the development of the academic staff at the MU - Varna after *internal defense.*

The dissertation work was prepared at the Department of Medical Genetics of the Varna Medical University "Prof. Dr. Paraskev Stoyanov" and Laboratory of Medical Genetics at UMBA L "St. Marina" EAD, Varna. According to the procedure, Dr. Yahya presented all the necessary documents materials following the requirements of PRAS of MU-Varna .

This review has been developed and presented in accordance with the requirements of the Law on the Development of the Academic Staff of the Republic of Bulgaria /ZRASRB/, the

Regulations for the Implementation of the ZRASRB and the Regulations on the Development of the Academic Staff at the Medical University /MU/ - Varna. I declare that I have no conflict of interest with the author of the dissertation.

Brief biographical data and professional qualifications

Dr. Dinnar Ali Yahya was born on 21.01.1994 in Isperih. She completed high education school "Alexander Pushkin" in the city of Varna in 2011, and university education in medicine - with honors at the Medical University "Prof. Dr. Paraskev Stoyanov" in Varna in 2017 (Diploma No. 00 5055 / 2017). In 2019, she started working as a full-time assistant in the Department of Medical Genetics of the Medical University of Varna and as a specializing medical doctor in the Medical Laboratory of the University hospital "St. Marina" Varna.

She acquires a specialty in Medical Genetics in 2023 (State Examination Commission in Sofia (Diploma No. 02 6565 / 2023), building laboratory and consulting competences for work in the hospital genetic service and teaching skills for students in medicine (Bulgarian and English language training), in pharmacy and "Medical Nurse" specialists.

The wide range of interests of Dr. Yahya is impressive, such as participation in 17 qualification *courses and specializations,* one of them under project No. BG05M2OP001-2.016-0025 "Creation of a multidisciplinary educational environment for the development of personnel with integral competencies in the field of biomedicine and health care", Constanta, Romania, 2023. In the same year, she is awarded as *a mentor for* a scientific exchange project of foreign students, (SCORE), organized by IFMSA (International Federation of Medical Students' associations).

Dr. Yahya is a co-author of a total of 6 **full-text** publications**,** 4 of which published in journals, indexed in Web of Science or Scopus **(***Romanian Journal of Internal Medicine, Cytology and Genetics, Pediatrics),* 1 in a journal with Impact Factor. Publications in **summary** - 22 issues, of which 11 in journals with IF *(Prenatal Diagnosis, European Journal of Human Genetics),* ECA newsletter, etc. She is the main contractor of 1 scientific project financed by the "Science" Fund of the University of Varna about the dissertation work.

She is a member of the Bulgarian Society of Human Genetics and Genomics, National Society of Hematology and Hemotransfusion, European Society of Human Genetics (ESHG), European Cytogeneticists Association (ECA), International Society for Prenatal Diagnosis (ISPD).

She speaks 4 languages, apart from maternal Bulgarian (fluent English, Turkish, Russian and basic Italian).

Structure of the dissertation

The dissertation is presented in 130 pages, contains 15 figures and 12 tables. It includes: contents (2 pp), abbreviations (3 pp), introduction (3 pp), literature review (43 pp), working hypothesis, aim and tasks (1 p), clinical contingent and methods (7 pp.), research results (14 pp.), discussion (22 pp.), summary and directions for future work (3 pp.), conclusions (1 pp.), contributions (1 pp.), bibliography (24 pp.) , Appendices 1,2 (5 pps), dissertation publications (1 page), acknowledgments The bibliography covers 201 literary sources, of which 5 are in Cyrillic and 196 are in Latin. The presented figures and photos follow the cited affiliation of the reference authors.

The extended summary,52 standard pages, is written in accordance with the dissertation.

Note: Scientific work is structured in the 8 standard sections, in an acceptable ratio with a dominant volume of the literature review.

Actuality of the subject of the dissertation

The dissertation submitted for review concerns *a current* topic related to the need for modern molecular-genetic diagnostic assessment in patients with health significant oncohematological disorder - Acute Myelogenous Leukemia (AML). Generally accepted international recommendations for the purpose of risk stratification include not only mandatory conventional chromosomal analysis (CCA), but also molecular genetic methods. The fluorescent in situ hybridization FISH method is increasingly being replaced by a molecular genetic method using a set of genes to even whole genome analysis. The genetic basis of the neoplastic process provides an affordable and reliable assessment and stratification by CCA at the cellular level and by a fast, easy to perform and applicable MLPA method (Multiplex Ligase-dependent Probe Amplification) to report molecular genetic markers specific to AML. In this way, a more complete and accurate idea of the underlying genetic mechanism is obtained, with a maximally personalized approach for a favorable outcome of the disease.

