S T A N D P O I N T

related to the dissertation of **Dr. SVILENA ANGELOVA ATANASSOVA**, a full-time doctoral student in the Haematology and Blood Transfusion Doctoral Program for awarding the educational and scientific degree "**Doctor**" in the field of higher education: 7. Health and Sports, professional field: 7.1. Medicine

on the topic:

ROLE OF SELECTED MICRO RNAs IN PLASMA AS DIAGNOSTIC AND PROGNOSTIC MARKERS IN MYELODYSPLASTIC SYNDROME

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Myelodysplastic syndromes, often known as MDS, are a diverse collection of clonal disorders that affect the pluripotent stem cell and are characterised by impaired haematopoiesis, cytopenia, and a higher risk of progression to acute myeloid leukaemia. The clinical course of these conditions varies, with elderly individuals being the most frequently observed cases. Because it requires a complex combination of clinical, morphological, and genetic evidence, the diagnosis of myelodysplastic syndrome (MDS) continues to be a clinical challenge. There is a significant correlation between age and morbidity, with males being more affected by the condition than women. For decades, the disease has posed several challenges in terms of diagnosis, risk stratification, clinical course, and treatment. The pathogenic mechanisms of the disease have not been fully understood yet. Many patients with cytopenia have genetic abnormalities that resemble myelodysplastic syndromes. However, these variations fail to meet the established diagnostic criteria. The development of the disease is influenced by cytogenetic and epigenetic abnormalities, disturbances in DNA methylation, apoptosis, and, quite often, immune system dysfunction. This phenomenon is reflected in the dysregulation of stem cells and the emergence of a pathological clone characterised by dysplasia and compromised functionality. Cytogenetic aberrations occur in about 50% of newly diagnosed patients, whereas the frequency increases to 80% in secondary MDS patients. Genomic abnormalities may include areas that contain multiple tumour suppressor genes, whose expression is critical to understanding the biology of the disease and risk stratification. Along with established and studied cytogenetic and molecular genetic abnormalities, current research has become more focused on the epigenetic regulatory mechanisms and interactions that precipitate genomic instability in a significant number of patients with MDS. The heterogeneity of

the disease, the differences in survival rates, and the unique aspects of the clinical course highlight the importance of identifying dependable biological prognostic markers. Recently, a considerable number of scientific publications have drawn the attention of researchers to small regulatory molecules, including microRNAs, which play a role in the expression processes of tumour suppressor genes and oncogenes. MicroRNAs are small non-coding RNA molecules, generally ranging from 19 to 25 nucleotides in length. They play a crucial role in regulating gene expression by binding to the 3'-untranslated areas of target messenger RNAs (mRNAs), which can result in translational repression or the degradation of mRNA. MicroRNAs have become known as significant regulators in the development of MDS, affecting essential cellular processes including differentiation, proliferation, and apoptosis.

In these specific respects, the dissertation thesis authored by Dr. Svilena Atanassova holds significant relevance. This study carefully examines and gathers novel data regarding the expression levels of five chosen microRNAs: miR-22, miR-144, miR-16, miR-451a, and let-7a. Additionally, it assesses the prognostic significance of the previously mentioned RNAs by comparing high-risk patients with MDS and extrapolating the data to the risk stratification of the various subtypes of MDS.

Formally, the dissertation thesis consists a total of 176 pages and includes 19 figures and 29 tables. The bibliography comprises 290 titles in the Roman alphabet, with 18% of the bibliographic citations published after 2019.

A literature review

The currently available classifications of the disease are described in detail: the 2016 WHO classification, the FAB classification, and the 2020 revised WHO classification of myeloid and lymphoproliferative malignancies. The risk factors and scales related to MDS have been examined in detail, with an emphasis on molecular genetic abnormalities and their prognostic significance. An analysis of the disease pathogenesis has been carried out focussing on the significance of prevalent chromosomal abnormalities in MDS: deletion of the long arm of chromosome 5 (del 5q), aberrations in chromosome 7, trisomy 8, and chromosomal aberrations involving chromosome 3. The importance of certain somatic mutations affecting genes involved in cellular processes associated with the differentiation and proliferation of haematopoietic cells, signal transduction, and epigenetic control is described. Significant emphasis is placed on specific epigenetic pathways involved in the pathogenesis of myelodysplastic syndrome.

1. DNA methylation and the role of DNA methyltransferases (DNMTs).
2. Histones and histone modifications that alter the chromatin structure and, as consequently, the expression of genes.
3. MicroRNAs (miRNAs, miRs), non-coding endogenous RNAs of 19–25 nucleotides in length, are complementary to the 3' untranslated region of target genes. The potential

of microRNAs as diagnostic and prognostic biomarkers, as well as treatment-response indicators in myelodysplastic syndromes (MDS), has been analysed.

The literature review is competently composed, showcasing the doctoral student's comprehensive understanding of literature sources and her strong analytical skills regarding the data presented by other authors. An analysis of the cited literature reveals that around 20% of the sources were published in the last five years, with a total of 290 cited sources.

The objective of the dissertation is articulated with precision. A total of nine clearly defined tasks have been established to accomplish the objective.

