MEDICAL UNIVERSITY "PROF. DR. PARASKEV STOYANOV" – VARNA

FACULTY OF MEDICINE

DEPARTMENT OF INTERNAL DISEASES, SECOND PART

ACADEMIC SECTOR OF HEMATOLOGY

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BIOMARKERS FOR PERSONALIZED TREATMENT APPROACH IN PATIENTS WITH MULTIPLE MYELOMA

THESIS SUMMARY

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The final meeting of the scientific jury for the defense of the dissertation work of Radi Evgeniev Lukanov MD will be held on 04.06.2025.

Defense materials are available at the Scientific Department of MU Varna and published on the website of MU "Prof. Dr. Paraskev Stoyanov" – Varna.

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1. Abbreviations

AKT – Ak strain transforming

ALCL – anaplastic large cell lymphoma

ALK – anaplastic lymphoma kinase

APP – amyloid beta precursor protein

ARID3B – AT-rich interactive domain 3B

ASF1B – anti-silencing function 1B histone chaperone

ASR – age-standardized rate

ATG12 – autophagy-related gene 12

B2M (β2- microglobulin) – β2- микроглобулин

BAFF – B-cell activating factor

BAK1 – Bcl2 antagonist/killer 1

BBC3 – Bcl2 binding component 3

Bcl-2 – B-cell lymphoma 2

BCL2L2 – Bcl-2-like protein 2

Bcl-xL – B-cell lymphoma-extra large

BCMA – B-cell maturation antigen

BiTE – bispecific T-cell engager

BMF – Bcl2 modifying factor

BTRC – beta-transducin repeat containing E3

CAR-T – chimeric antigen receptor T-cel

CBFB – core-binding factor subunit beta

CCND3 – cyclin D3

CCNE1 – cyclin E1

ccRCC – clear-cell renal cell carcinoma

CDK6 – cyclin dependent kinase 6

CdkI – cycline dependent kinase

CDKN1C – cyclin dependent kinase inhibitor 1C

CR – complete response

CRC – colorectal cancer

CT – Computed tomography

CXCR4 – chemokine receptor type 4

DAP – death-associated protein

DGCR8 – diGeorge syndrome critical region 8

DICER – Rna endonuclease 3

DKK1 – Dickkopf-1

DNM3os – dynamin 3 opposite strand

DNMT1 – DNA methyltransferase 1

DROSHA – RNAase 3

DSS – Durie-Salmon система

E2F3 – early region 2 binding factor 3

EGFL7 – epidermal growth factor-like domain-containing gene 7

EGFR – epidermal growth factor receptor

eGFR – estimated glomerular filtration rate

EMD – extramedullary disease

ERBB2/3 – erythroblastic oncogene B

EVL – enah/VASP-like

EZH2 – enhancer of zeste homolog 2

FAP – fibroblast activation protein-alpha

FAS – FS-7-associated surface antigen

FasL – Fas ligands

FBXW7 – F-box and WD repeat domain containing 7

FDG – 18F fluoro-deoxyglucose

FISH – Fluorescence in-situ hybridization

FLC – free light chains

FRZB – frizzled related protein

GEM-PANC-1 – gemcitabine resistance pancreatic cancer

HCC – hepatocellular carcinoma

Hepatocyte growth factor

HHV-8 – Human herpesvirus 8

HIF-1a/VEGF – hypoxia-inducible factor 1α/ vascular endothelial growth factor

IGF1R – insulin like growth factor 1 receptor

IgH – immunoglobulin heavy chains

IKKβ – inhibitory kappa B kinase beta

IL10RA – interleukin 10 receptor subunit alpha

IL2RB – interleukin 2 receptor subunit beta

IL-3 – Interleukin-3

IL-6 – Interleukin-6

IL6R – interleukin 6 receptor

IRF4 – interferon regulatory factor 4

ISS – International staging system

JAK/STAT – Janus kinase/signal transducers and activators of transcription

JNK/SAPK – stress-activated protein kinase/c-Jun NH(2)-terminal kinase

LANA-1 – latency-associated nuclear antigen

LDH – lactate dehydrogenase

LIN28B – cell lineage abnormal 28 B

MAF – musculoaponeurotic fibrosarcoma oncogene homolog

MAF-B – musculoaponeurotic fibrosarcoma oncology family, protein B

MAP kinase – mitogen activated protein kinase

MAP3K11 – mitogen-activated protein kinase kinase kinase 11

Mcl-1 – myeloid cell leukemia-1

MGUS – monoclonal gammopathy of undetermined significance

MIP-1 alpha – macrophage inflammatory protein-1 alpha

MiR – micro ribonucleic acid

miRISC – miR-induced silencing complex

MLK3 – mixed lineage kinase 3

MMP13 – matrix metalloproteinase-13

MMSET – multiple myeloma set domain

MMSET/FGFR3 – multiple myeloma SET domain/ fibroblast growth factor receptor 3

MRI – magnetic resonance imaging

mTOR – mammalian target of rapamycin

MYC – myelocytoma

MYCBP – c-Myc-binding protein

NF kappa B – nuclear factor-kappa B

NK – natural killer

OPG –osteoprotegerin

OS – overall survival

OSCC – oral squamous cell carcinoma

p27kip – cyclin dependent kinase inhibitor 1B

PAMPs – pathogen-associated molecular patterns

PDRG1 – p53 and DNA damage regulated 1

PET/CT – positron emission tomography omputed tomography

PFS – progression-free survival

PI3K – phosphatidylinositol 3-kinase

PIAS3 – protein inhibitor of activated STAT3

PR partial response – partial response

PRC2 – polycomb repressive complex 2

PRDM1 –PR domain zinc finger protein 1

primary miRNA, pri-miRNA

PSMD10 – proteasome 26S Subunit, Non-ATPase 10

PTEN – phosphatase and TENsin homolog

PTEN/AKT/GSK – PTEN/Akt/glycogen synthase kinase-3

RANKL – receptor activator of nuclear factor kappa-B ligand

R-ISS – revised international staging system

SD – stable disease

SDF-1 alpha – stromal derived factor 1α

SFRP2 – secreted frizzled related protein 2

Smad9 – mothers against decapentaplegic homolog 9

SP1 – specificity protein 1

STARD13 – star-related lipid transfer domain protein 13

SMM – smoldering multiple myeloma

TGF-β – transforming growth factor-beta

TKI – tyrosine kinase inhibitors

TLRs – toll-like receptors

TNFR – tumor necrosis factor receptor

TNFSF11 – tumor necrosis factor superfamily member 11

Tp – tumor protein p53

TP53INP1 – tumour protein p53-induced nuclear protein 1

USP27X/Bim – ubiquitin specific peptidase 27 X-linked/ Bcl-2 interacting mediator of cell death

VEGF – vascular endothelial growth factor

VGPR – very good partial response

Wnt – wingless-type MMTV integration site family

WNT3A – wingless-type MMTV integration site family, member 3A

αSMA – alpha smooth muscle actin

2. Introduction

Multiple myeloma (MM) is the second most common malignant hematological disease, characterized by clonal proliferation of plasma cells in the bone marrow and various systemic manifestations. Despite significant advances in diagnostic methods and the development of more effective therapeutic regimens over the past decade, MM remains a disease with high morbidity and mortality, especially in age groups over 65 years. The significant heterogeneity of the disease results in diverse cytogenetic and molecular profiles, clinical presentations, and inconsistent therapeutic responses.

The traditional diagnostic approach in MM is based on a combination of morphological, cytogenetic, immunological, and imaging methods. However, numerous challenges remain, from the lack of sufficiently sensitive markers for early detection and risk stratification to monitoring treatment response and timely detection of relapse. Recently, the scientific community's attention has shifted toward micro ribonucleic acids (miR)—short non-coding

RNA molecules that critically regulate gene expression, cell proliferation, apoptosis, and interaction with the tumor microenvironment. Various studies have demonstrated that certain miRs are overexpressed or suppressed in different malignancies, including MM, making them promising diagnostic and prognostic biomarkers as well as potential therapeutic targets.

In this context, new approaches for a more precise understanding of biological processes in multiple myeloma are becoming increasingly important. Key research directions include studies on the role of miRs as critical regulators of gene expression and various epigenetic factors influencing malignant cell growth. Also of significant importance is the interaction between myeloma cells and the bone marrow tumor microenvironment, including immunomodulation and angiogenesis processes. These directions may aid in identifying new biomarkers for early diagnosis, more accurate prognosis, and optimal monitoring of therapeutic responses, potentially leading to more precise risk stratification and improved clinical outcomes.

Future research in multiple myeloma is likely to focus on deeper molecular profiling and extensive application of advanced methods for analyzing circulating biomarkers, including various miR profiles. Advanced technologies and artificial intelligence in processing large databases open new opportunities for precise disease evaluation and developing personalized therapeutic strategies. Such advancements could enhance the effectiveness of existing therapeutic regimens or lead to the creation of new, more selective drugs, reducing the frequency of relapses and sustainably improving patient survival and quality of life.

3. Research groups and methods

3.1. Aim of the research

The current study investigates the expression levels of selected circulating miRs in patients newly diagnosed with MM and healthy controls. The patient group will be reassessed after 6 months. The main objective is to demonstrate the specific expression of pre-selected miRs at diagnosis and after 6 months of treatment. The results will be correlated with treatment response, changes in clinical status, and laboratory parameters.

3.2. Tasks:

1. Select newly diagnosed MM patients, stratify them according to demographic and clinicopathological characteristics, monitor disease progression, and evaluate treatment effectiveness over six months.

2. Determine serum levels of miR-214-3p, miR-199a-5p, miR-497-5p, miR-373-3p, and miR-126-5p in patients consenting to participate in the biomarker study.
3. Reassess serum levels of miR-214-3p, miR-199a-5p, miR-497-5p, miR-373-3p, and miR-126-5p in patients who remain in the study after the 6-month follow-up period.
4. Analyze expression levels of miR-214-3p, miR-199a-5p, miR-497-5p, miR-373-3p, and miR-126-5p in newly diagnosed patients compared to healthy controls.
5. Analyze and compare expression levels of miR-214-3p, miR-199a-5p, miR-497-5p, miR-373-3p, and miR-126-5p in newly diagnosed patients, patients after 6 months of treatment, and healthy controls.
6. Assess serum levels of miR-214-3p, miR-199a-5p, miR-497-5p, miR-373-3p, and miR-126-5p in healthy volunteers with similar demographic characteristics.
7. Analyze serum levels of miR-214-3p, miR-199a-5p, miR-497-5p, miR-373-3p, and miR-126-5p in newly diagnosed MM patients according to ISS stage.
8. Analyze serum miR levels based on the severity of bone disease in newly diagnosed MM patients.
9. Analyze serum miR levels based on the cytogenetic profile in newly diagnosed MM patients.
10. Analyze serum miR levels according to the presence of hypercalcemia in newly diagnosed MM patients.
11. Compare levels of miR-214-3p, miR-199a-5p, miR-497-5p, miR-373-3p, and miR-126-5p in untreated patients during treatment according to therapy response, compared to healthy controls.
12. Establish correlations between miR expression levels and various clinicopathological parameters in treatment-naive patients and post-treatment.
13. Determine the specificity and sensitivity of miRs as potential diagnostic biomarkers.
14. Analyze the predictive role of miR-199a-5p, miR-126-5p, miR-497-5p, miR-214-3p, and miR-373-3p as biomarkers in relation to overall survival (OS) and progression-free survival (PFS).

4. Materials and Methods

4.1. Materials

4.1.1. Facilities for dissertation implementation

- Clinical Hematology Clinic, UMHAT “St. Marina” EAD – Varna
- Clinical Immunology Laboratory at the Department of Medical Genetics, Medical University and University Hospital "St. Marina" – Varna

4.1.2. Patient population

A prospective, non-interventional, single-center clinical trial was conducted between 2022 and 2024, involving 56 patients who met inclusion criteria without exclusion criteria, and 12 healthy volunteers.

A total of 100 samples were collected:

- 56 primary samples (newly diagnosed, treatment-naive MM patients)
- 32 secondary samples (newly diagnosed MM patients after 6 months of treatment according to NCCN and ESMO international guidelines, from whom primary samples were initially collected)
- 12 samples from healthy volunteers matched to the patient population by demographic characteristics.

The clinical trial was conducted following ethical approval by the Research Ethics Committee of Medical University "Prof. Dr. Paraskev Stoyanov" – Varna (decision №129 dated 06.04.2023), in compliance with the Declaration of Helsinki.

Inclusion criteria:

- Age above 18 years
- Patients diagnosed with multiple myeloma according to IMWG 2014 criteria
- Expressed willingness to participate by signing an informed consent form

Exclusion criteria:

- Patients under 18 years of age
- Patients not meeting inclusion criteria
- Expressed unwillingness to participate in the study
- Disease progression after 6 months of treatment

All 56 patients included in the study were diagnosed with multiple myeloma according to the IMWG criteria from 2014.

Diagnosis and treatment were conducted at the Clinical Hematology Clinic of UMHAT "St. Marina" – Varna, and patients were recruited from May 2023 to April 2024. Before enrollment in the clinical trial, all patients signed informed consent for participation and separate informed consent for the collection of biological material (plasma/serum). Patient recruitment was carried out exclusively at the Clinical Hematology Clinic of UMHAT "St. Marina" – Varna. All patients who agreed and signed informed consent for follow-up (a total of 56) and for the collection of biological material (a total of 56) participated in the study.

The following assessments were performed on all patients:

- Clinical examination, including medical history and examination
- Routine laboratory tests – peripheral blood count parameters, standard biochemical panel (urea, creatinine, lactate dehydrogenase (LDH), total protein levels, liver enzymes, uric acid, beta-2 microglobulin, electrolytes, serum calcium levels, peripheral blood smear)
- Creatinine clearance
- Quantitative and qualitative assessment of immunoglobulins, serum protein electrophoresis, protein immunofixation, serum and 24-hour urine free light chain analysis
- Bone marrow aspiration, morphological assessment, flow cytometry of bone marrow aspirate, conventional metaphase cytogenetics, and fluorescence in situ hybridization (FISH)
- Whole-body low-dose computed tomography (WBLDCT) or positron emission tomography (PET-CT)

The study also included 12 healthy volunteers serving as controls for analyzing biological markers – miR-214-3p, miR-199a-5p, miR-497-5p, miR-373-3p, and miR-126-5p.

Patient staging was performed according to the International Staging System (ISS).

Patient Medical History

For each patient in the study, a medical record was created documenting the following information:

Demographic data:

- Names (initials)
- ID number (trial enrollment number)
- Age
- Gender: male/female

Medical history:

- Performance status/ECOG: Each patient's general condition was initially assessed according to ECOG as 0, 1, or 2.
- Comorbidities

Multiple myeloma information:

- Type of multiple myeloma
- ISS staging
- Cytogenetic profile of the disease by metaphase cytogenetics
- Presence of del 17p determined by FISH
- Presence of extramedullary formations determined by PET-CT or WBLDCT
- Date of initiation of first-line treatment
- Survival at the 6th month of treatment
- Response at the 6th month of treatment

4.1.3. Biological material:

All patients included in the study, who did not drop out, had samples collected twice – at diagnosis and after 6 months:

• 2 x EDTA blood tubes (3 ml each) for molecular analysis at screening.

• 1 x serum vacutainer (8 ml)

4.2. Methods

4.2.1. Laboratory methods

The laboratory method used in this study is RNA isolation and Real-time PCR performed using the miRNeasy Serum/Plasma Kit (Qiagen).

The analysis was conducted according to the manufacturer's protocol, involving two

stages – RNA isolation and polymerase chain reaction.

Stage 1 – Isolation of small RNA molecules

Protocol for isolation of small RNA molecules: The material used for miR testing is blood serum. Blood serum was obtained by venipuncture using a closed BD Vacutainer™ SST™ II Advance 5 ml system, catalog number 367955 (Becton Dickinson, USA). Following sampling, blood was allowed to clot at room temperature for 30 minutes, then centrifuged at 1500 × g for 15 minutes at room temperature. Serum was then separated, aliquoted into 500 µl portions, and stored at −80 °C until analysis.

MiR isolation was performed from 200 µl of serum using the commercial miRNeasy Serum/Plasma Kit (50), catalog number 217184 (QIAGEN, Germany) according to the manufacturer's protocol. For normalization control, 3.5 µl (1.6×108 copies per µl) control miR C. elegans miR-39: miRNeasy Serum/Plasma Spike-In Control, catalog number 219610 (QIAGEN, Germany), was added to each serum sample. Samples were eluted in 25 µl RNase-free water. The isolation procedure included the following steps:

1. Samples were thawed at room temperature (15-25°C).

2. 1000 µl (5 volumes) of QIAzol Lysis Reagent was added to 200 µl of sample. Samples were mixed by vortexing.

3. The tube containing the lysate was incubated at room temperature (15-25°C) for 5 min. To each sample, 3.5 µl (1.6×108 copies per µl) control miR C. elegans miR-39 was added.

4. 200 µl chloroform (equal to the initial sample volume) was added to the lysate. Vortex vigorously for 15 s until fully mixed.

5. The tube containing the lysate was incubated at room temperature for 2–3 minutes.

6. Samples were centrifuged for 15 minutes at 12,000 × g at 4°C. After centrifugation, the sample was separated into 3 phases: a clear upper aqueous phase containing RNA, a white interphase, and a red organic lower phase.7. 600 µl of the upper aqueous phase was transferred to a new tube, avoiding the interphase. 900 µl (1.5 volumes) of 100% ethanol was added and mixed well by pipetting.

8. Up to 700 µl of the sample, including any precipitate formed, was transferred to an RNeasy MinElute spin column in a 2 ml collection tube. Centrifuged at 8000 × g for 15 s at room temperature, discarding the flow-through.

9. Step 8 was repeated until the entire sample had passed through, discarding flow-through.

10. 700 µl Buffer RWT was added to the RNeasy MinElute spin column. Centrifuged

for 15 s at 8000 × g to wash the column, discarding the flow-through.

11. 500 µl Buffer RPE was added to the RNeasy MinElute column. Centrifuged for 15 s at 8000 × g to wash the column, discarding the flow-through.

12. 500 µl of 80% ethanol was added to the RNeasy MinElute column. Centrifuged for 2 minutes at 8000 × g to wash the spin column membrane. The collection tube was discarded.

13. The RNeasy MinElute column was placed into a new 2 ml collection tube. The spin column lid was opened and centrifuged at maximum speed (25,000 × g) for 5 minutes to dry the membrane. The collection tube was discarded.

14. The RNeasy MinElute column was placed into a new 1.5 ml tube. 25 µl RNase-free water was added directly to the spin column membrane center. Centrifuged for 1 minute at full speed (25,000 × g) to elute RNA.Протокол за PCR

Stage 2 – Polymerase chain reaction.

PCR Protocol

Each sample was subsequently subjected to reverse transcription using the miRCURY LNA RT Kit (catalog No. 339340, QIAGEN, Germany) following the manufacturer's protocol: 1.0 µl eluted miR was mixed to a final volume of 10 µl by adding 9.0 µl mastermix, composed of 2.0 µl 5× reaction buffer, 6.0 µl RNase-free water, and 1.0 µl 10× enzyme mix. Samples were incubated at 42°C for 60 minutes, followed by enzyme inactivation at 95°C for 5 minutes.