The literary review impresses with its volume; illustrated with 4 figures and 6 tables. The author thoroughly systematizes the scientific information on the topic in two devisions: 1) definition and molecular bases of AML; genetic characterization and classification of AML; risk stratification by ELN; and 2) Most commonly used molecular genetic markers and methods for their detection.

A rich scientific bibliography is presented, which shows the most up to date, (2023 incl.) knowledge of the use of the genetic characteristics of AML. Five papers of Bulgarian authors in the practical and applied field of the disease are included.

The *summary* systematizes the scientific data and logically leads to the construction of *a working hypothesis* with reasonable arguments for the choice of the investigation: MLPA is a suitable method for the study of molecular genetic markers and it can be recommended to be implemented in the routine assessment of the genetic basis in patients with newly diagnosed AML in parallel with cytogenetic analysis.

The **goal** of the study is clearly formulated - To evaluate the applicability of the MLPA (Multiplex Ligase-dependent Probe Amplification) method for reporting AML-specific molecular genetic markers in the routine clinical-diagnostic activity of evaluating patients with newly diagnosed AML. To achieve this goal, **5** *tasks have been formulated*, which are logically connected and in a synthesized form.

1. To introduce a molecular genetic method for the identification of significant molecular genetic markers associated with AML.
2. To select patients with newly diagnosed AML meeting the criteria for inclusion in the prospective study.
3. To conduct a molecular genetic analysis of DNA isolated from leukocytes from venous blood from patients with newly diagnosed AML before treatment and from a control group of healthy individuals.
4. To compare the data with those from a parallel KCA, and to summarize and analyze the results of the conducted molecular genetic research.
5. To summarize the role of the used molecular genetic method in the initial genetic screening and to derive guidelines for improving the genetic evaluation of the contingent of newly diagnosed patients with AML.

I give particular emphasis to the third task related to the possibility of introducing the use of molecular genetic methods in hematological diseases in our country and the fifth task summarizing the data to figure out guidelines for work in patients with AML.

Material and methods - the section is presented in sufficient volume. The study extends to a period of 16 months (February 2022 - May 2023) and is prospective in its nature. The patient contingent is registered at the University clinic of hematology of UMBAL "St. Marina" Varna, and the genetic studies are conducted at the Medical Genetics Laboratory of the same hospital.

The inclusion and exclusion criteria for the selection of patients and the reason for this are listed. The procedure for recruiting the group is clearly described - personal contact of the doctoral student for the purpose of obtaining Informed Consent from each patient (Appendix 1 and 2). The control group of participants is also described. Patients have beebn diagnosed according to generally accepted diagnostic and risk stratification criteria of the International Leukemia Working Group. The documentary method includes the use of information from the hospital's electronic database.

The laboratory-genetic methods (DNA isolation, MLPA - Multiplex Ligase-dependent Probe Amplification, and Conventional cytogenetic analysis) mastered and applied by the doctoral student are described in detail in laboratory protocols. A panel with a set of 4 groups of statistical methods with specific groups of questions and software products, personal work of the doctoral student, is used.

Note – the section has to give the numbers of examined patient goups for both methods and the principle of division by indications, beyond their characterization in the Results section.

The results are presented in response to the set tasks and are illustrated with 14 figures and 5 tables, the appendix of which does not duplicate the text. According to the type of questions asked for resolution and the studies conducted, the results are grouped into:

- Descriptive – epidemiological characteristics of the patients and volunteers included in the study (age, sex, indications)
- Presentation and analysis of the results of conducted genetic studies (MLPA Multiplex Ligase-dependent Probe Amplification and Conventional cytogenetic analysis KCA)

During the study period **61 patients and 21 controls** are examined.

MLPA testing is performed in all 61 individuals, and conventional cytogenetic analysis - in 53 patients, the last achieving a success rate in 38 (71.7%) **(**normal *methodical success rate* for extraction and analysis of bone marrow cultures in patients with AML*)*. I consider Table 12 to be very appropriate, reflecting the concordance between the findings of MLPA and KCA.