The "Materials and Methods" section is elaborated with considerable detail. The facilities of the University Hospital “St Marina” in Varna were used, including the Clinic of Clinical Haematology, Central Clinical Laboratory, Immunology Laboratory, and Genetic Laboratory. A total of 50 participants were prospectively analysed, comprising 40 patients diagnosed with myelodysplastic syndrome and 10 healthy controls. The research project took place at the Clinical Haematology Clinic within the University Hospital “St. Marina” Varna, following the approval from the Research Ethics Committee (REC) at the Medical University “Prof. Dr. Paraskev Stoyanov” – Varna. Participants were enrolled after completing informed consent procedures. Criteria for the inclusion of patients with MDS, which were widely available and had been previously specified, were utilised. Routine laboratory tests alongside highly specific molecular genetic tests were conducted. The data from the study was processed using specialised statistical software (IBM SPSS Statistics v.24, GraphPad Prism, version 10.4.1, Python v.3.8, and Google Sheets). The main characteristics of the data were summarised by calculating the means, standard deviations, medians, and percentage distributions. Methods were used to check the normality of the data distribution in a given group, the non-parametric Mann-Whitney test for comparing medians between two independent groups. Spearman's correlation coefficient is used to evaluate the relationship between two quantitative variables, regardless of the data's distribution. Regression analysis was employed to evaluate the linear relationship between two variables. Survival was assessed using Kaplan-Meier curves. Data with a significance threshold of $\alpha=0.05$ were deemed statistically reliable. The methods are thoroughly detailed, ensuring complete reproducibility and demonstrating the dissertation candidate's active involvement in their execution.

The personal research has been conducted appropriately**. The "Results" section** covers the complete course of scientific advancement. The text is well-illustrated with 19 figures, providing a clear visual representation**.**

. It is systematised into ten subsections, which sequentially present results from the relevant research and thoroughly analysed data. In conclusion, the results are analysed at each stage, and a logical transition is made to the next stage. The conducted studies outline several areas of interest:

I. An evaluation has been done on the diagnostic potential of specific plasma microRNAs in patients with MDS compared to healthy controls. Notable distinctions have been found regarding microRNA 144, microRNA 16, Let 7a, and microRNA 451a.

II. A comparative analysis has been performed on the levels of microRNAs across various subtypes of MDS. The potential role of Let 7a as a biomarker has been established.

III. A correlation analysis has been conducted to examine the relationships among the microRNAs, revealing a statistically significant positive correlation among all analysed RNAs.

IV. A statistically significant correlation exists between microRNA levels, LDH values, ferritin levels, and the percentage of blasts in the bone marrow; however, no significant predictive relationships have been identified.

V. The role of **miR-22** and **miR-451a** as predictors for myelodysplastic syndrome patients is emphasised, with these two microRNAs identified as the most significant. Subsequent simple and multivariate analyses confirm their strong predictive value. **miR-22** demonstrates a positive correlation with disease probability, whereas **miR-451a** exhibits an inverse correlation, indicating a potential protective effect. The models exhibit high accuracy and stability, thereby reinforcing the diagnostic relevance of these two microRNAs.

VI. The prognostic value of **let-7a** has been determined, establishing it as the most robust predictor among the examined microRNAs. Elevated let-7a levels correlated with a higher likelihood of classification within the high blast MDS group.

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The dissertation concludes with an in-depth discussion of the results obtained. It demonstrates Dr. Svilena Atanassova's excellent literary awareness, as well as the correct and competent interpretation of the results obtained. It also provides guidelines for future research.

The six conclusions presented encapsulate the key findings of the dissertation. They consistently adhere to the established objectives.

he dissertation work of Dr. Svilena Atanassova has significantly advanced our understanding of certain epigenetic interactions that play a crucial role in the development of myelodysplastic syndrome.

1. A study examining plasma microRNAs in patients with myelodysplastic syndrome has been conducted in Bulgaria for the first time.

2. The relationship between the levels of specific microRNAs and some biochemical indicators reflecting erythropoiesis and iron metabolism in MDS has been analysed. The potential of microRNAs for additional information on the pathological processes occurring in the bone marrow has been proven.

3. The correlation between let-7a and the percentage of bone marrow blasts and R-IPSS risk has been confirmed. The potential function of risk stratification in myelodysplastic syndrome has been revealed.

4. The non-invasiveness of plasma microRNAs underscores their potential as a complement to conventional diagnostic methods, especially in cases where histopathological examination poses difficulties.

In addition to being up-to-date and dissertable, the dissertation has been developed with great precision. Results and conclusions have been reached based on the original findings.

The dissertation reflects the individual effort of the doctoral candidate. A list of scientific publications, comprising four titles related to the dissertation, indicates that the research has been thoroughly publicised.

The abstract corresponds to the content of the dissertation.

In conclusion, the dissertation presented to me for peer review is meticulously planned and executed. Contributions have been achieved in both scientific and practical fields. I strongly recommend that the members of the Esteemed Scientific Jury vote in favour of awarding **Dr. SVILENA ANGELOVA ATANASSOVA** the scientific degree "Doctor" in the field of higher education 7. Health and Sports, professional field: 7.1. Medicine, as part of the Haematology and Blood Transfusion doctoral program

PEER REVIEWEI
Col. Assoc. Prof. I

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