Quantitative Real-Time PCR was performed on each sample using the miRCURY LNA SYBR Green PCR Kit (200) and (600), catalog numbers 339345 (200) and 339346 (600) (QIAGEN, Germany), and pre-designed primers miRCURY LNA miRNA PCR Assay, catalog number 339306 (QIAGEN, Germany). According to the manufacturer's protocol, 3.0 µl complementary DNA (cDNA), diluted 30-fold, was mixed with 7.0 µl mastermix (5.0 µl 2× miRCURY SYBR® Green Master Mix, 0.05 µl ROX dye, 1.0 µl primer mix, and 0.95 µl RNase-free water) in 10 µl reactions performed in triplicates for 6 target miRs in 384-well plates. The used miRCURY LNA miRNA PCR Assay primers (QIAGEN, Germany, GeneGlobe reference numbers in brackets) were as follows: cel-miR-39-3p (YP00203952), hsa-miR-214-3p (YP00204510), hsa-miR-199a-5p (YP00204494), hsa-miR-497-5p (YP00204354), hsa-miR-126-5p (YP00206010), hsa-miR-373-3p (YP00204604).The thermal cycling conditions were as follows: Enzyme activation at 95°C for 2 minutes; 40 cycles of 95°C for 10 seconds; 56°C for 60 seconds with fluorescence detection; Melting curve analysis for amplification specificity: initial denaturation at 95°C for 15 seconds, cooling to 60°C for 60 seconds, and heating to 95°C at a rate of +0.05°C per second with fluorescence detection. Analysis was

performed using a QuantStudio Dx instrument by Applied Biosystems (USA), and the threshold cycle (Ct) was recorded for each sample.

Relative concentrations of target miRs were calculated using the ΔΔCt method relative to reference miR—normalization control C. elegans miR-39 and relative to a reference sample (the arithmetic mean Ct value of all individuals). Calculations were done using Microsoft Office Excel 2016, and results were presented as ratios to the reference sample.

4.2.2. Statistical methods

For statistical processing, specialized software for statistical analysis was used (IBM SPSS Statistics, GraphPad Prism for Windows).

The following methods were applied:

• Descriptive analysis to present the frequency distribution of qualitative variables, including absolute values (n) and relative frequencies (%).

• Shapiro–Wilk's test to evaluate the normality of distribution for continuous variables. Results are normally distributed when there is no statistical significance according to the Shapiro–Wilk's test ($p>0.05$) and skewness values fall within the permissible range (-1/+1). Kurtosis is considered not to influence the results of statistical tests, thus there is no specific rule for its interpretation.

• Variation analysis of quantitative variables to assess central tendency characteristics and dispersion of results. Data reflecting normally distributed continuous variables are presented as mean ± standard deviation (SD). Continuous variables with non-Gaussian distribution are presented using the positional measure median and interquartile range (IQR), extending between the 25th and 75th percentiles.

• Comparative analysis using Student-Fisher's t-test to compare two independent groups with normally distributed results, and the non-parametric Mann–Whitney and Kruskal–Wallis tests for comparison of two or more independent groups, respectively, with non-Gaussian distribution results.

• Correlation analysis to identify relationships between two quantitative traits—Pearson correlation analysis for normally distributed results and Spearman rank correlation analysis for non-Gaussian distribution results. The correlation strength was evaluated based on the correlation coefficient using a 7-level scale:

$0 < R \leq 0.1$ – very weak to negligible correlation

$0.1 < R \leq 0.3$ – weak correlation

$0.3 < R \leq 0.5$ – moderate correlation

$0.5 < R \leq 0.7$ – moderately strong correlation

$0.7 < R \leq 0.9$ – strong correlation

$0.9 < R < 1$ – very strong correlation

$R = 1$ – perfect correlation

• Linear regression analysis to determine the degree of dependence and to predict values of the dependent variable.

• Receiver-Operating Characteristic (ROC) analysis to evaluate the diagnostic reliability of studied indicators and determine their threshold (cut-off) values.

• Kaplan–Meier curves were used to assess time-to-event data, such as survival or progression.

• Graphical analysis for visual representation of obtained results.

For all statistical analyses, the critical level of significance was set at $\alpha=0.05$.

The null hypothesis was rejected, and statistical significance was established at $p<0.05$.

5. Results

5.1. Patient characteristics

A total of 56 patients and 12 healthy controls participated in the study, with a slight predominance of females: 33 women (58.9%) compared to 23 men (41.1%) (Fig. 1). The male-to-female ratio is 1:1.43, which is identical to that of the control group.

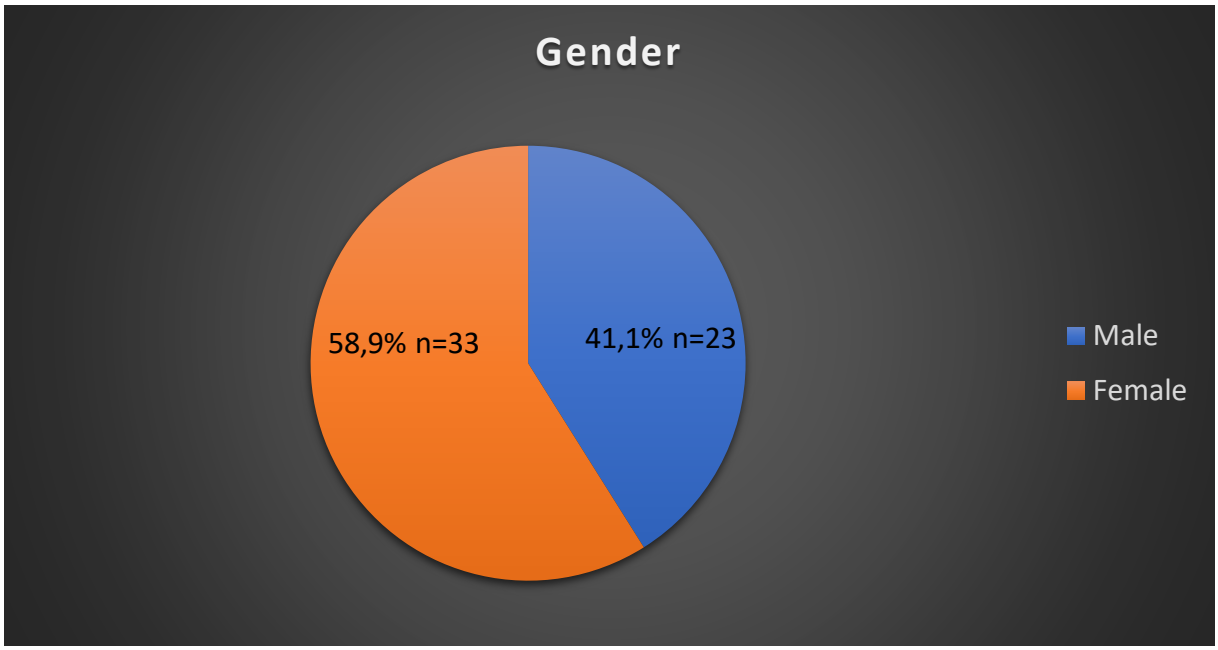

Figure 1: Gender distribution

The average age of the patients included in the study is 66 years (range 36–90), with 51.8% (n=29) being over 65 years of age (Fig. 1). The average age of the control group was matched to that of the patient population.

The age distribution is presented in Figure 2. The youngest patient is a 36-year-old woman, and the oldest is a 90-year-old woman. The highest incidence occurs in the 65–69 age group (25.45%), and this holds true for both sexes. The mean age of the study group at the time of diagnosis is 66 years.

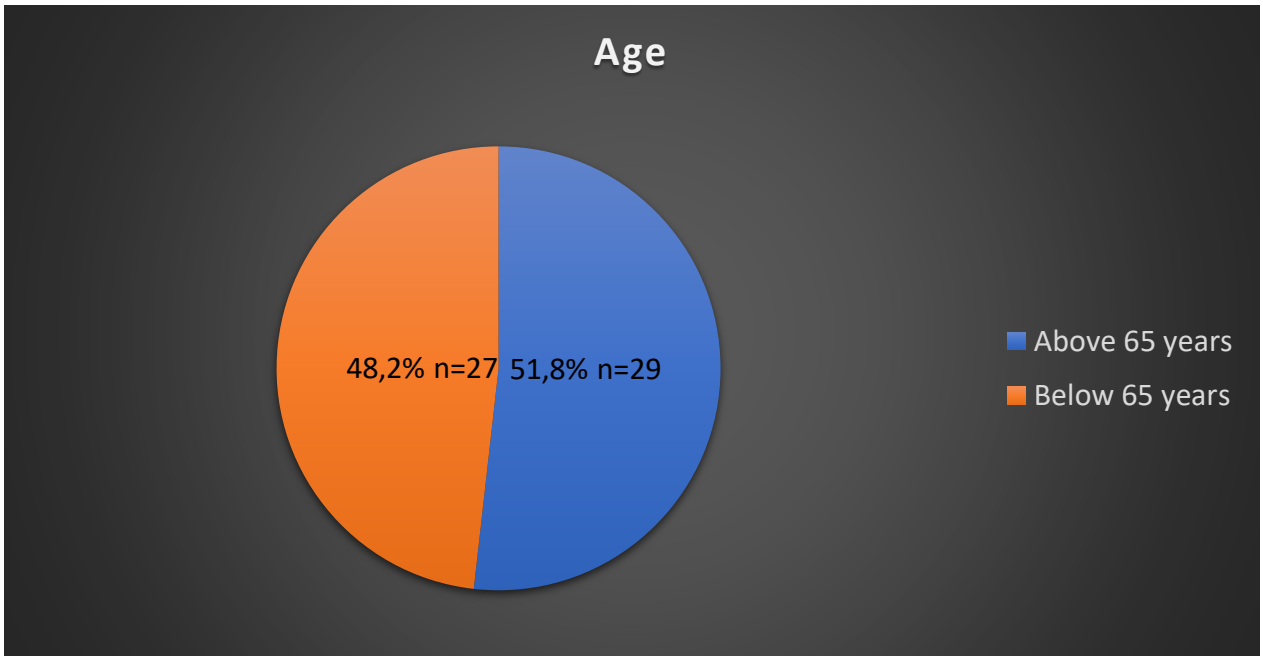

Figure 2: Age distribution

The distribution of the immunological myeloma type (Fig. 3) is as follows: 33.9% (n=19) are IgG kappa, 25% (n=14) are IgG lambda, 10.7% (n=6) are IgA kappa, 5.3% (n=3) are IgA lambda, 10.7% (n=6) are light chain kappa, 7.1% (n=4) are light chain lambda, 3.5% (n=2) have non-secretory multiple myeloma, and 3.5% (n=2) have primary plasma cell leukemia.

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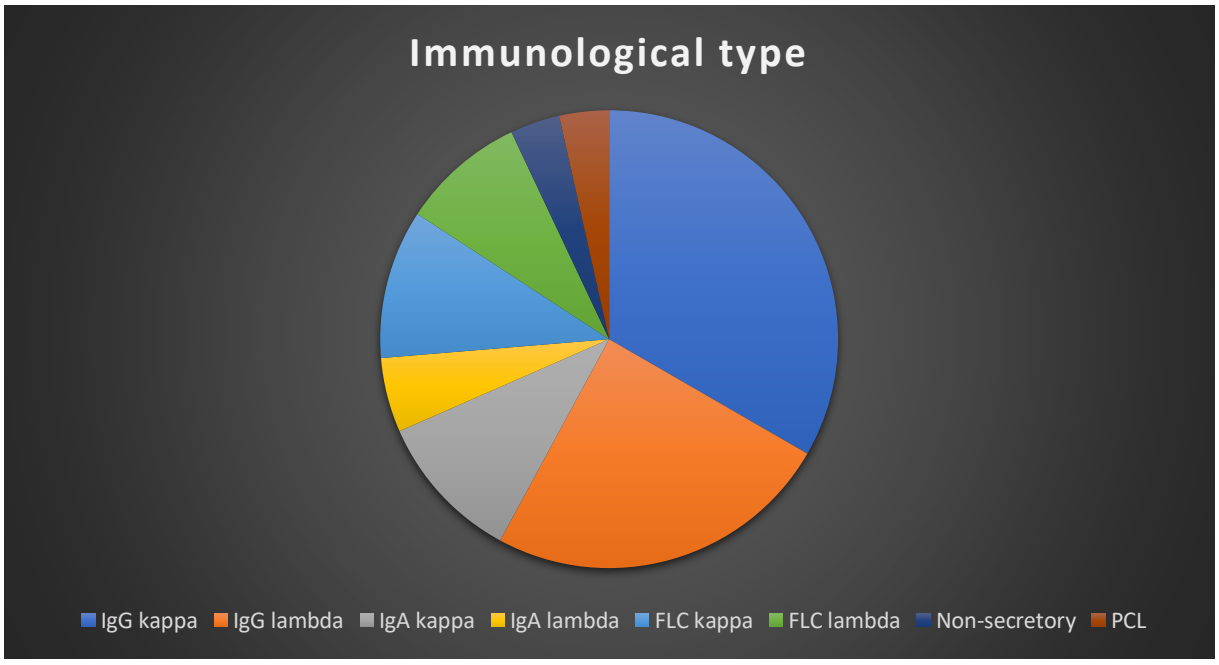


Figure 3: Immunological type distribution

The patients were staged according to the International Staging System (ISS) of the International Myeloma Working Group (IMWG) from 2005 (Fig. 4): ISS I – 31.5% (n=17), ISS II – 16.6% (n=9), and ISS III – 51.8% (n=28) of the patients.

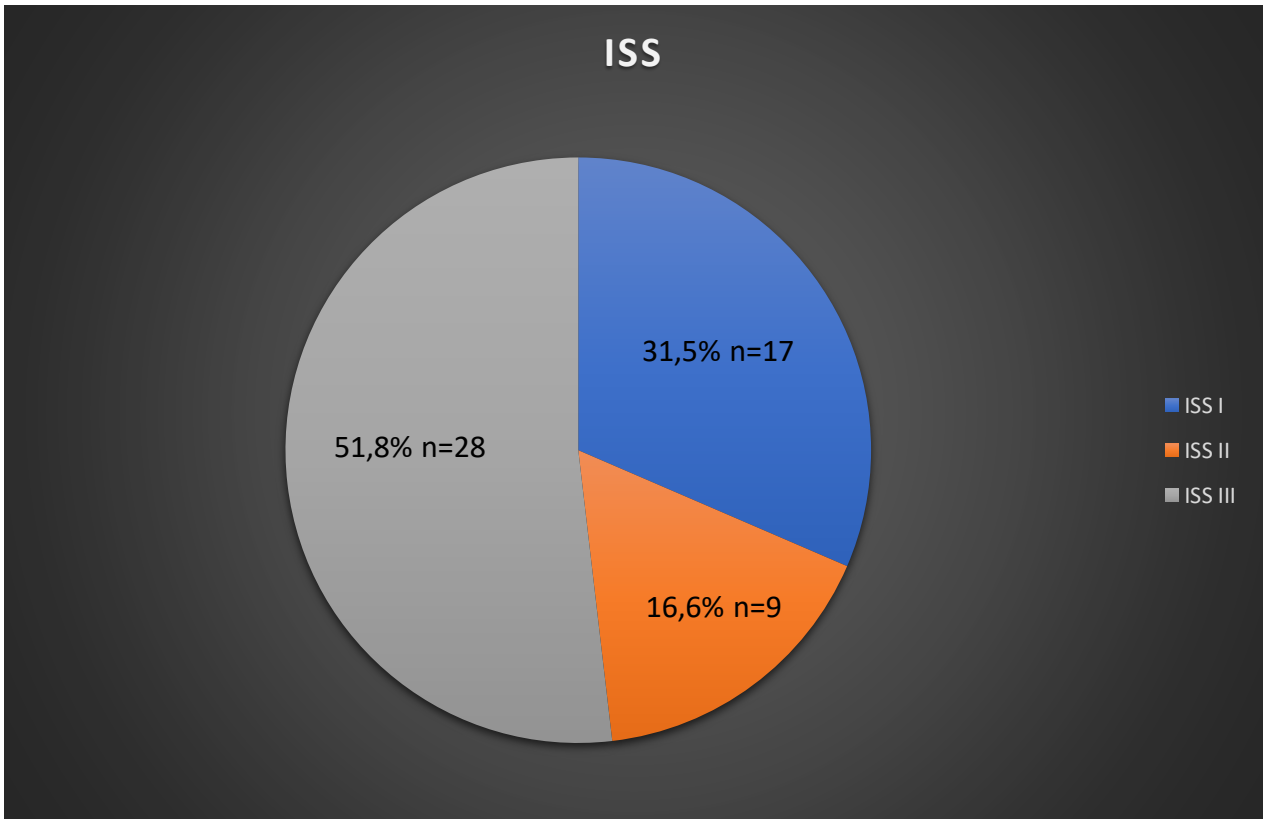


Figure 4: ISS distribution

According to their clinical and laboratory characteristics, the patients were classified based on several indicators: 82.1% (n=46) of the study population had bone disease, defined as one or more osteolytic lesions detected by X-ray, computed tomography, or positron emission tomography (Fig. 5). Renal insufficiency (serum creatinine >177 mmol/L or creatinine clearance <40 mL/m) was found in 37% (n=17) (Fig. 6). Hypercalcemia (Fig. 7), defined as a level above 2.75 mmol/L, was recorded in 19.6% (n=11). Symptomatic anemia (Fig. 8), defined as a hemoglobin level below 100 g/L, was observed in 48.2% (n=27) of the patients.