The comparison of the two methods in terms of their overall success rate and informativeness (graphically presented in Figure 11) highlights the comparability of the two genetic methods. Regarding *the success rate* of the application of both methods, it is in favor of MLPA over KCA, with great statistical significance ($p<0.00001$, Chi-square test). Regarding *the detection rate of pathology*, no statistically significant difference has been found - 55.7% for MLPA and 39.6% for KCA. ($p=0.08544$, Chi-square test). Very important, however, is the reported total detectability from the application of a combination of the two methods for 48 (78.7%) patients. Moreover, in 18% (n=11) of all examined, MLPA provides information on findings missed by KCA.

Attention is drawn to the assessment made by the doctoral student of the average survival rate of patients divided into risk groups according to ELN 2022 (Kaplan-Meyer test, GraphPad Prism), by gender and by age.

I consider the observation related to the stratification by subtype of AML according to WHO 2022 to be very important. In practice, it turns out that the group of *unclassified* patients is the most numerous - 37.7% (n=23) either due to the absence of a detected genetic change by both methods or due to the inability to detect the genetic change presence. Of *the classified* patients, the group of MDS-related changes is leading - 23% (n=14).

I accept the stated results and their statistical processing as adequate to the set goals and objectives.

The discussion is the main section for any dissertation, in which the doctoral student analyzes the results obtained in the context of his knowledge of the literature review and looks for a logical analysis in evaluation and comparability.

The results are dicussed in two subsections: descriptive-epidemiological characterization of the participants in the study, results of the genetic tests conducted through MLPA and KCA.

The results of the study indicate that the combination of KCA and MLPA significantly increases the amount of detected genetic markers.

Regarding the application of *MLPA* – the method allows the detection of monogenic and some chromosomal changes missed by CCA; monogenic markers (in the *NPM1, IDH2, DNMT3A genes)* new to practice in our country are detected. The limitations of the method related to the target success rate are also analyzed: a) target nature, determined by the type of included probes, b) different sensitivity regarding monogenic variants, deletions and duplications, respectively 5-10%, 20% and 40%, c) choice of studied biological material in myeloid neoplasias - bone marrow or peripheral blood, d) limited number of publications on methodologically similar studies.

Regarding the application of *KCA*, the study confirms that this method retains its leading role by now for the detection of numerical an gross structural chromosomal changes. Sensitivity to low-grade branches and balanced rearrangements further stabilizes its role in the initial evaluation of patients with AML. Also discussed are the disadvantages of KCA related to a) low resolution and unrecognized translocations, b) requirement for call cultivation and risk of lack of metaphase plates for analysis c) technical problems related to the source of biological material, (“dry” punction, bone marrow biopsy impossibility/refusal to be performed.

The author concludes that the near future belongs to rapid and wide-ranging genetic studies visualizing a large range of structural and numerical genetic changes. Such is a molecular-genetic *whole-genome* method, Optical Genome Mapping (OGM) (Bionano Genomics, USA), disadvantaged so far by the high price and interpretation of extracted large amounts of information requiring complex bioinformatics processing by specialized personnel.

In the discussion, the doctoral student evaluates the revealed genetic findings in relation to the clinical stage, applied to a relatively small group of patients; she goes beyond the genetic laboratory knowledge and seeks an interdisciplinary approach to hematologically affected patients, not used in mass clinical practice.

The rate of development and the possibility of reaching patients with AML for initial diagnostic evaluation and therapy are commented. These factors affecting the patient's disease

outcome address the need for public awareness for effective prevention and general improvement of the mass health culture.

Note -An extremely thorough, understandable, logiac and up-to-date main section of the dissertation.

Inferences of the scientific work are based on the received data and correspond to the set goals and objectives.

1. Among the selected patients with newly diagnosed AML, there was a clear preponderance (more than three quarters - 78.7%) of those without other previous hematological (including malignant) diseases.
2. MLPA allowed detection of monogenic and chromosomal alterations in more than half (55.7%) of patients, which contributed to their successful classification and stratification of most of them according to risk according to current international classifications.
3. Through the method used, new for us, monogenic markers new to our practice were diagnosed (in the NPM1, IDH2, DNMT3A genes), which were reported in one third (34.4%) of all examined patients.
4. The MLPA method allowed additional detection of chromosomal changes compared to CCA, with MLPA providing information on findings missed by CCA in one fifth (18%) of all examined.
5. In nearly one third (27.9%) of the patients, conventional cytogenetic analysis revealed a pathology without an analogous result from the molecular genetic method.
6. The combination of the two methods - cytogenetic and molecular genetic - provided information for the majority (78.7%) of our patients.

The contributions of the dissertation are correctly organized into 3 groups.

Original contributions

1. For the first time in our country, systematized information, and analysis of the results of the application of the MLPA method with an assessment of its contribution to the care of newly diagnosed AML patients over the age of 18 is performed and presented. The obtained results are a basis for comparative studies at the national and international level.
2. The present work is one of the few prospective studies in our country examining and comparing the diagnostic success rate of genetic laboratory methods - in this case, CCA and MLPA, in relation to this contingent of patients, drawing conclusions for routine clinical-diagnostic practice.

Confirmatory contributions

1. The lasting role of the cytogenetic method as a practical and tested one in specifying significant numerical and large structural chromosomal markers included in modern classifications and algorithms for therapeutic behavior has been confirmed.
2. The need to upgrade the application of conventional cytogenetics with modern high-resolution molecular genetic methods to prevent frequent problems with the latter and specify more detailed, including monogenic, somatic changes in these patients has been confirmed.
3. The leading role of the combination of cytogenetic and molecular genetic methods in laboratory diagnostics for revealing the genetic basis of the disease in patients with AML has been confirmed.

Practical contributions

1. A method for molecular genetic analysis - MLPA, was introduced for genetic screening of patients with newly diagnosed AML, in parallel with the routinely conducted cytogenetic analysis within the scope of the Laboratory of Medical Genetics, Varna.
2. Guidelines for the selection of genetic laboratory methods, biological material and genetic markers have been derived to improve the genetic evaluation of the contingent of newly diagnosed patients with AML.

Note - I accept the author's self-assessment and consider it is reasonable and possible to combine two points of a confirmatory (2 and 3) and an applied (1 and 2) contribution type.

Published Papers and Scientific summaries in connection with the dissertation.

Dr. Yahya has presented 3 full-text publications in scientific journals in *Romanian Journal of Internal Medicine* (2022, 2023), *Varnenski* journal *Medical Forum* (2022) (required for the defense of a project at NIMU Varna); and 4 publications of abstracts in *European*

Journal of Human Genetics (2022), 13th *European cytogenomics conference* ECA newsletter, XII National Congress of Hematology (2023) All of them are ***primarily*** her authorship and *up-to-date*.

Critical notes, commentary and recommendations to the dissertation:

My overall view of the scientific study is that it essentially presents acquired laboratory (personal skill and design) and analytical expertise for molecular genetic testing of newly diagnosed AML patients to aid clinical oncohaematological practice.

I find it particularly appropriate and valuable the inclusion of a section "Conclusion and prospects for future work", in which the doctoral student briefly reflects the essence of the research work and derives guidelines for action - against the background of limited funding *at present, the introduction of MLPA* as a screening method in newly diagnosed patients with AML, with expansion of the contingent (pediatric patients) and duration of follow-up, and using bone marrow as a DNA source. I heartily recommend her scientific and clinical development in the field of oncohematology.

On a personal level - I have known Dr. Yahya since she joined the Department of Medical Genetics in 2019. As an assistant and specialist, she has very good theoretical and practical knowledge in medical genetics, which she daily applied in her practice as a doctor and teacher. She is a collaborative colleague, quickly acquired skills to work with both the department and laboratory staff, uses four languages and has technical skills.

Conclusion:

Dr. Yahya's dissertation demonstrates in-depth literary knowledge on a current topic, very good possibilities for handling laboratory and statistical research methods, skills for written presentation and discussion of scientific data in the field of genetic sciences with clinical application. The doctoral student meets the academic criteria in accordance with the rules for academic development of MU-Varna for awarding the scientific and educational degree "Doctor". I strongly recommend to the scientific jury to award a scientific and educational degree "doctor" in a scientific specialty " Genetics " by **Dr. Dinnar Ali Yahya .**

28 .05. 2024 **Reviewer:**

PROF. DR LUDMILA ANGELOVA, MD, PhD