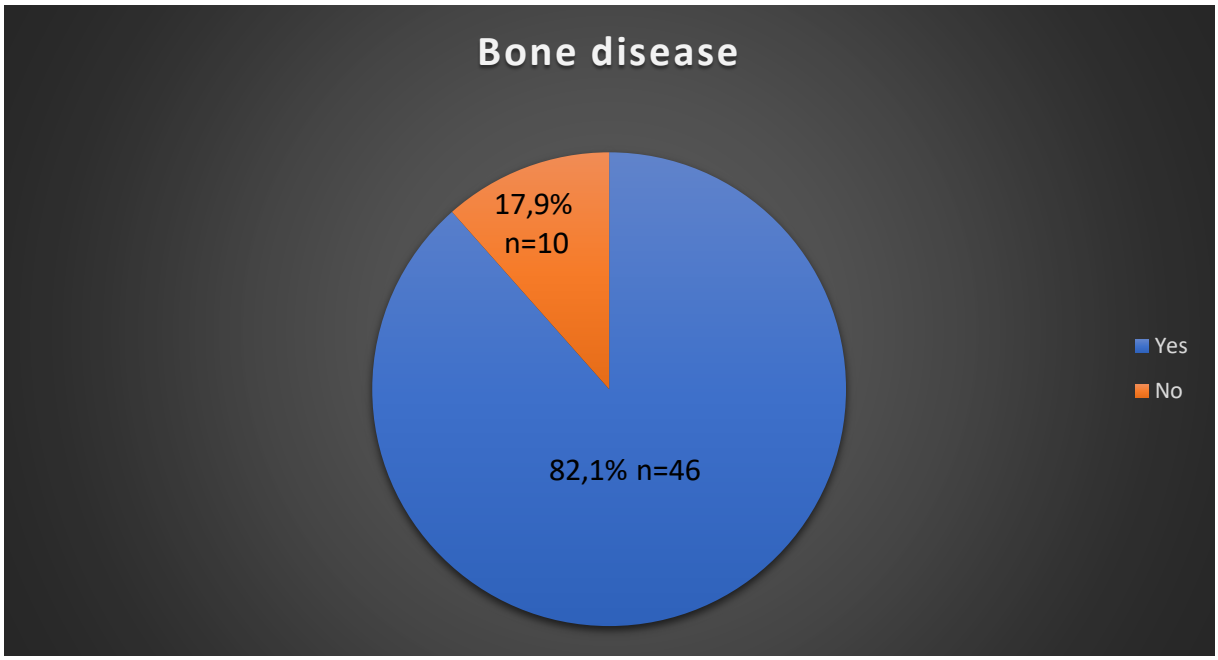


Figure 5: Presense of bone disease

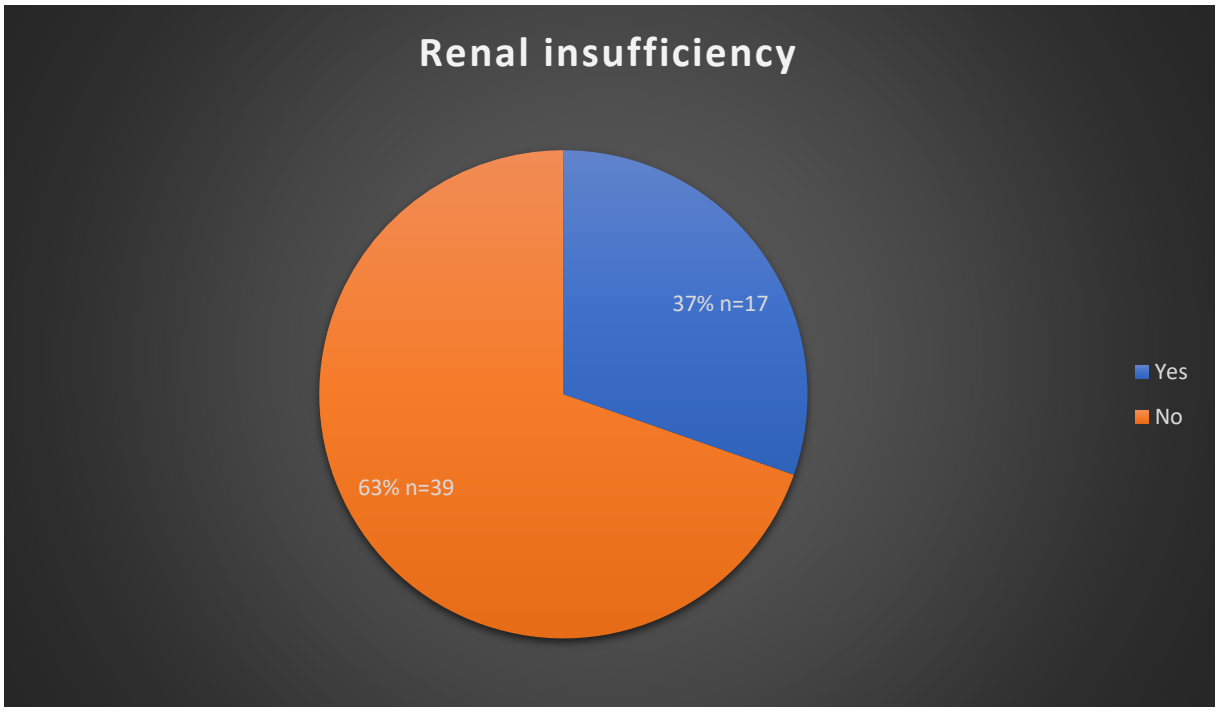


Figure 6: Patients with renal insufficiency

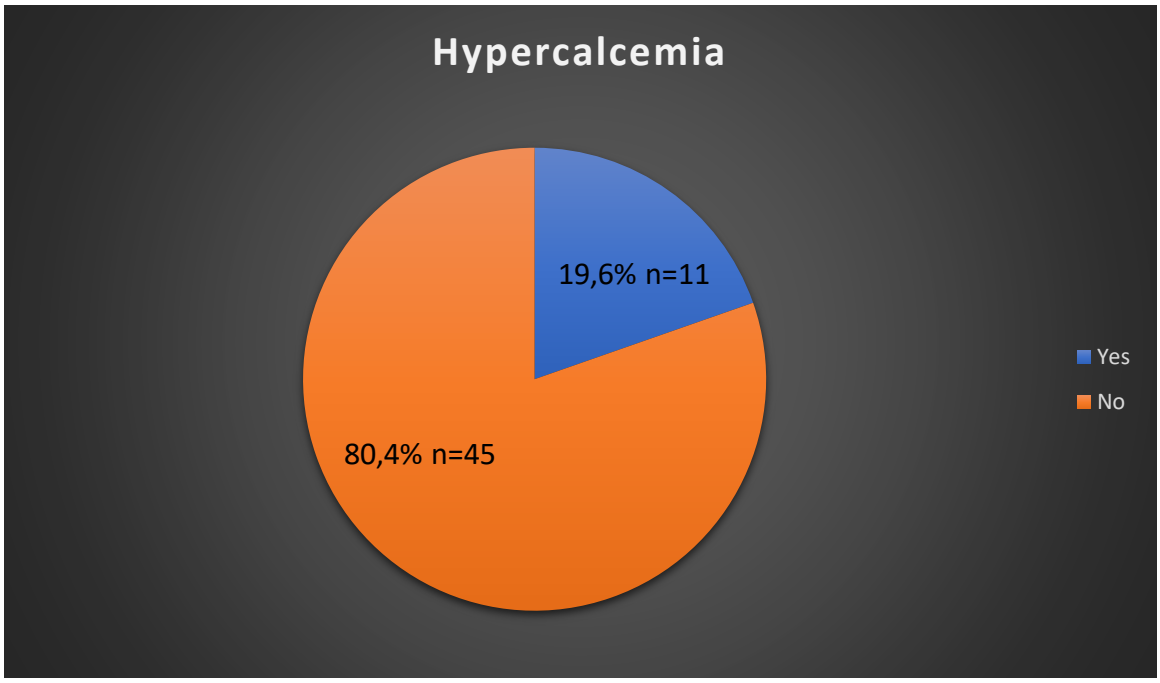


Figure 7: Patients with hypercalcemia

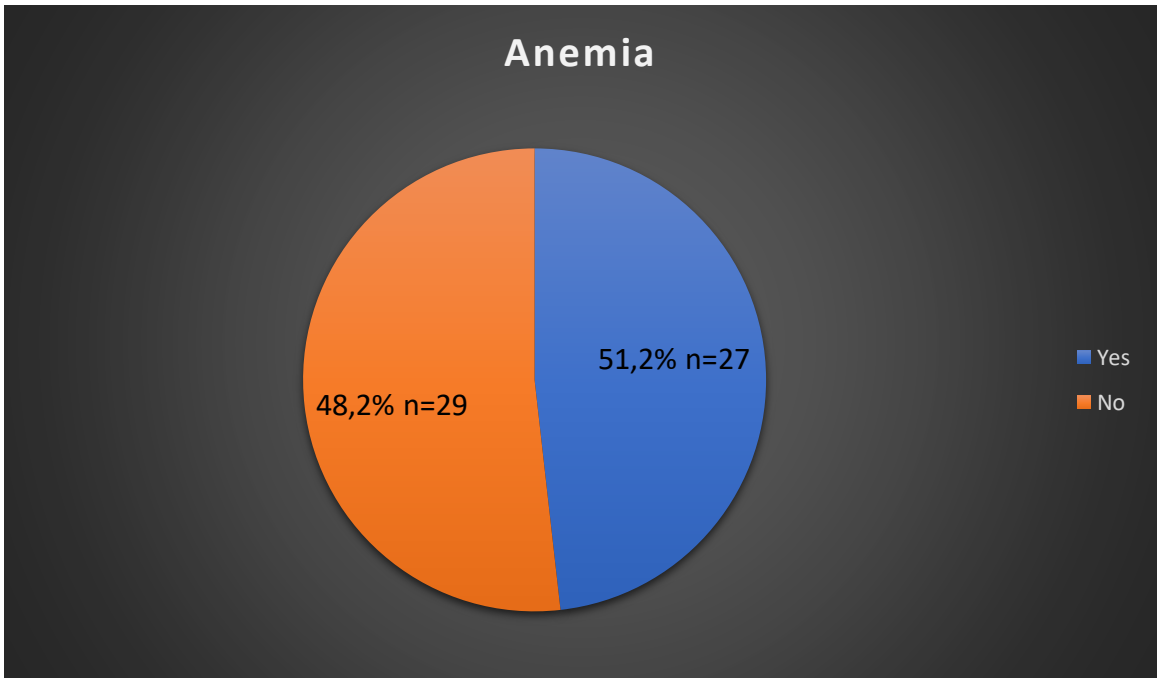


Figure 8: Patients with symptomatic anemia

A conventional cytogenetic analysis was performed on all patients; however, in 37.5% (n=21) of them, no metaphases were detected for analysis. Of the 35 successfully analyzed, 74% (n=26) had a normal karyotype and 26% (n=9) had an abnormal karyotype.

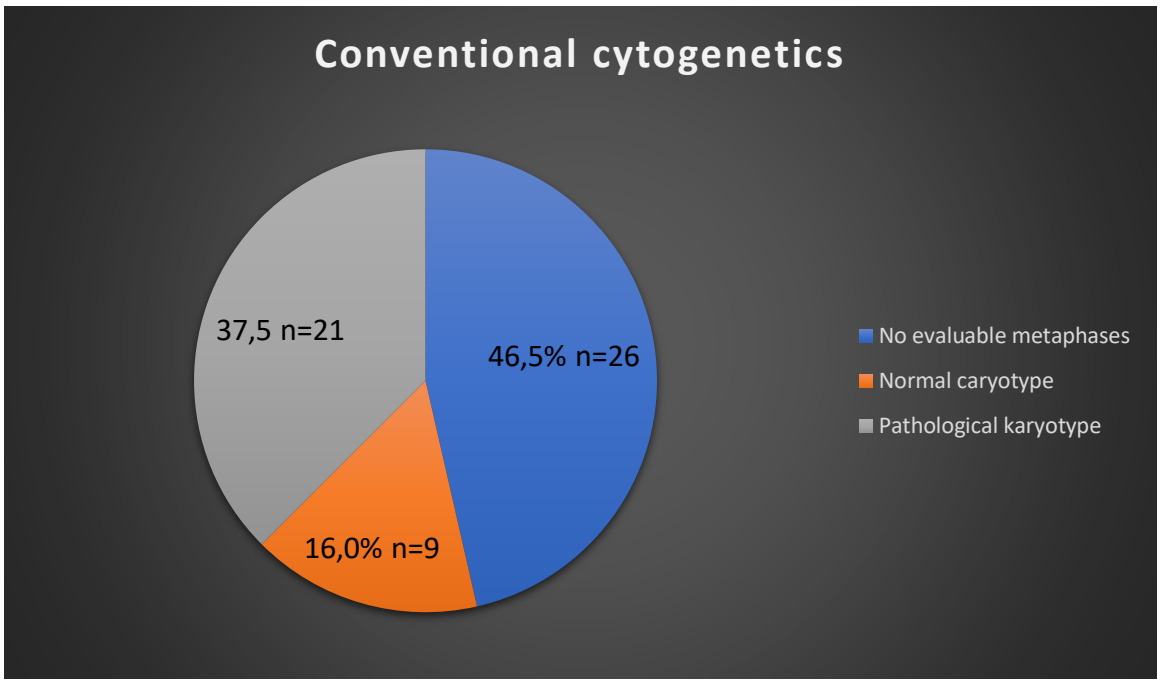


Figure 9: Cytogenetic profile

Of the 56 patients enrolled in the study, 32 (57%) reached the second (follow-up) sample after 6 cycles of therapy according to the VCD protocol. The reasons for patient dropout are as follows:

- Death – 13 patients
- Refusal to continue participation in the study – 5 patients
- Loss to follow-up – 4 patients
- Disease progression – 2 patients

The remaining 32 patients are categorized into 2 groups: 20 out of 32 (62.5%) achieved a complete response (CR) or a very good partial response (VGPR) – Group 1. The other 12 out of 32 (37.5%) achieved a partial response (PR) or exhibited stable disease (SD) – Group 2.

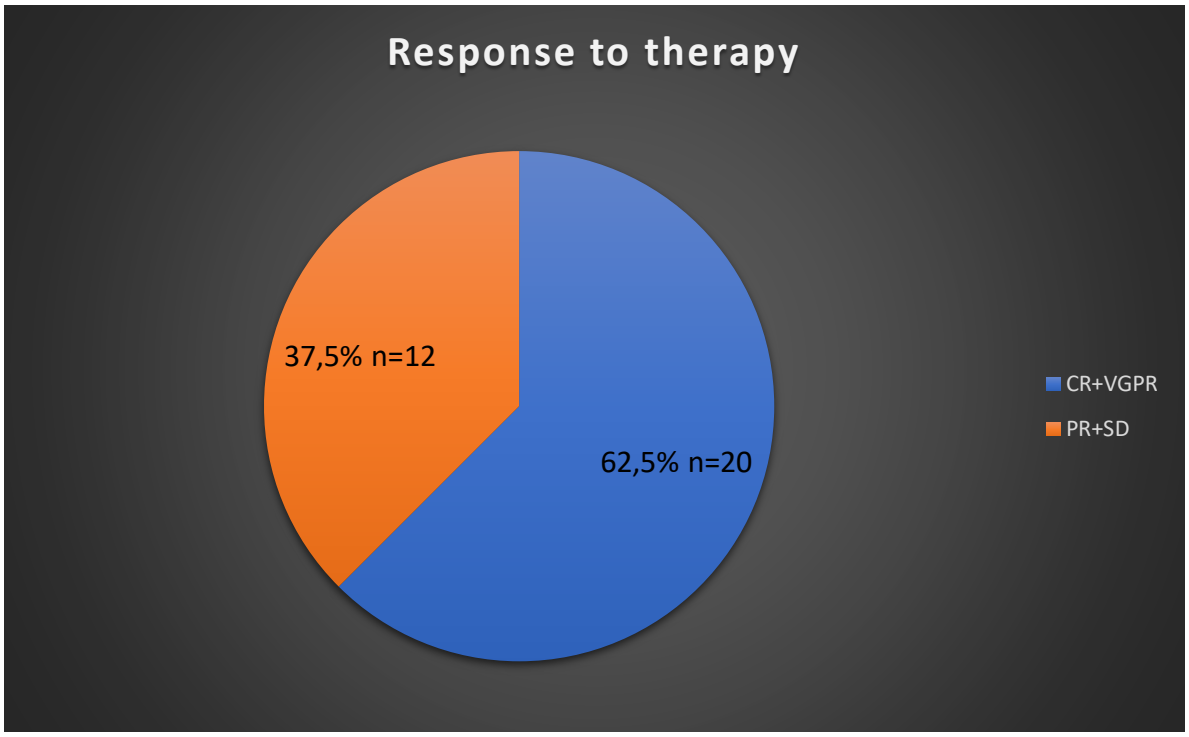


Figure 10: Response to therapy after 6 months treatment

All demographic and clinical-laboratory characteristics of the patients are presented in Table 1.

Table 1. Demographic and clinical-laboratory characteristics of the patients

Indicator		**N (count) / %**
Age	Mean	66 (36-90)
	>65 years	29/51,8%
Gender	Male	23/41,1%
	Female	33/58,9%
ISS	I	17/31,5%
	II	9/16,6%
	III	28/51,8%
Bone disease	yes	46/82,1%
	no	10/17,9%
Renal insufficiency	yes	17/37,0%

	no	29/63,0%
hypercalcemia	yes	11/19,6%
	no	45/80,4%
Anemia	yes	27/48,2%
	no	29/51,8%
Conventional cytogenetics	No evaluable metaphases	21/37,5%
	Normal caryotype	26/46,5%
	Pathological karyotype	9/16%
Response to therapy	Droppouts	24/42,8%
	CR+VGPR	20/35,7%
	PR+PD	12/21,4%
Hemoglobin	Mean ± CI (range)	103,0 ± 9,81
Creatinine	Mean ± CI (range)	117,4 ± 37,03
Beta-2 microglobulin	Mean ± CI (range)	5,494 ± 1,244
M-protein	Mean ± CI (range)	40,56 ± 8,68
miR-214-3p		Mean ± CI (range)
	Primary sample	2,128 ± 1,078
	Secondary sample	1,276 ± 0,523
	Controls	1,448 ± 0,6709
miR-497-5p		Mean ± CI (range)
	Primary sample	1,360 ± 0,303
	Secondary sample	1,136 ± 0,365
	Controls	1,505 ± 0,485
miR-126-5p		Mean ± CI (range)
	Primary sample	0,9721 ± 0,1257
	Secondary sample	1,326 ± 0,272
	Controls	1,800 ± 0,78
miR-199a-5p		Mean ± CI (range)
	Primary sample	1,168 ± 0,2934
	Secondary sample	1,365 ± 0,348
	Controls	3,018 ± 1,513
miR-373-3p		Mean ± CI (range)
	Primary sample	N/A

	Secondary sample	N/A
	Controls	N/A

5.2. Investigation of miR in patients with untreated and treated multiple myeloma, as well as in healthy controls

A study of specific miRs was conducted in all 56 patients with newly diagnosed multiple myeloma, with follow-up (paired) samples taken from 32 of these patients after 6 months of treatment. The same panel was also used for testing all 12 healthy volunteers. Five types of miRs, associated with various malignant, autoimmune, and inflammatory diseases, were examined.

Because of the experimental nature of the investigation and the lack of sufficient scientific data worldwide regarding normal miR expression in human serum, no reference values have been described. In this study, an averaged value was used.

The relative quantity (RQ) of miR expression in the serum samples of the patients was normalized to an external control, cel-miR-39. The RQ value was determined using the 2^-ΔΔCt method via StepOne Software v2.0.

Actual values were obtained in 95% of the samples for 4 of the miRs tested. Only miR-373-3p exhibited subthreshold expression, with detectable values in only 19% of the samples. This result does not allow for statistical analysis (Table 2).

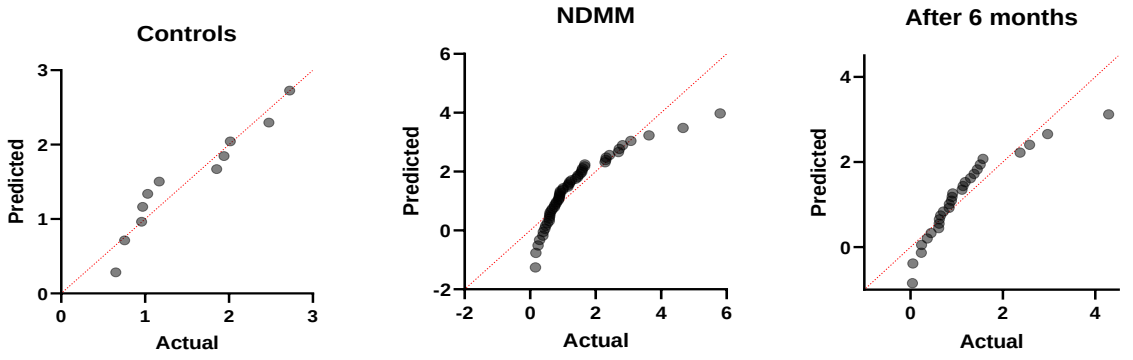
Table 2: Presence of suprathreshold expression levels in the studied miRs

	Primary sample (n / %)	Secondary sample (n / %)	Controls (n / %)
miR-214-3p	54/56 (96%)	30/32 (93,7%)	11/12 (91,6%)
miR-497-5p	54/56 (96%)	28/32 (87,5%)	12/12 (100%)
miR-126-5p	56/56 (100%)	31/32 (96,8%)	11/12 (91,6%)
miR-199a-5p	54/56 (96%)	29/32 (90%)	10/12 (83,3%)
miR-373-3p	13/56 (23,2%)	4/32 (12,5%)	2/12 (17,7%)

5.2.1. Comparative analysis of miR-199a-5p, miR-126-5p, miR-497-5p, miR-214-3p, and miR-373-3p values in patients before treatment, after treatment, and in healthy controls

A Shapiro-Wilk test for normality was performed on the four miRs. For none of the analyzed miRs did the normality test meet the criteria for at least two of the groups. The results are shown in the following figures:

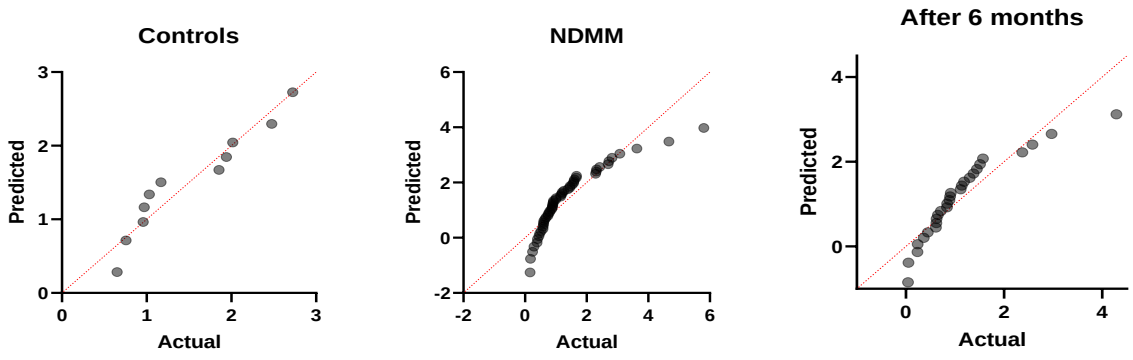
MiR-214-3p



p<0,0001 p<0,0001 p=0,2008

Figure 11: Normality plot of the population for miR-214-3p

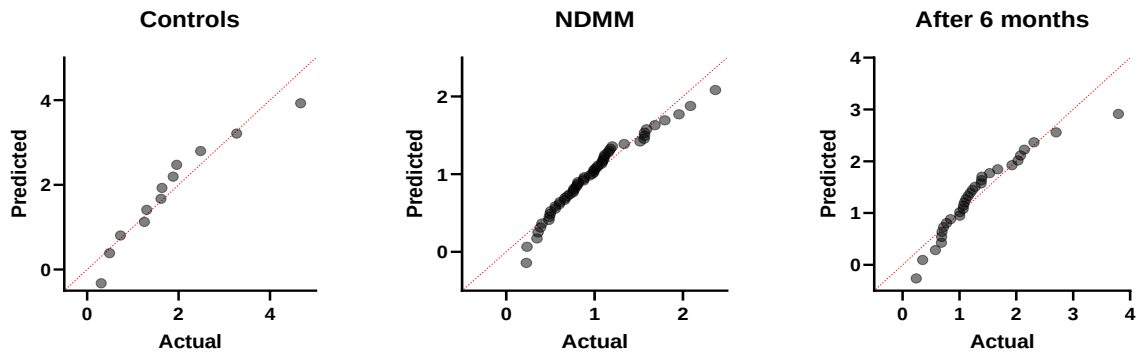
MiR-497-5p



p=0,1864 p=0,0006 p<0,0001

Figure 12: Normality plot of the population for miR-497-5p

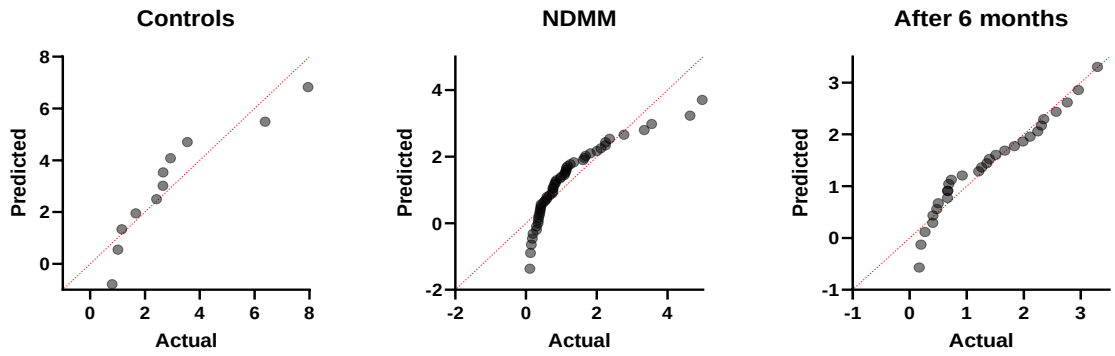
MiR-126-5p



p=0,2252 p=0,0188 p=0,0072

Figure 13: Normality plot of the population for miR-126-5p

MiR-199a-5p



p=0,0299 p<0,0001 p=0,0592

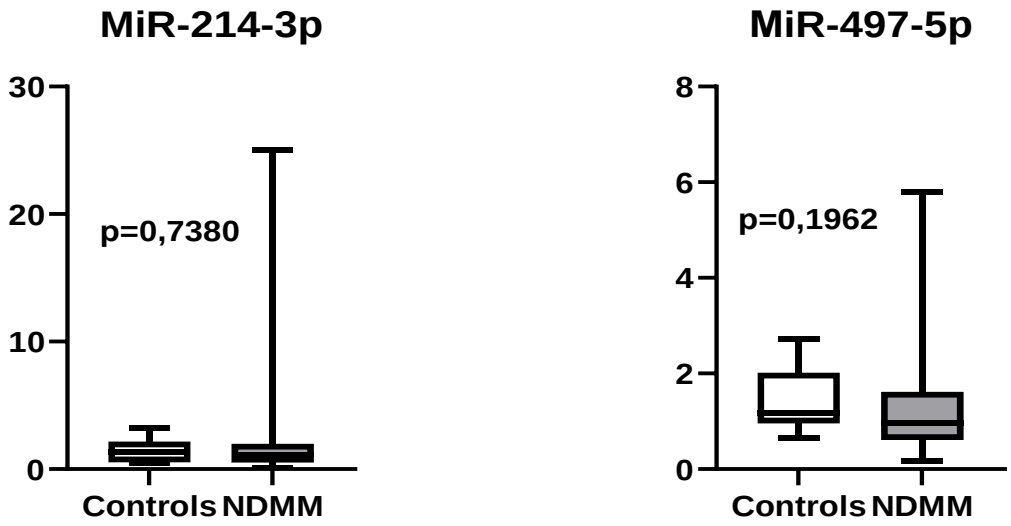
Figure 14: Normality plot of the population for miR-199a-5p

Average values of the studied miRs in newly diagnosed MM patients before treatment and in healthy controls are presented in table 3.

Table 3: Average values of the studied miRs in patients before treatment and in healthy controls, and statistical significance

	NDMM	Controls	p
miR-214-3p	2,128 ± 1,078	1,448 ± 0,6709	0,7380
miR-497-5p	1,360 ± 0,303	1,505 ± 0,485	0,1962
miR-126-5p	0,9721 ± 0,1257	1,800 ± 0,78	**0,0096**
miR-199a-5p	1,168 ± 0,2934	3,018 ± 1,513	**0,0003**
miR-373-3p	N/A	N/A	N/A

From the analysis (Fig. 14) comparing the two populations, miR-126-5p and miR-199a-5p showed significantly lower levels in the MM group versus healthy controls (non-parametric Mann-Whitney test). These findings suggest their role in the pathogenesis of MM.



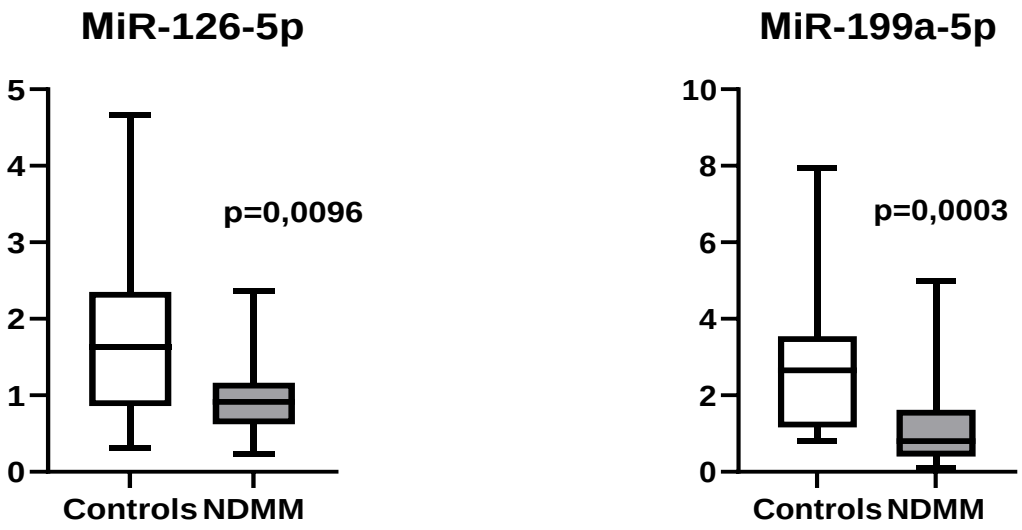


Figure 15: Serum levels of miR-214-3p, miR-497-5p, miR-126-5p, and miR-199a-5p in healthy controls and patients before treatment

5.2.2. Comparative Analysis of miR-199a-5p, miR-126-5p, miR-497-5p, miR-214-3p, miR-373-3p Values in Newly Diagnosed Patients According to ISS, Number of Osteolytic Lesions, Cytogenetic Profile, and Presence of Hypercalcemia

5.2.2.1. According to ISS

Table 4 shows the average values of the studied miRs in relation to the disease stage according to ISS. The Kruskal-Wallis test revealed no significant association with ISS for any of the selected biomarkers (Fig. 15).

Table 4: Mean values of the studied miRs in patients before treatment, categorized by ISS

	ISS I (n=17)	ISS II (n=9)	ISS III (n=27)	p
miR-214-3p	1,244 ± 0,603	1,014 ± 0,565	3,022 ± 2,048	0,2710
miR-497-5p	1,199 ± 0,491	0,825 ± 0,101	1,631 ± 0,504	0,1474
miR-126-5p	1,151 ± 0,29	0,99 ± 0,186	0,866 ± 0,166	0,1824
miR-199a-5p	1,29 ± 0,58	1,071 ± 0,500	1,165 ± 0,467	0,7843
miR-373-3p	N/A	N/A	N/A	N/A

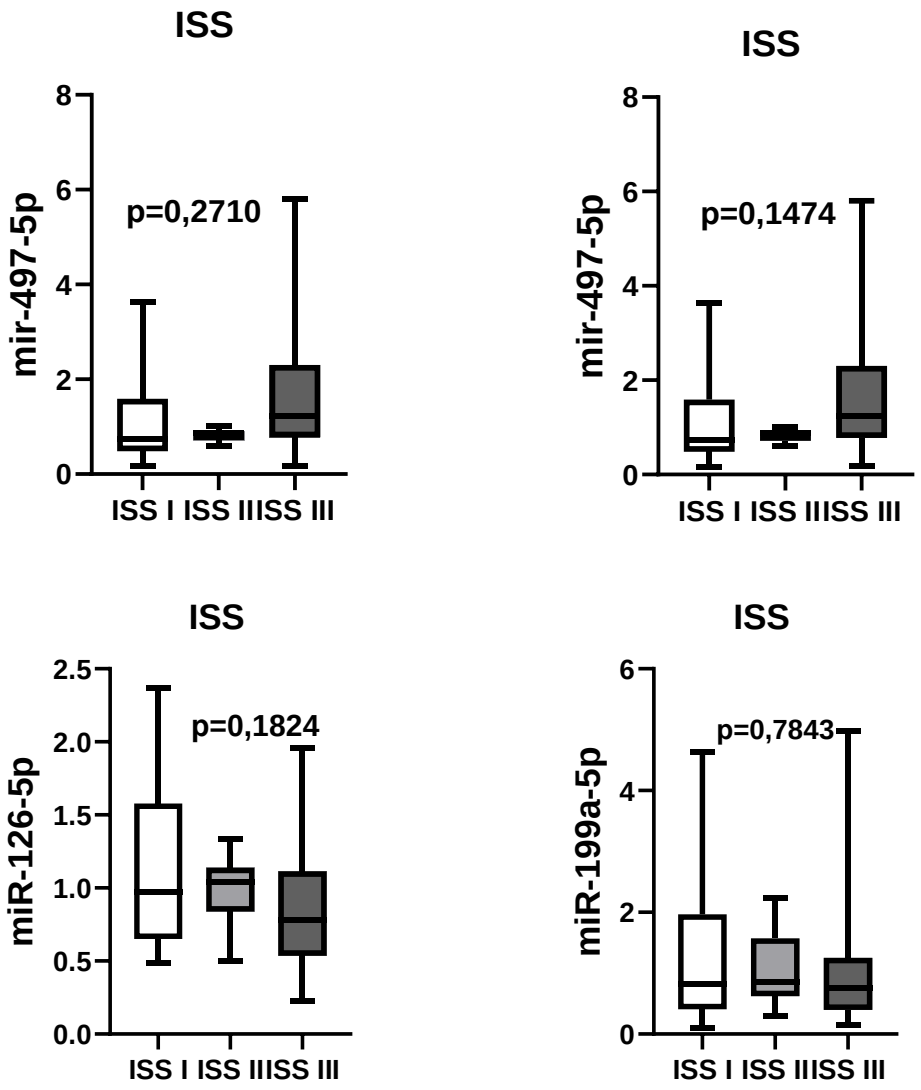


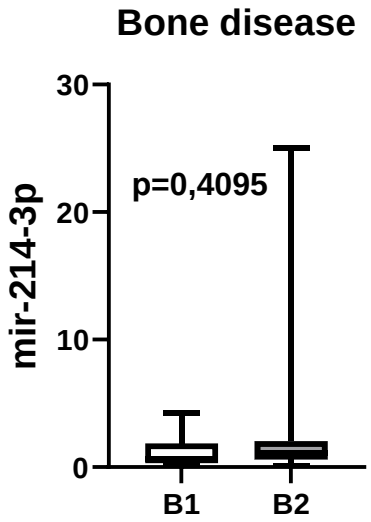
Figure 16: Comparative analysis of the values of miR-214-3p, miR-497-5p, miR-126-5p, and miR-199a-5p in newly diagnosed multiple myeloma patients stratified according to ISS.

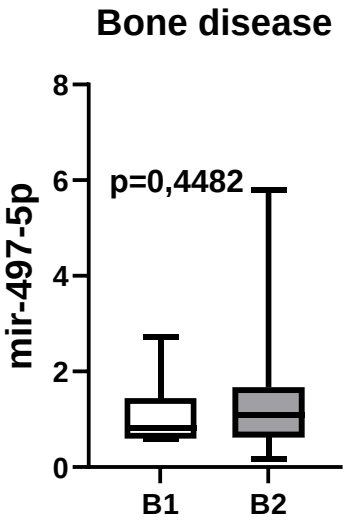
5.2.2.2. According to the Number of Osteolytic Lesions

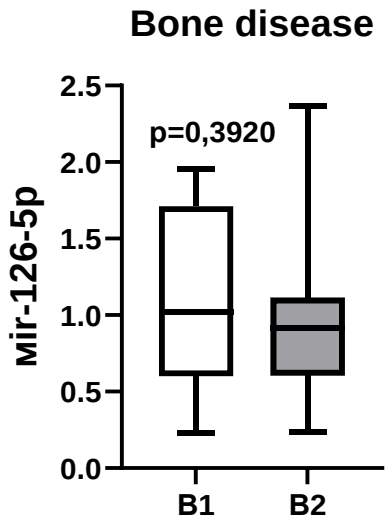
Based on the number of bone lesions detected by WBLDCT or PET-CT, the patient population was divided into two groups (B1: 0–3 lesions, B2: >3 lesions). A comparative analysis was then performed on the average miR values, depending on the presence and number of registered osteolytic lesions (Table 5). No significant differences were found for miR-214-3p, miR-497-5p, or miR-126-5p. For miR-199a-5p, there was a trend toward lower levels in patients with more pronounced bone disease (Fig. 16).

Table 5: Average values of the studied miRs in patients before treatment, divided into groups based on the number of osteolytic lesions

	B1 (n=10)	B2 (n=44)	p
miR-214-3p	1,238 ± 0,901	2,330 ± 1,313	0,4095
miR-497-5p	1,070 ± 0,485	1,426 ± 0,359	0,4482
miR-126-5p	1,116 ± 0,436	0,941 ± 0,129	0,3920
miR-199a-5p	1,650 ± 0,822	1,080 ± 0,321	0,0862
miR-373-3p	N/A	N/A	N/A







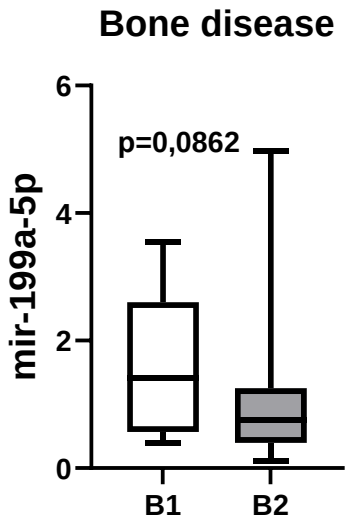


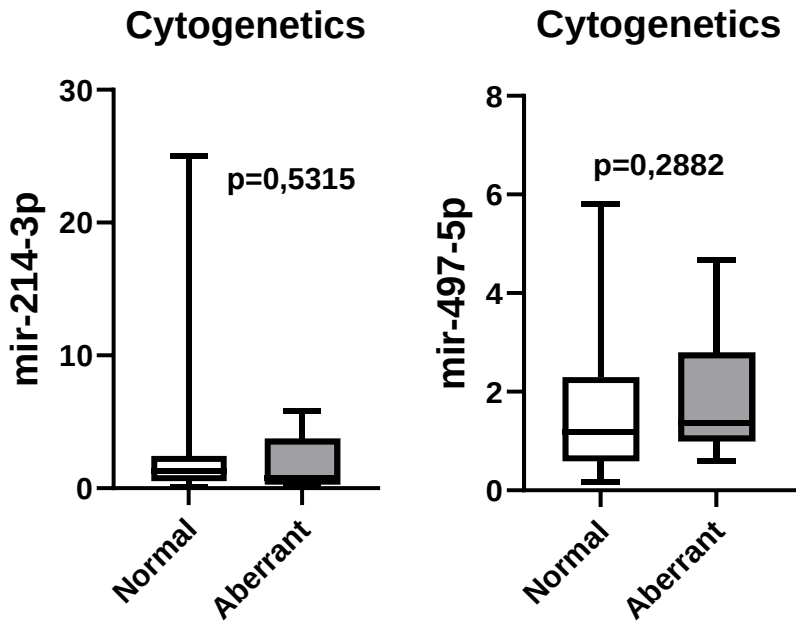
Figure 17: Comparative analysis of the values of miR-214-3p, miR-497-5p, miR-126-5p, and miR-199a-5p in newly diagnosed multiple myeloma patients stratified by the number of osteolytic lesions.

5.2.2.3. According to Cytogenetic Profile

Depending on the cytogenetic profile (established via conventional metaphase cytogenetics), patients were divided into two groups (normal and abnormal karyotype). We analyzed the average values of the studied miRs according to karyotype.

Таблица 6: Table 6: Average values of the studied miRs in patients before treatment, divided into groups based on cytogenetic profile

	Normal	Aberrant	p
miR-214-3p	2,490 ± 1,94 **(n=26)**	1,777 ± 1,989 **(n=7)**	0,5315
miR-497-5p	1,502 ± 0,498 **(n=26)**	1,958 ± 1,135 **(n=8)**	0,2882
miR-126-5p	0,973 ± 0,208 **(n=26)**	1,177 ± 0,357 **(n=9)**	0,1596
miR-199a-5p	1,251 ± 0,507 **(n=25)**	1,474 ± 0,911 **(n=9)**	0,5594
miR-373-3p	N/A	N/A	N/A



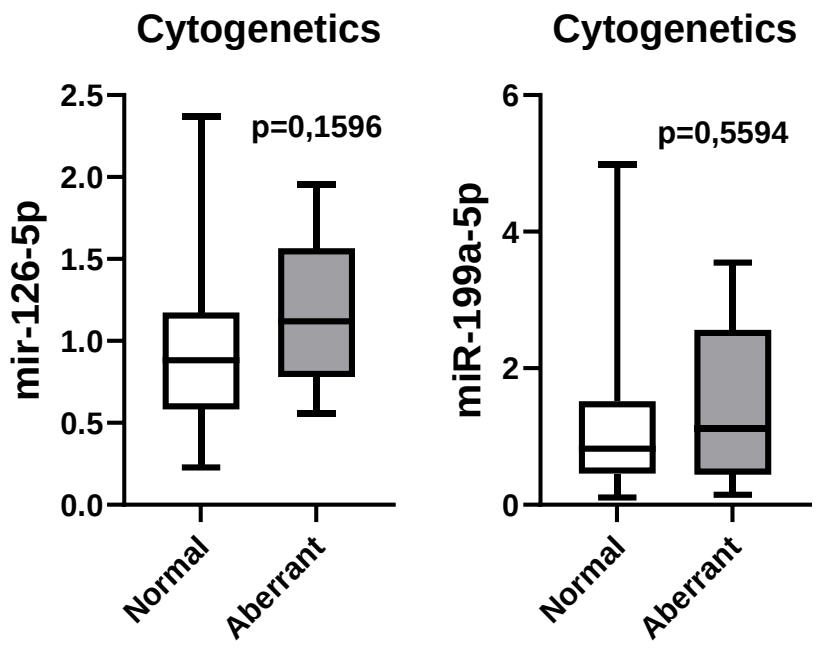


Figure 18: Comparative analysis of the values of miR-214-3p, miR-497-5p, miR-126-5p, and miR-199a-5p in newly diagnosed multiple myeloma patients according to cytogenetic profile.

5.2.2.4. According to the presence of hypercalcemia

The role of the selected miRs in the pathogenesis of hypercalcemia was also evaluated. We analyzed the average values (Table 7) of miRs in patients with elevated calcium levels at diagnosis, compared to those in whom this complication was not documented.

Table 7: Average values of the studied miRs in patients before treatment, divided into groups based on the presence of hypercalcemia

	Normal (n=42)	Hypercalcemia (n=11)	p
miR-214-3p	1,731 ± 0,781	3,678 ± 4,855	0,4076
miR-497-5p	1,233 ± 0,293	1,86 ± 1,04	0,1649
miR-126-5p	0,964 ± 0,145	1,00 ± 0,285	0,6253
miR-199a-5p	1,085 ± 0,29	1,582 ± 1,013	0,4679
miR-373-3p	N/A	N/A	N/A

Our results did not reveal any significant deviations in the performed analysis and cannot reject the null hypothesis.

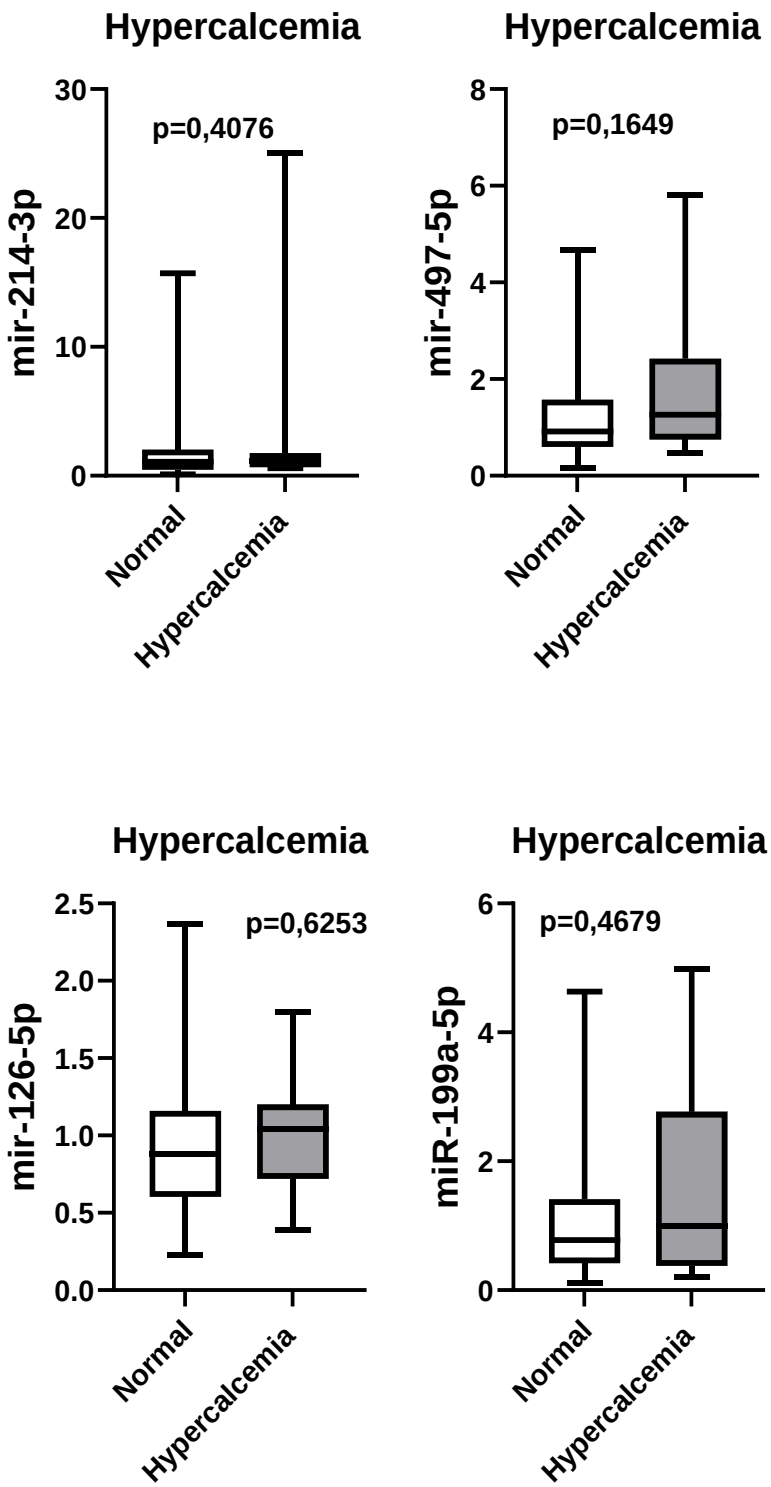


Figure 19. Comparative analysis of the values of miR-214-3p, miR-497-5p, miR-126-5p, miR-199a-5p in newly diagnosed multiple myeloma patients depending on their cytogenetic profile.

5.2.3. Correlation analysis of miR-199a-5p, miR-126-5p, miR-497-5p, miR-214-3p, miR-373-3p with the patients' clinical laboratory values

A Spearman correlation analysis was conducted to evaluate the relationship between the potential biomarkers and routine laboratory tests commonly used in clinical practice (Table 8).

Table 8: Spearman correlation coefficient and p-values for the studied miRs and hemoglobin, serum creatinine, beta-2 microglobulin, and M-protein levels

	Hemoglobin p; r	Creatinine p; r	Beta-2 mcg p; r	M-protein p; r
miR-214-3p	0,9708; 0,0069	0,3703;-0,1666	0,7075; 0,0702	0,8228; 0,0462
miR-497-5p	0,4732;-0,1338	0,2829;-0,1991	0,5848;-0,1021	0,6866;-0,0831
miR-126-5p	0,1255; 0,2765	0,1626;-0,2529	**0,0025;-0,5159**	0,3922;-0,1716
miR-199a-5p	**0,0210; 0,4127**	0,3020;-0,1915	**0,0425;-0,3667**	0,7513; 0,0653
miR-373-3p	N/A	N/A	N/A	N/A

A statistically significant moderate negative correlation was found between the RQ levels of miR-126-5p and beta-2 microglobulin (Fig. 20). A weak correlation was also observed with hemoglobin, creatinine, and paraprotein values (Figs. 21, 22, 23). miR-199a-5p had a moderate correlation with hemoglobin (Fig. 24) and beta-2 microglobulin (Fig. 25), and a weak correlation with serum creatinine (Fig. 26).

For miR-214-3p and miR-497-5p, no significant proportional relationships were detected with the parameters under study.

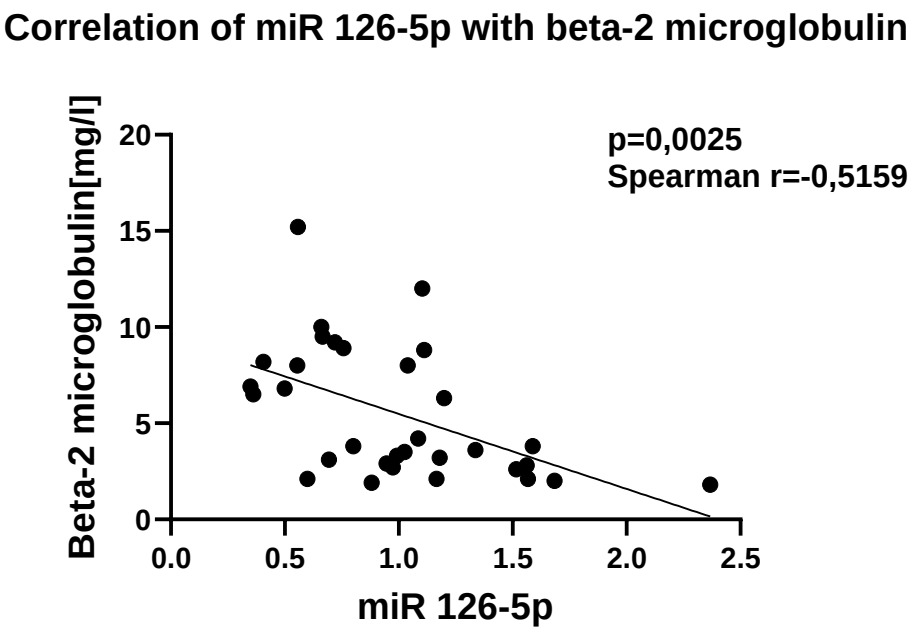


Figure 20: Correlation analysis between miR-126-5p and baseline beta-2 microglobulin values

A significant negative correlation was demonstrated between miR-126-5p levels and beta-2 microglobulin (Spearman correlation coefficient = –0.5159; p=0.0025).

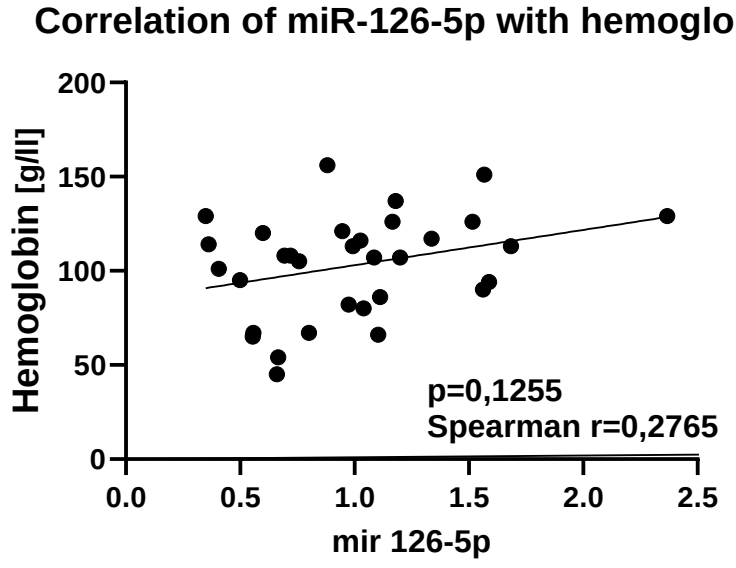


Figure 21: Correlation analysis between miR-126-5p and baseline hemoglobin values

The graph shows a weak positive correlation between miR-126-5p and baseline hemoglobin (Spearman correlation coefficient = 0.2765; p=0.1255).

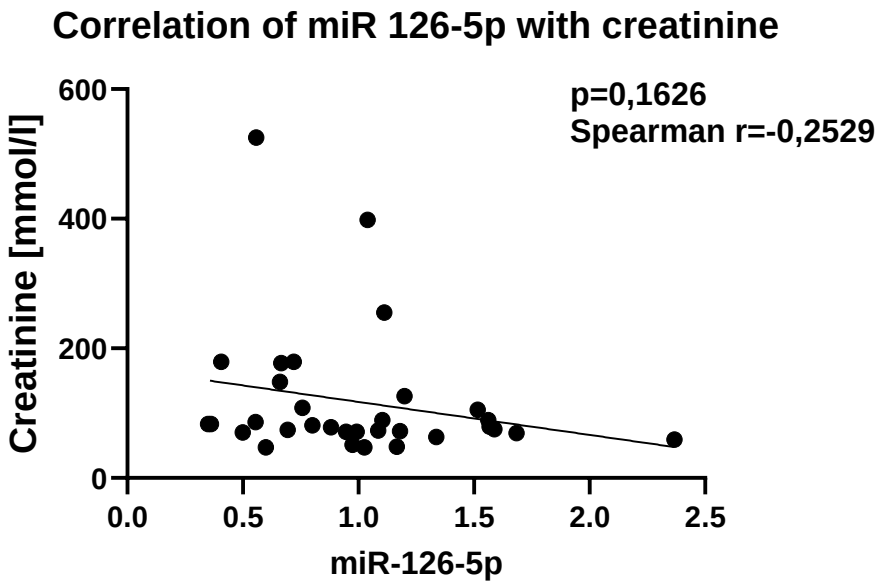


Figure 22: Correlation analysis between miR-126-5p and baseline serum creatinine values

A slight negative correlation was found between miR-126-5p and baseline serum creatinine (Spearman correlation coefficient = –0.2529; p=0.1626).

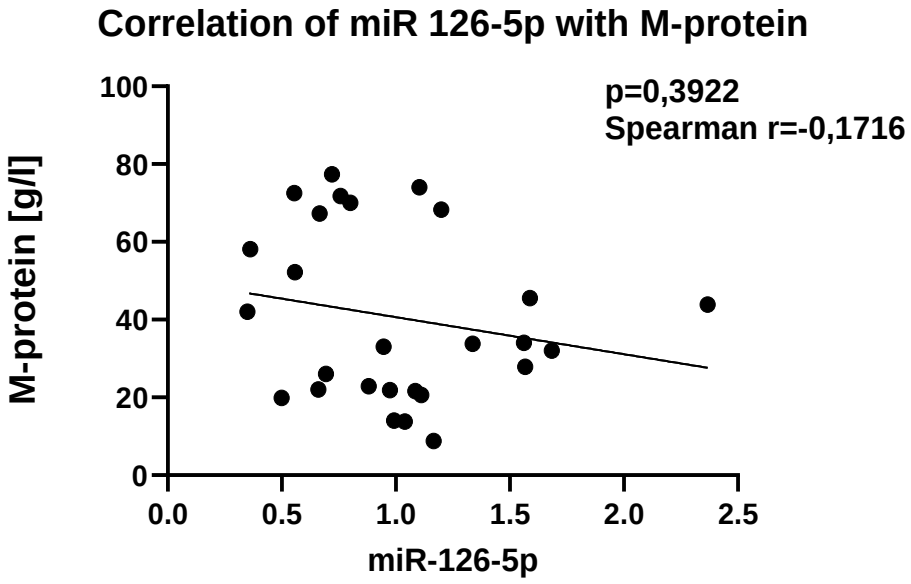


Figure 23: Correlation analysis between miR-126-5p and baseline M-protein values

A mild negative correlation was observed between miR-126-5p and baseline M-protein (Spearman correlation coefficient = –0.1716; p=0.3922).

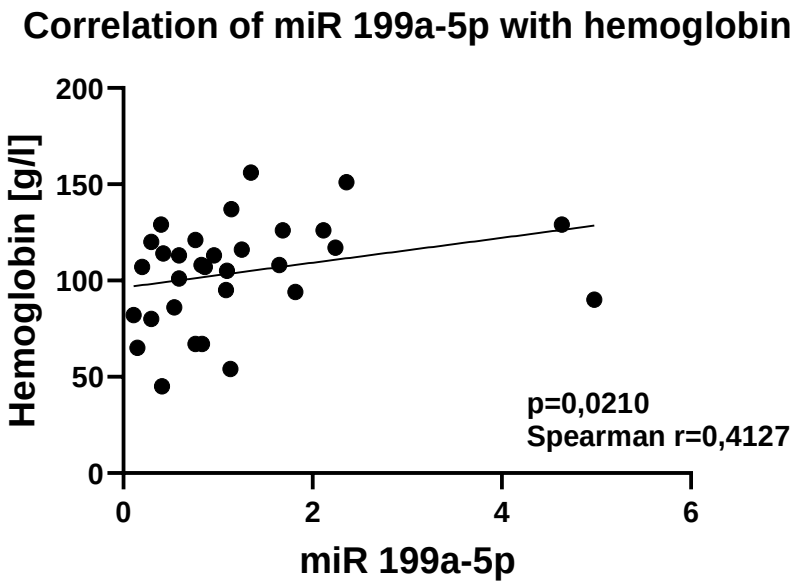


Figure 24: Correlation analysis between miR-199a-5p and baseline hemoglobin values

A moderately strong and statistically significant correlation was demonstrated between miR-199a-5p levels and hemoglobin (Spearman correlation coefficient = 0.4127; p=0.0210).

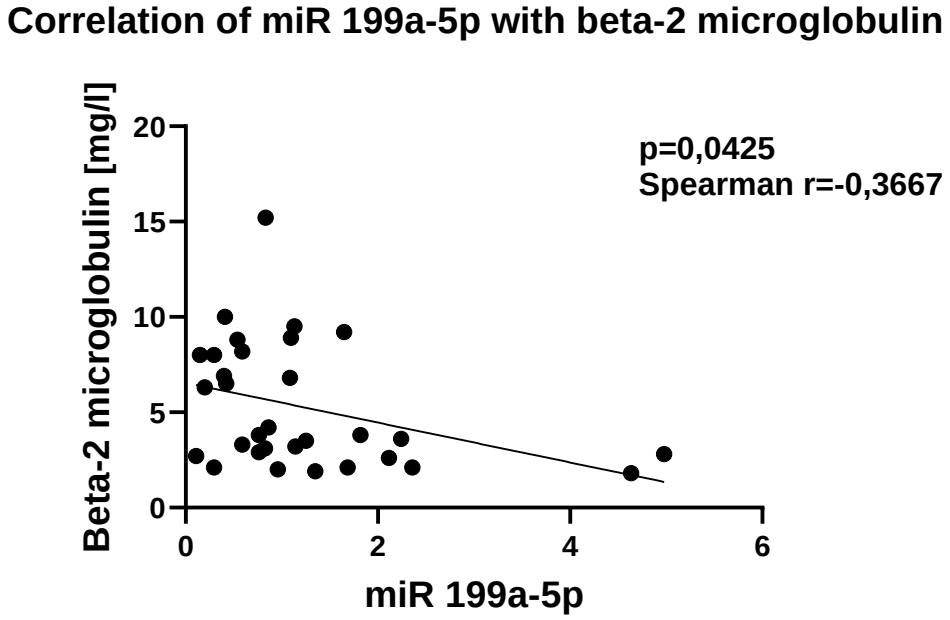


Figure 25: Correlation analysis between miR-199a-5p and beta-2 microglobulin

The graph shows a moderate negative correlation between miR-199a-5p expression levels and beta-2 microglobulin (Spearman correlation coefficient = –0.3667; p=0.0425).

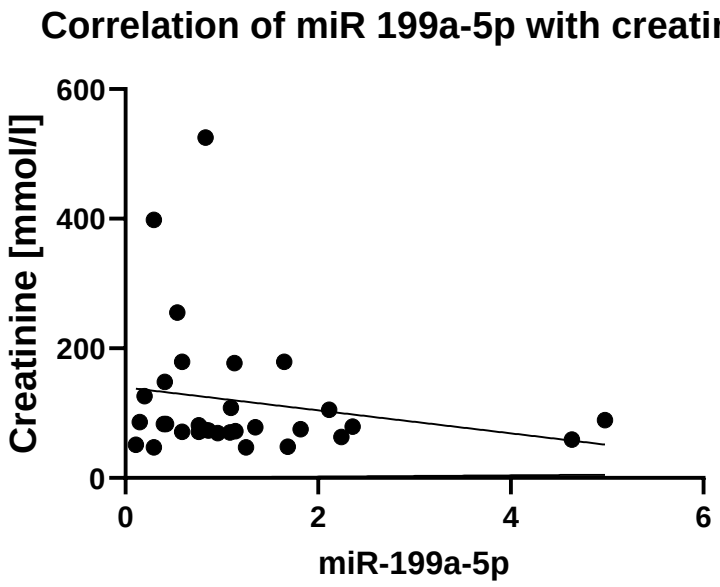


Figure 26: Correlation analysis between miR-199a-5p and serum creatinine

A slight negative correlation was found between miR-199a-5p and creatinine levels (Spearman correlation coefficient = –0.1915; p=0.3020).

5.2.4. Assessment of miR-199a-5p, miR-126-5p, miR-497-5p, miR-214-3p, miR-373-3p levels in patients who underwent a second examination, and their paired samples during therapy and treatment response, compared to healthy controls

Table 9: MiR-199a-5p, miR-126-5p, miR-497-5p, miR-214-3p, and miR-373-3p values in patients who had a follow-up sample taken before starting treatment and paired samples taken after 6 months of therapy

	Pre treatment values	Post treatment values	p
miR-214-3p	1,293 ± 0,397 **(n=31)**	1,276 ± 0,523 **(n=30)**	0,7212
miR-497-5p	1,112 ± 0,279 **(n=31)**	1,136 ± 0,365 **(n=28)**	0,2061
miR-126-5p	0,999 ± 0,163 **(n=32)**	1,326 ± 0,272 **(n=31)**	**0,0246**
miR-199a-5p	1,211 ± 0,419 **(n=31)**	1,365 ± 0,348 **(n=29)**	0,3386
miR-373-3p	N/A	N/A	N/A

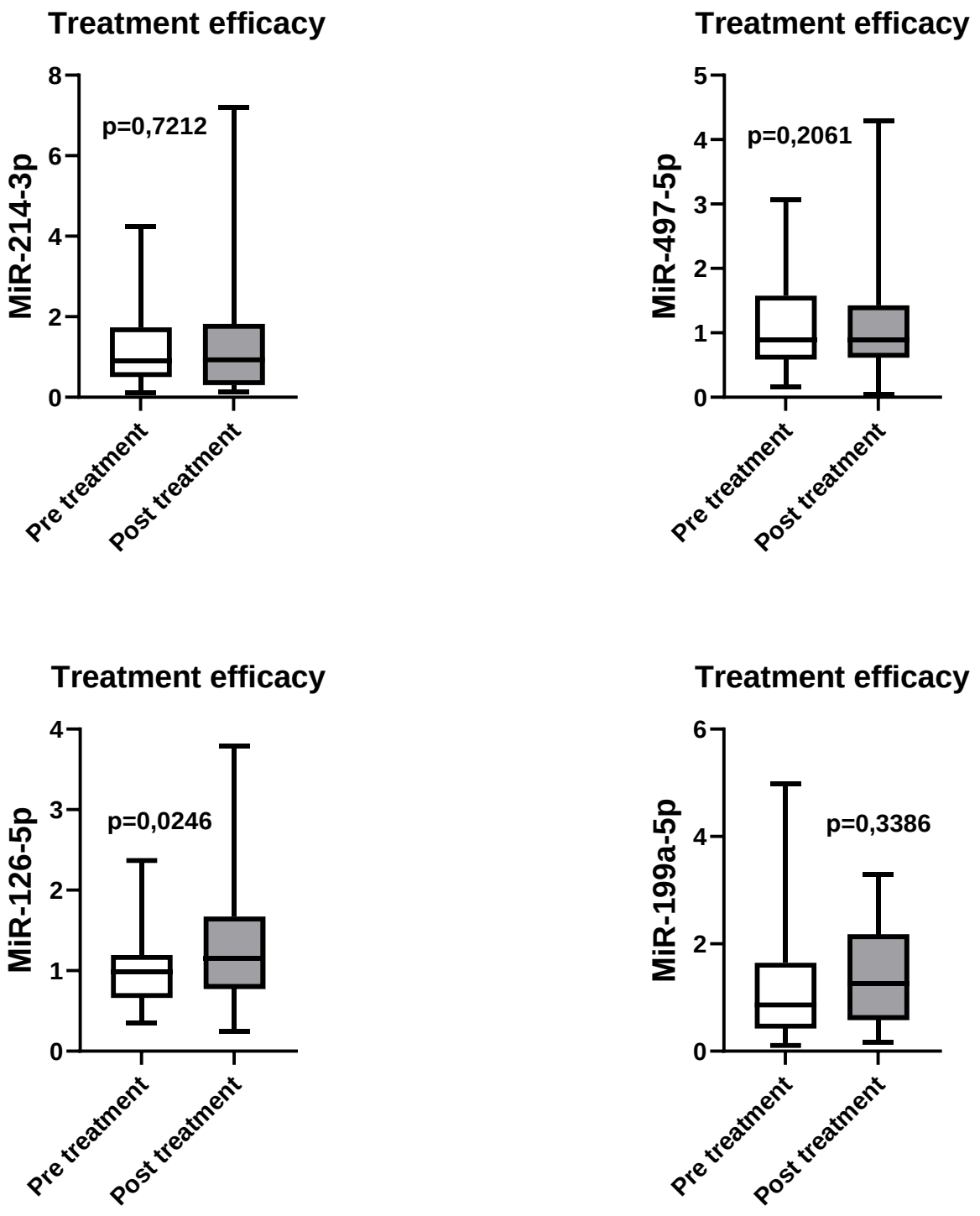


Figure 27: Wilcoxon signed-rank test for paired samples of miR-199a-5p, miR-126-5p, miR-497-5p, and miR-214-3p

Significantly higher miR-126-5p levels were observed in patients following treatment, approaching control values (Fig. 28, Table 10). No significant changes in the other studied biomarkers were demonstrated during the course of therapy.

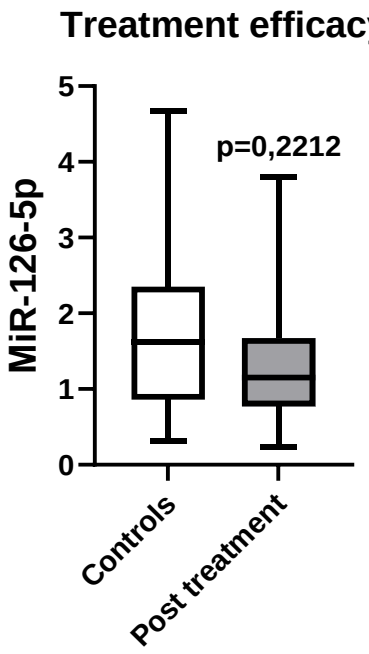


Figure 28: Comparison of miR-126-5p expression levels in healthy controls vs. patients after 6 months of therapy (non-parametric Mann-Whitney test)

Table 10: Mean values of miR-126-5p in healthy controls vs. patients after 6 months of therapy

	Controls	Post treatment values	p
miR-126-5p	1,800 ± 0,78 **(n=12)**	1,326 ± 0,272 **(n=31)**	0,2212

5.2.5. Analysis of miR-199a-5p, miR-126-5p, miR-497-5p, miR-214-3p, miR-373-3p levels during treatment according to therapy response, and comparison with healthy controls

Of the 56 patients included in the study, 32 (57%) reached the second (follow-up) sample point after 6 cycles of VCD protocol therapy. Among these, 20 out of 32 (62.5%) achieved complete response (CR) or very good partial response (VGPR), while the remaining 12 out of 32 (37.5%) attained partial response (PR) or exhibited stable disease (SD).

Subsequently, a Kruskal-Wallis test was performed to compare the results of four independent groups.

Table 11 shows the mean values of the studied biomarkers, categorized by treatment response.

Table 11: Mean values of miR-199a-5p, miR-126-5p, miR-497-5p, and miR-214-3p in healthy controls, patients before treatment, and patients after 6 months of therapy according to treatment response

	Controls	NDMM	CR+VGPR	PR+SD	P; ε^2
miR-214-3p	1,448 ± 0,671 **(n=10)**	2,128 ± 1,078 **(n=54)**	1,349 ± 0,828 **(n=18)**	1,166 ± 0,589 **(n=12)**	0,6705
miR-497-5p	1,505 ± 0,485 **(n=11)**	1,360 ± 0,303 **(n=54)**	1,145 ± 0,399 **(n=18)**	1,121 ± 0,863 **(n=10)**	0,3407
miR-126-5p	1,8 ± 0,78 **(n=12)**	0,9721 ± 0,126 **(n=56)**	1,486 ± 0,379 **(n=19)**	1,073 ± 0,387 **(n=12)**	**0,0056; 0.029**
miR-199a-5p	3,018 ± 1,513 **(n=11)**	1,168 ± 0,293 **(n=54)**	1,522 ± 0,51 **(n=18)**	1,108 ± 0,448 **(n=11)**	**0,0038; 0.076**
miR-373-3p	N/A	N/A	N/A	N/A	N/A

A post hoc Dunn's test for multiple comparisons was conducted, and the direct comparison results are presented in Table 12.

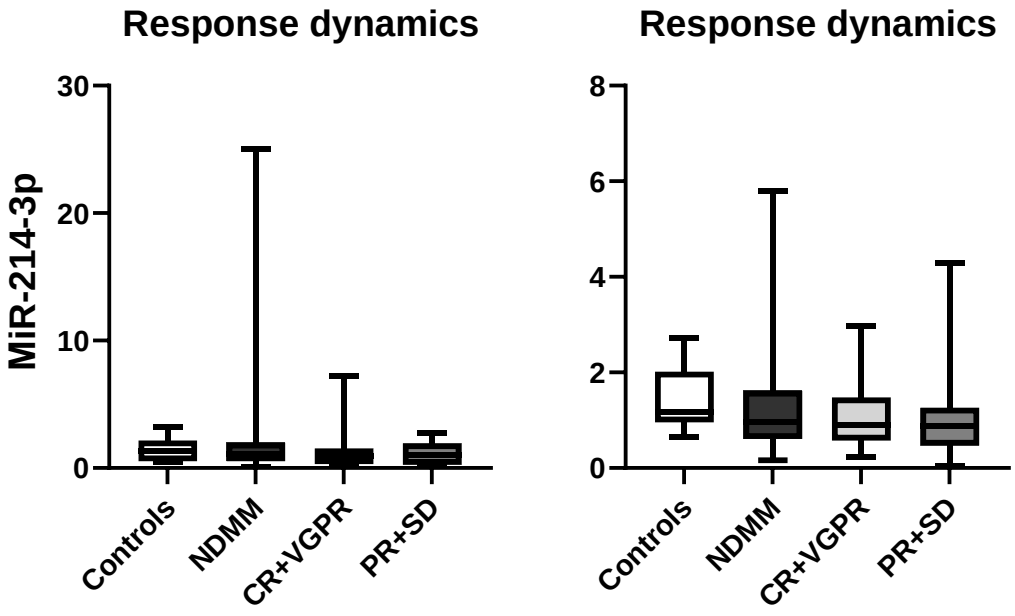
Table 12: Dunn's Post Hoc Analysis for Multiple Comparisons

	Controls v.s. NDMM	Controls v.s. CR+VGPR	Controls v.s. PR+SD	NDMM v.s. CR+VGPR	NDMM v.s. PR+SD	CR+VGPR v.s. PR+SD
miR-214-3p	p>0,9999	p>0,9999	p>0,9999	p>0,9999	p>0,9999	p>0,9999
miR-497-5p	p>0,9999	p=0,7646	p=0,5717	p>0,9999	p>0,9999	p>0,9999
miR-126-5p	**p=0,0418**	p >0,9999	p=0,5167	**p=0,0320**	p>0,9999	p=0,6871
miR-199a-5p	**p=0,0022**	p=0,2561	p=0,0751	p=0,8366	p >0,9999	p >0,9999
miR-373-3p	N/A	N/A	N/A	N/A	N/A	N/A

MiR-199a-5p levels are significantly lower in patients with newly diagnosed multiple myeloma (NDMM) compared to healthy controls, suggesting its role as a potential diagnostic biomarker. On the other hand, its expression does not change significantly based on therapy response (CR+VGPR vs. PR+SD). It can thus be inferred that miR-199a-5p is more informative for diagnosis than for evaluating treatment efficacy.

MiR-126-5p also shows significant deviations. Its expression levels are considerably lower in untreated patients and can be restored to near-control levels in cases of a satisfactory treatment effect (CR+VGPR). In contrast, in patients with partial response or stable disease, miR-126-5p levels do not differ statistically from those before treatment.

The study results indicate no significant differences in the RQ values of miR-497-5p and miR-214-3p in any of the subpopulations investigated.



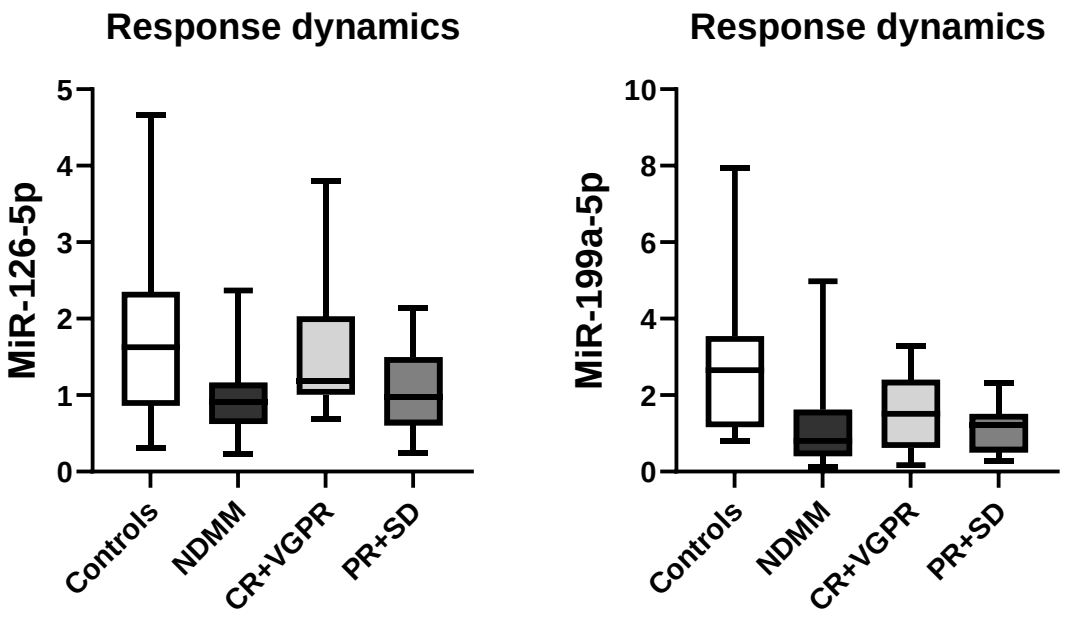


Figure 29: Analysis of miR-199a-5p, miR-126-5p, miR-497-5p, and miR-214-3p levels in healthy controls, untreated patients, those with CR+VGPR, and those with PR+SD

5.2.6. Diagnostic value of miR-199a-5p, miR-126-5p, miR-497-5p, miR-214-3p, miR-373-3p as biomarkers

A ROC (Receiver Operating Characteristic) analysis was conducted to assess the diagnostic potential of the studied biomarkers (Table 13).

Table 13: Results of the ROC Analysis of the Studied miRs as Biomarkers for Distinguishing Multiple Myeloma Patients from Healthy Controls

	AUC, p	Cut-off	Sensitivity % (CI)	Specificity % (CI)
miR-214-3p	0,5343, p=0,7323	0.4435	18.52 (10.38-30.84)	100.00 (72.25-100.00)
miR-497-5p	0,6254, p=0,1924	0.9405	50.00 (37.11-62.89)	81.82 (52.30-96.77)
miR-126-5p	**0,7359, p=0,0108**	1.1900	78.57 (66.18-87.29)	75.00 (66.18-87.29)
miR-199a-5p	**0,8325, p=0,0005**	2.2430	87.04 (75.58-93.58)	63.64 (35.38-84.83)
miR-373-3p	N/A	N/A	N/A	N/A

Among the four successfully analyzed miRs, miR-126-5p and miR-199a-5p demonstrate the most promising characteristics as diagnostic biomarkers—reflected in their high AUC values and significant p-values. miR-199a-5p exhibits the highest sensitivity, while miR-126-5p shows more balanced specificity and sensitivity. miR-214-3p and miR-497-5p have limited diagnostic value, lower AUC values, and a lack of statistical significance (Fig. 29).

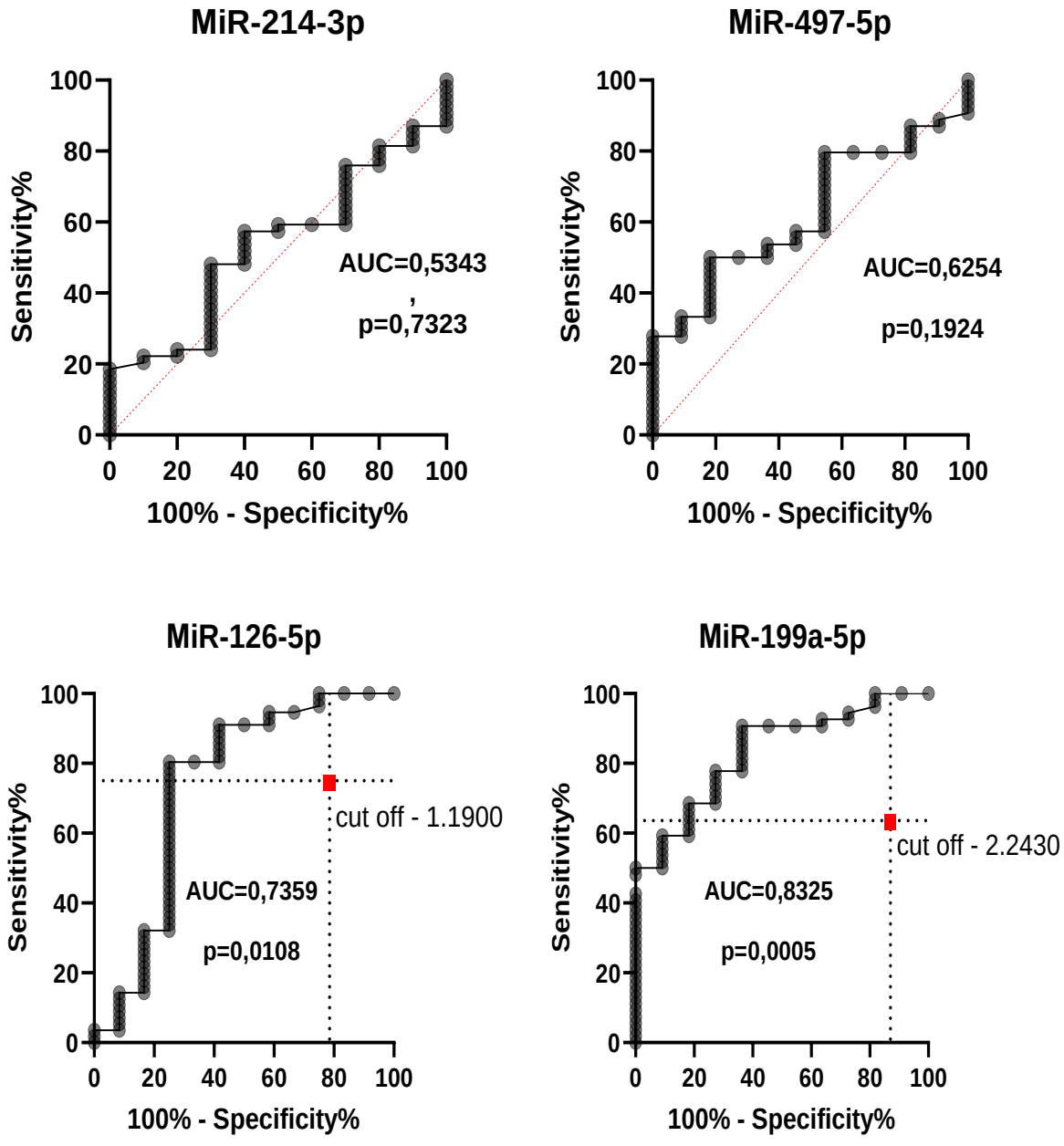


Figure 30: ROC Analysis of the Studied miRs as Biomarkers for Distinguishing Multiple Myeloma Patients from Healthy Controls

5.2.7. Overall survival (OS), Progression-free survival (PFS), and the predictive role of miR-199a-5p, miR-126-5p, miR-497-5p, miR-214-3p, miR-373-3p as biomarkers

5.2.7.1. Overall survival (OS) and progression-free survival (PFS) in the entire study population

The median OS for the entire group was not reached by the 38th month of follow-up. The large number of early deaths within the first 2 months after diagnosis is notable, with a moderate decline in survival over 9 months, after which a plateau is reached. Beyond the 16th month, no new deaths were recorded, and by the end of the follow-up period, 72.7% of patients remained alive (fig. 30).

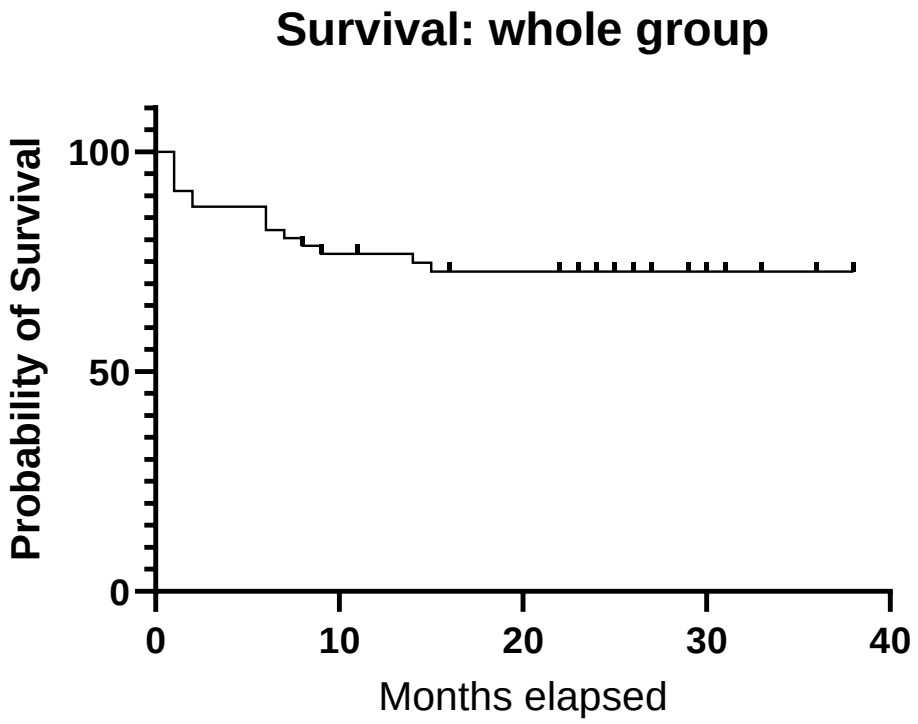


Figure 31: Kaplan-Meier curve of overall survival (OS)

With regard to PFS, the median was likewise not reached by the end of the follow-up period. A more pronounced decline in the curve was observed over the first 10 months, reaching 67.7%. After the 25th month, PFS stabilized with half the patients remaining event-free (Fig. 31).

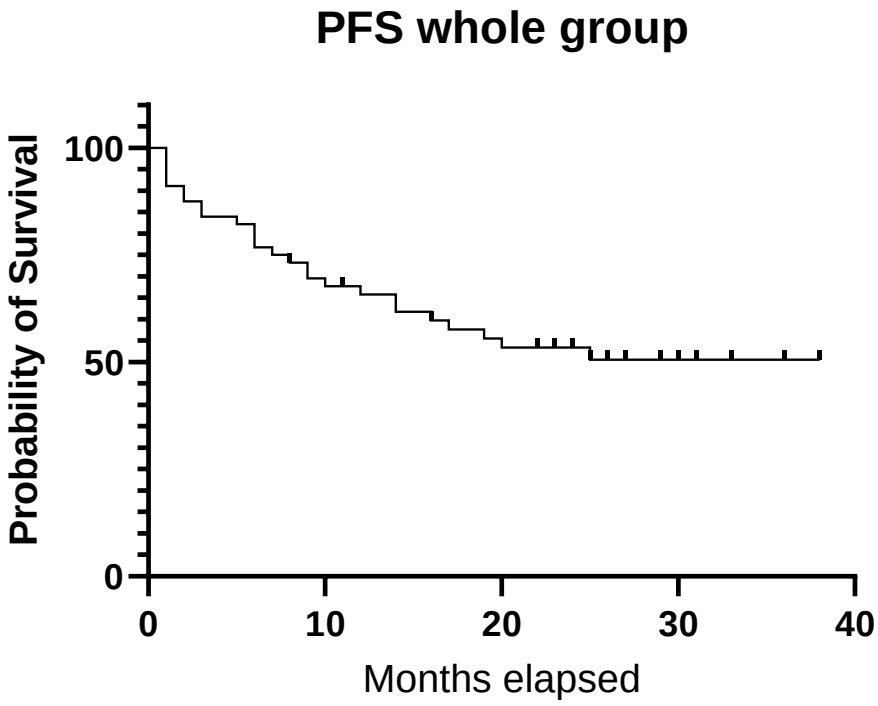


Figure 32: Kaplan-Meier curve of progression-free survival (PFS)

5.2.7.2. Overall Survival (OS) and Progression-Free Survival (PFS) According to ISS

A significant difference (p=0.0134) was detected when comparing OS directly across patient subgroups stratified by ISS. During the follow-up period, there were no deaths among patients in ISS I, compared to 2 in ISS II and 11 in ISS III. The median was not reached in any subgroup (Fig. 32).

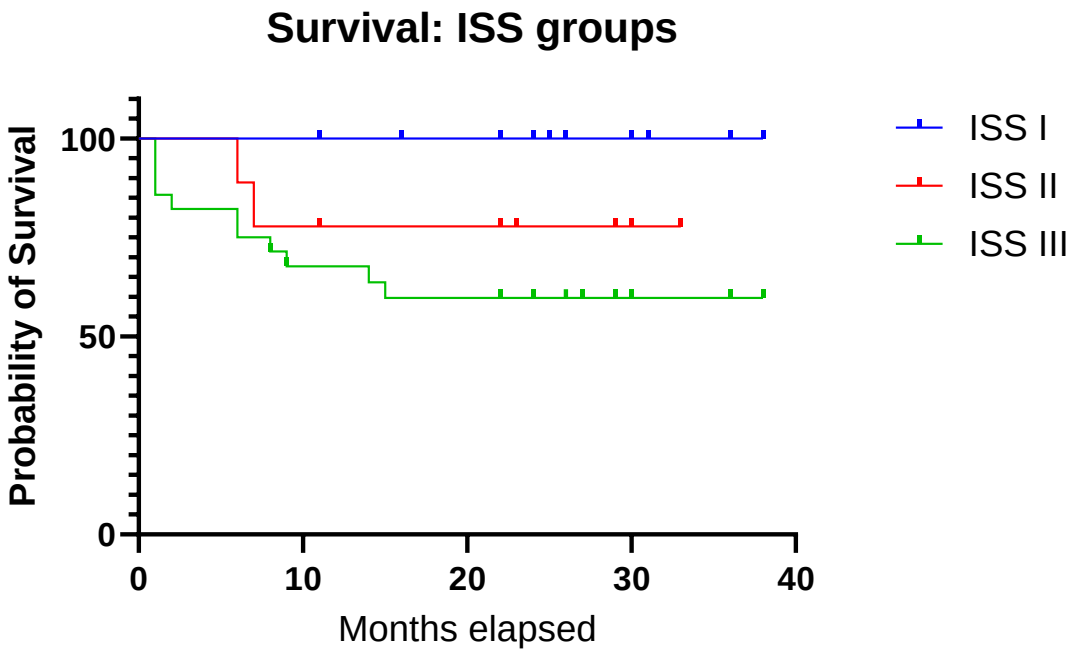


Figure 33: Survival analysis by ISS

A similar pattern was observed in the PFS curve analysis (p=0.0005). There were no deaths among ISS I patients, while 2 patients in ISS II and 11 in ISS III died during the follow-up period. Only the high-risk group exhibited a median PFS of 11 months (Fig. 33).

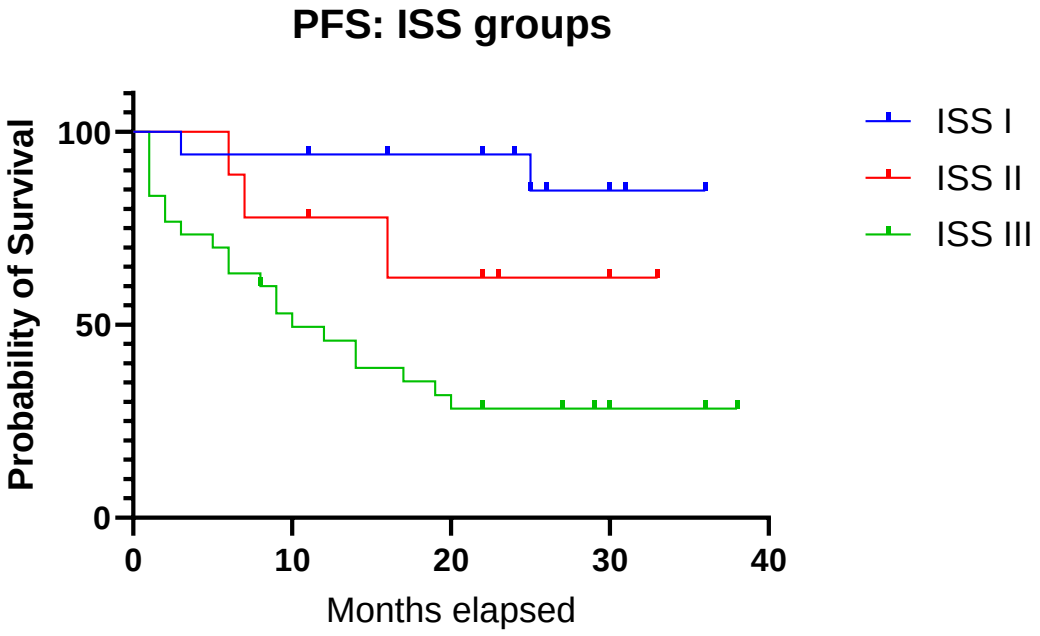


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Figure 34: PFS Analysis by ISS

5.2.7.3. Predictive Role of miR-199a-5p, miR-126-5p, miR-497-5p, miR-214-3p, miR-373-3p as biomarkers for overall survival (OS) and progression-free survival (PFS)

To assess the individual roles of miR-199a-5p, miR-126-5p, miR-497-5p, miR-214-3p, and miR-373-3p in OS and PFS, a Cox regression analysis was performed (Tables 14 and 15).

Table 14: Cox Regression Analysis for miR-199a-5p, miR-126-5p, miR-497-5p, miR-214-3p, miR-373-3p (OS)

	β coefficient	CI β коеф.	Hazard Ratio	CI HR
miR-214-3p	0.27	**0.03 до 0.50**	1.31	**1.03 до 1.65**
miR-497-5p	0.95	**0.19 до 1.66**	2.57	**1.22 до 5.25**
miR-126-5p	- 1.16	- 3.33 до 0.76	0.31	0.04 до 2.13
miR-199a-5p	- 0.26	- 1.26 до 0.53	0.77	0.28 до 1.70
miR-373-3p	N/A	N/A	N/A	N/A

For OS, miR-214-3p and miR-497-5p emerged as statistically significant potential predictive markers for overall survival and risk of early mortality. Each unit increase in miR-214-3p was associated with a 31% increase in the risk of death. The results for miR-497-5p were even more pronounced, as higher levels were linked to more than a twofold increase in HR. miR-126-5p and miR-199a-5p did not demonstrate significant results, though a protective role was hinted at for miR-126-5p.

Table 15: Cox Regression Analysis for miR-199a-5p, miR-126-5p, miR-497-5p, miR-214-3p, miR-373-3p (PFS)

	β coefficient	CI β коеф.	Hazard Ratio	CI HR
miR-214-3p	0.32	**0.06 до 0.61**	1.38	**1.07 до 1.84**
miR-497-5p	0.99	**0.24 до 1.72**	2.7	**1.28 до 5.60**
miR-126-5p	- 1.21	- 3.33 до 0.64	0.3	0.04 до 1.90
miR-199a-5p	- 0.3	- 1.28 до 0.48	0.74	0.28 до 1.62
miR-373-3p	N/A	N/A	N/A	N/A

A similar pattern was observed for PFS, again highlighting miR-214-3p and miR-497-5p as statistically significant predictive biomarkers (Table 15). Elevated miR-214-3p expression implies up to a 38.1% higher risk of disease progression. Higher miR-497-5p levels are likewise associated with over a twofold increase in the risk of progression. miR-199a-5p and miR-126-5p did not reach the threshold for statistical significance, though miR-126-5p again showed a potential protective function.

6. Discussion

6. 1. Introduction

Multiple myeloma (MM) is a heterogeneous malignant hematologic disease with a complex etiology, characterized by clonal proliferation of malignant plasma cells in the bone marrow. Despite advances in therapeutic strategies, MM remains incurable, and patients often experience multiple relapses due to the development of drug resistance [Rajkumar SV et al. 2024] The identification of reliable biomarkers for early diagnosis, prognosis, and monitoring of treatment response is crucial for improving patient outcomes. In this study, we investigated the expression levels of the specific circulating miRs: miR-126-5p, miR-199a-5p, miR-214-3p, miR-497-5p, and miR-373-3p in newly diagnosed MM patients, both before treatment and after six months of therapy, comparing them with healthy controls. Our findings provide information about the potential role of these miRs as diagnostic and prognostic biomarkers in MM, as well as their association with clinical parameters and treatment response.

6.2. Decrease in miR-126-5p and miR-199a-5p in patients with MM

The present results found that miR-126-5p and miR-199a-5p were significantly decreased in newly diagnosed MM patients compared to healthy controls. This outcome aligns with several studies underscoring the tumor-suppressive roles of these miRs in MM and other diseases [Zhao Y et al. 2020, Wei D et al. 2019, Liu X et al. 2018]. Research on miR-126 shows that it is a key regulator of signaling pathways related to the epidermal growth factor receptor (EGFR), as well as angiogenesis and adhesion [Zhao Y et al. 2020]. In a range of solid tumors, including hepatocellular carcinoma (HCC) [Zailaie SA et al. 2022] and breast cancer [Msheik ZS et al. 2022], low miR-126 levels correlate with more aggressive behavior and reduced patient survival. When its expression is restored (via agonist application), there is a distinct suppression of cell proliferation, angiogenesis, and metastasis [Du C et al. 2014]. Thus, miR-126 acts as a tumor suppressor, blocking pro-oncogenic signals, suggesting that its therapeutic

restoration might limit tumor progression, including in multiple myeloma (MM) (Zhao Y. et al. 2020).

In turn, miR-199a also stands out as a potent tumor suppressor in various malignant diseases, such as oral squamous cell carcinoma (OSCC) and ovarian carcinoma [Wei D et al. 2019]. In these cases, miR-199a is significantly reduced compared to normal tissues; when its levels are restored, tumor cells lose their capacity for intensive proliferation and invasion, partly due to the blockade of NF-κB–mediated signaling pathways (for example, via direct regulation of IKKβ or NF-κB1) [Markopoulos GS et al. 2018]. Furthermore, miR-199a reduces the expression of matrix metalloproteinases (MMP-2, MMP-9) [Lou Z et al. 2018], which are key in the metastatic process. These observations confirm that miR-199a directly suppresses critical factors in tumor growth and metastasis, making it a potential target for molecularly targeted therapy [Wei D. Et al. 2019], [Liu X. et al. 2018].

6.2.1. MiR-126-5p as a tumor suppressor agent

In our patient cohort, we found that miR-126 concentration decreases in cases with more advanced clinical stage, greater tumor burden, and markers associated with poorer prognosis and aggressive disease. This is consistent with previous studies emphasizing the prognostic significance of miR-126 in various neoplasms. For instance, in clear cell renal cell carcinoma (ccRCC), low miR-126 expression is associated with a higher tumor grade and poorer survival [Vergho D et al. 2014, White NM et al. 2011]. Similar observations in non-small cell lung cancer and colorectal cancer (CRC) indicate that reduced miR-126 levels are closely linked to disease progression, metastasis, and lower overall survival [Jusufović E et al. 2012, Liu Y et al. 2014, Zhang Y et al. 2013].

The functional activity of miR-126 is known to target key molecules involved in angiogenesis, proliferation, and the tumor microenvironment. In the context of MM—where progression is tightly linked to the bone marrow microenvironment and neovascularization—its documented angiogenic and growth-regulatory properties could explain the reported findings. Numerous studies have shown that miR-126 modulates the VEGF/VEGFR axis, PI3K/AKT signaling, and other critical pathways [Kong R et al. 2016, Jusufović E et al. 2012]. Our data, indicating lower miR-126 levels in more aggressive cases, align with experiments in renal carcinoma and melanoma, where restoration of miR-126 suppresses cell proliferation and migration [Li N et al. 2013, Felli N et al. 2013]. Although these mechanisms are less explored

in MM, the parallels suggest that miR-126 may inhibit growth and signaling pathways in MM as well, thereby limiting disease aggressiveness.

Additionally, miR-126 influences immune regulation and stromal interactions, which are known to be crucial in MM. Similar miR-126 alterations have been associated with immune imbalances in acquired aplastic anemia and myelodysplastic syndromes, where miR-126 levels reflect disease severity [Giudice V et al. 2011]. By analogy, in the MM microenvironment, immune regulation and vascular growth may be partially shaped by miR-126–dependent changes, including cytokine signaling, endothelial integrity, and adhesion molecules.

6.2.2. The protective role of miR-199a-5p

In the present study, among the examined miRs, miR-199a-5p stands out as particularly significant. We found significantly lower levels of miR-199a-5p in patients with newly diagnosed MM (NDMM) compared to healthy controls. This aligns with previous reports linking miR-199a-5p to tumor-suppressive functions in various solid and hematological malignancies [Byrnes KA et al. 2016, Yu X et al. 2022].

MiR-199a-5p has been shown to influence numerous neoplasm-associated mechanisms by directly targeting genes involved in cell proliferation, survival, and angiogenesis [Wang L et al. 2020, Wang SH et al. 2014]. One of its validated targets in other tumor models is mitogen-activated protein kinase 11 (MAP3K11), known as MLK3—a key element in MAP kinase signaling [Song G et al. 2010]. In several solid tumors, low levels of miR-199a-5p correlate with increased expression of MAP3K11, which, in turn, promotes proliferation and invasion [Hong, S.A et al. 2023]. Although the present study did not directly evaluate MAP3K11 in MM, extrapolations from other malignancies suggest a potential mechanism by which reduced miR-199a-5p contributes to MM pathogenesis, allowing for uncontrolled MAP kinase signaling.

Additionally, miR-199a-5p is associated with modulation of various oncogenic pathways, including mTOR and NF-κB, in other neoplastic processes [Callegari E et al. 2018, Wu C et al. 2016]. Its decreased expression in NDMM indicates that it plays a fundamental role in malignant transformation. This aberration may pave the way for disease progression long before clinical symptoms appear.

6.3. Correlation with clinical parameters

In this study, a correlation analysis was conducted between five selected miRs and several routine laboratory parameters (hemoglobin, creatinine, beta-2 microglobulin, and

paraprotein levels). These clinically significant tests are regularly used in patients with multiple myeloma (MM) and reflect key aspects of hematological and functional status—including bone marrow infiltration, the degree of anemia, and kidney involvement. The main objective of their inclusion in the analysis was to identify possible relationships between the levels of the examined miRs and the clinical manifestations of myeloma disease.

The analysis found that reduced levels of miR-126-5p are closely associated with higher values of beta-2 microglobulin—a marker widely used in clinical practice to assess disease severity and renal dysfunction in MM. This finding suggests that deficient miR-126-5p expression reflects a more advanced disease course and a higher tumor burden, as elevated beta-2 microglobulin levels generally correlate with more pronounced bone marrow infiltration and/or worsened renal function. In the context of personalized medicine, miR-126-5p could be investigated as an additional prognostic indicator to aid more precise patient staging, especially when combined with other criteria such as ISS or R-ISS.

Regarding hemoglobin, its correlation with miR-126-5p ($r=0.2765$; $p=0.1255$), although indicative of a trend toward a positive relationship, did not reach statistical significance ($p>0.05$). The pro-apoptotic function of miR-126 is also addressed by [Fourdinier, O et al. 2019], which found an independent positive association between this miR and hemoglobin levels in patients with chronic kidney disease.

Within the context of chronic hematological disorders in MM and the data from Fourdinier's publication, it can be hypothesized that the reduction in miR-126-5p plays a role in the pathogenesis of anemia, but this effect is likely influenced by other factors (e.g., erythropoietin production, bone marrow infiltration, inflammatory processes).

As for creatinine values ($p=0.1626$; $r=–0.2529$), they also did not achieve statistical significance, but the negative correlation observed mirrors the trend in Fourdinier's study, in which lower miR-126 levels are often associated with worsened renal function and a lower eGFR. Although renal function assessment in MM is multifaceted (including proteinuria, beta-2 microglobulin, etc.), this tendency toward a negative relationship supports the hypothesis that decreased miR-126-5p reflects or exacerbates kidney problems, or simultaneously signals disease progression.

Moreover, higher miR-199a-5p levels exhibit a positive correlation with better hematological parameters, particularly higher hemoglobin values, and an inverse correlation with beta-2 microglobulin. Anemia is one of the most common clinical manifestations of

multiple myeloma, resulting from bone marrow infiltration by malignant plasma cells and the subsequent suppression of normal erythropoiesis. Therefore, the positive association between miR-199a-5p and hemoglobin suggests that higher expression of this miR could indicate less extensive bone marrow infiltration and a better-preserved hematopoietic function. Thus, miR-199a-5p may prove to be a valuable biomarker, providing additional insights into the patient's condition and the potential severity of the disease.

In summary, the data from this study support the potential of miR-126-5p and miR-199a-5p as biomarkers for risk stratification and therapeutic response assessment in newly diagnosed multiple myeloma patients. Their expression levels can provide an additional layer of information on top of routinely used clinical parameters (such as serum calcium, LDH, hemoglobin, beta-2 microglobulin, ISS stage), thus enabling a more precise evaluation of patient status. This is particularly important in the context of an individualized therapeutic approach, where the early identification of patients with a poorer prognosis could lead to modifications in the choice or intensity of treatment. The available literature data on the role of these miRs are still limited, especially in national studies, which underscores the need for more extensive and in-depth research aimed at validating and implementing miR-based biomarkers in clinical practice.

6.4. Changes in miR levels after treatment

After six months of treatment, patients who achieved a complete response (CR) or very good partial response (VGPR) showed a significant increase in miR-126-5p levels, approaching those of the healthy controls. This finding suggests that effective therapy can restore miR-126-5p expression, potentially disrupting the pro-angiogenic environment and slowing further progression. At present, MM risk stratification primarily relies on cytogenetic abnormalities, serum markers, and imaging studies. Including miR-126 in prognostic models may offer a new molecular level of stratification. As in colorectal cancer and clear cell renal carcinoma [Selven H et al. 2021, Khella HW et al. 2015], where low miR-126 levels are associated with reduced survival and are considered part of integrative prognostic models, miR-126 in MM could similarly sharpen risk accuracy and aid in clinical decision-making for more individualized therapies.

Patients with a partial response (PR) or stable disease (SD) did not show significant changes in miR-126-5p levels, indicating that miR-126-5p expression correlates with treatment

effectiveness. This result suggests that miR-126-5p could serve as a biomarker for monitoring therapeutic response and dynamically assessing tumor burden.

Interestingly, miR-199a-5p levels did not change substantially in our cohort after treatment, nor did they strongly correlate with response categories such as CR/VGPR vs. PR/SD. This implies that although miR-199a-5p is significant for establishing or maintaining the malignant phenotype at diagnosis, its role as a dynamic marker for short-term therapeutic response is not confirmed. Such a more static role at diagnosis, rather than a fluctuating biomarker during treatment, is not without precedent; certain genetic and epigenetic changes reflect the baseline biology of the disease rather than therapy-induced shifts.

Nevertheless, among the patients who reached CR or VGPR, there was a tendency toward higher miR-199a-5p levels. Based on this, one may infer that miR-199a-5p also responds to effective treatment, although to a lesser degree than miR-126-5p. The lack of statistical significance may be due to the small sample size or the need for a longer follow-up period to observe meaningful changes.

6.5. MiR-214-3p and miR-497-5p as prognostic biomarkers

MiR-214-3p and miR-497-5p did not show significant differences between MM patients and healthy controls at diagnosis. However, higher levels of these miRs were associated with worse overall survival (OS) and progression-free survival (PFS), suggesting their potential role as prognostic biomarkers.

6.5.1. MiR-214-3p in disease progression

MiR-214-3p is implicated in promoting disease progression by targeting PTEN, leading to activation of the PI3K/AKT pathway, which enhances cell survival, proliferation, and resistance to apoptosis (Liu J et al. 2017). The association of elevated miR-214-3p levels with worse outcomes in our study supports its potential role as a prognostic biomarker in MM. Higher miR-214-3p levels may contribute to disease aggressiveness and therapy resistance. In their study, Misiewicz-Krzeminska I et al. reported similar results, demonstrating that restoring miR-214 in myeloma cell cultures led to reduced proliferation and induced apoptosis.

6.5.2. MiR-497-5p and its complex manifestation

MiR-497-5p has been reported to act as a tumor suppressor in various diseases by targeting genes involved in cell cycle regulation and apoptosis, such as B-cell lymphoma 2

(BCL2) and cyclin D2 (CCND2) (Wu R et al. 2016). However, our findings suggest that higher levels of miR-497-5p may be associated with unfavorable outcomes in MM. This discrepancy might be due to the complex regulatory interactions in MM, where miR-497-5p could have context-dependent functions, potentially acting as an oncogene under certain cellular conditions.

New research indicates that miR-497-5p may be involved in the regulation of angiogenesis and can have a dual role depending on the disease type and microenvironment [Luo G. 2021]. In MM, elevated miR-497-5p levels might contribute to pathological angiogenesis or disrupt apoptotic pathways in a way that promotes the survival of malignant cells.

6.6. Lack of significant findings for miR-373-3p

MiR-373-3p was undetectable or present at very low levels in most of our samples, limiting the possibility of deriving meaningful statistical data. Although miR-373-3p has been reported to exhibit both suppressive and oncogenic properties in various diseases [Wei F et al. 2015, Langroudi L et al. 2015], its role in MM remains unclear. The low detection levels in our study could be due to technical constraints, such as the sensitivity of detection methods, or might reflect a minimal involvement of miR-373-3p in MM pathophysiology. Further research with more sensitive detection methods or analysis of larger cohorts could help clarify the potential role of miR-373-3p in MM.

6.7. Diagnostic potential of miR-126-5p and miR-199a-5p

Analysis of receiver operating characteristic (ROC) curves demonstrated that miR-126-5p and miR-199a-5p show potential as diagnostic biomarkers for MM. miR-199a-5p exhibited high sensitivity (87.04%) but moderate specificity (63.64%), making it suitable for screening purposes aimed at minimizing false negatives. In a disease often requiring invasive bone marrow examinations and complex imaging methods for diagnosis, a miR-based serum biomarker could simplify early detection and potentially identify patients at higher risk of rapid progression. Although miR alone does not replace established diagnostic algorithms, its integration with serum free light chain analysis, β2-microglobulin, and protein electrophoresis could enhance diagnostic specificity and sensitivity.

A similar role for miR-199a as a diagnostic biomarker has been described by Zhang W et al. in the context of patients with non-small cell lung cancer [Zhang W et al. 2022], though to date there are no comparable studies in the area of multiple myeloma.

MiR-126-5p showed balanced sensitivity (78.57%) and specificity (75%), indicating good diagnostic accuracy. This balance suggests that miR-126-5p may be a reliable marker for distinguishing MM patients from healthy individuals. Including miR-126-5p measurement in diagnostic protocols could reduce the need for invasive procedures, such as bone marrow biopsy, and facilitate early detection.

These miR may complement existing diagnostic methods, such as serum protein electrophoresis, immunofixation, and free light chain assays, providing a non-invasive and accessible way to detect MM.

6.8. Therapeutic potential of miR expression modulation

The therapeutic potential of modulating miR expression in MM is an exciting prospect. Restoring the levels of tumor-suppressive miRs, such as miR-126-5p and miR-199a-5p, may inhibit disease growth and angiogenesis, as well as increase sensitivity to chemotherapy.

6.8.1. Restoration of miR-126-5p

Therapeutic strategies aimed at increasing miR-126-5p levels can suppress the expression of VEGFA, reducing angiogenesis and disease vascularization. Moreover, restoring miR-126-5p levels may enhance the effectiveness of existing treatments by increasing the sensitivity of MM cells to chemotherapeutic agents.

6.8.2. Restoration of miR-199a-5p

Similarly, restoring miR-199a-5p levels can suppress key factors such as HIF-1α and mTOR, reducing cell proliferation and inducing apoptosis. This may also overcome drug resistance, as miR-199a-5p has been shown to enhance the sensitivity of MM cells to proteasome inhibitors like bortezomib [Robak P et al. 2020].

6.8.3. Targeting oncogenic miRs

Conversely, inhibiting oncogenic miRs such as miR-214-3p can suppress anti-apoptotic pathways and overcome drug resistance. Antisense oligonucleotides or miR sponges can be used to inhibit miR-214-3p, potentially restoring PTEN function and inhibiting the PI3K/AKT pathway [Wang W et al. 2014].

6.8.4. Challenges and outlook

Developing effective delivery systems for miR mimics or inhibitors remains a significant challenge. The delivery mechanism must protect miRs from degradation, direct them specifically to MM cells, and minimize immune responses. Advances in nanotechnology—such as lipid nanoparticles, exosomes, and viral vectors—may facilitate the clinical application of miR-based therapies.

In addition, potential off-target effects and toxicity associated with miR modulation must be carefully evaluated through preclinical and clinical studies.

6.9. Study limitations

Our study has several limitations:

Sample size and single-center design: The relatively small sample size, particularly for patients who reached the six-month follow-up, may limit the generalizability of our findings and reduce statistical power. Larger cohorts from multiple centers are necessary to validate the current results and ensure their applicability to diverse patient populations.

Technical challenges: The low detection levels of miR-373-3p highlight the need for standardized protocols and more sensitive miR detection methods. Variations in RNA extraction, reverse transcription, and quantification techniques can affect miR measurements and comparability across studies.

Lack of functional validation: Although we observed associations between miR levels and clinical parameters, we did not perform functional studies to elucidate the mechanisms through which these miRs influence MM pathogenesis. Functional validation through in vitro and in vivo models would strengthen our understanding of their roles and therapeutic potential.

MM heterogeneity: MM is a highly heterogeneous disease with various genetic and molecular subtypes. Our study did not stratify patients based on specific cytogenetic abnormalities or molecular profiles, which can influence miR expression patterns. Future studies should consider such stratifications to explore miR associations according to MM subtypes.

6.10. Future directions

Future research should focus on:

Validation of findings in larger cohorts: Multicenter studies with larger patient populations will enhance the statistical reliability and general applicability of the results.

Long-term studies: Extended follow-up periods could provide insights into the long-term prognostic value of these miRs and their potential for predicting relapse or therapy resistance.

Functional studies: Investigating the molecular mechanisms by which these miRs influence MM pathogenesis is crucial. In vitro and in vivo studies can clarify their targets, relevant signaling pathways, and interactions with other molecular factors.

Integration with other biomarkers: Combining miR profiling with other molecular markers—such as genetic mutations, epigenetic changes, and protein biomarkers—may improve diagnostic and prognostic models, leading to more personalized treatment approaches.

Development of miR-based therapies: Preclinical studies evaluating the efficacy, safety, and delivery methods of miR mimics or inhibitors in MM models are required. Subsequent clinical trials could then assess their therapeutic potential in patients.

6.11. Conclusion conclusion

In conclusion, our study adds to the growing body of evidence supporting the role of circulating miRs as biomarkers in MM. The significant reduction of miR-126-5p and miR-199a-5p in MM patients, along with their potential as diagnostic markers and indicators of treatment response, underscores their clinical importance. The normalization of miR-126-5p levels after effective therapy further confirms its potential as a biomarker for monitoring therapeutic efficacy.

The association of elevated miR-214-3p and miR-497-5p levels with poorer outcomes suggests their possible utilization for prognosis and risk stratification. These miRs may aid in identifying patients at higher risk of disease progression who could benefit from more aggressive or alternative therapeutic strategies.

Our findings reinforce the significance of miRs in MM pathogenesis—well-documented in the international literature—and their potential as targets for therapeutic intervention. Further research is needed to validate these findings, elucidate the underlying mechanisms, and explore the development of miR-based therapies for improving MM management and patient outcomes.

6.12. Key findings

1. Significantly higher values of miR-126 and miR-199a were found in multiple myeloma patients compared to healthy controls.
2. A significant inverse relationship was demonstrated between the levels of miR-126-5p and miR-199a and beta-2 microglobulin.
3. A moderate positive correlation was observed between miR-199a-5p expression levels and hemoglobin.
4. Significantly higher miR-126-5p levels were recorded in patients who achieved (CR+VGPR) after treatment compared to untreated patients, a result that approaches the values of the control group.
5. MiR-126-5p and miR-199a-5p showed significant characteristics as diagnostic biomarkers in ROC analyses.
6. Elevated levels of miR-214-3p and miR-497-5p are associated with a higher risk of disease progression and early mortality.

7. Contributions

7.1. Original contributions

1. For the first time, a study was conducted on plasma levels of miR-126-5p, miR-199a-5p, miR-214-3p, miR-497-5p, and miR-373-3p in a large cohort of patients with a rare hematological neoplasm such as MM.
2. For the first time, the relationship of miR-126-5p, miR-199a-5p, miR-214-3p, miR-497-5p, and miR-373-3p with various clinical-laboratory and molecular genetic factors in newly diagnosed multiple myeloma patients was examined.
3. For the first time, the dynamics of serum levels of miR-126-5p, miR-199a-5p, miR-214-3p, miR-497-5p, and miR-373-3p during therapy was investigated, and their dependence on treatment response was analyzed.
4. For the first time in Bulgaria, the diagnostic role of miR-126-5p, miR-199a-5p, miR-214-3p, miR-497-5p, and miR-373-3p in MM patients was studied.
5. For the first time in Bulgaria, the predictive role of miR-126-5p, miR-199a-5p, miR-214-3p, miR-497-5p, and miR-373-3p for disease progression and early mortality in MM patients was evaluated.

7.2. Contributions with applied scientific value

1. MiR-126-5p, miR-199a-5p, miR-214-3p, miR-497-5p, and miR-373-3p could be integrated into the diagnostic algorithm and risk stratification of newly diagnosed MM patients.
2. MiR-126-5p, miR-199a-5p, miR-214-3p, miR-497-5p, and miR-373-3p could be used to evaluate the stage and progression of MM.
3. MiR-126-5p, miR-199a-5p, miR-214-3p, miR-497-5p, and miR-373-3p could serve as additional parameters for assessing the response of NDMM patients to applied therapy.
4. The results of this dissertation could serve as a starting point for the development of new targeted drugs.

8. Scientific publications on the topic

1. БИОМАРКЕРИ И ПЕРСОНАЛИЗИРАН ПОДХОД ПРИ ДИАГНОСТИКАТА И ЛЕЧЕНИЕТО НА МНОЖЕСТВЕНИЯ МИЕЛОМ Герчева Л. и Луканов Р ISSN 23677864 Hematology (Bulgaria) Volume 56, Issue 1, Pages 18 – 25 2020
2. CONVENTIONAL CYTOGENETIC ANALYSIS IN PATIENTS WITH MULTIPLE MYELOMA – SINGLE CENTER STUDY Author(s): Radi Lukanov, Stela Dimitrova, Valentina Miteva, Ilina Micheva EHA Library. Lukanov R. 06/09/21; 324359; PB1686
3. MiR-199 and miR-126 as potential biomarkers for newly diagnosed patients with multiple myeloma: Lukanov Radi, Chervenkov Trifon, Grudeva-Popova Zhanet, Micheva Ilina and Teneva Maria; American Journal of Hematology Volume 99, Issue S2 Supplement: Abstracts from the 2024 Lymphoma Leukemia and Myeloma Congress October 16-19, 2024 https://doi.org/10.1002/ajh.27491