Medical University –Varna
„Prof. Dr. Paraskev Stoyanov"
Faculty of Medicine
DEPARTMENT OF INTERNAL DISEASES II

MERLIN EROL EFRAIM

CLINICAL-BIOLOGICAL AND GENETIC MARKERS IN RISK STRATIFICATION IN PATIENTS WITH MYELODYSPLASTIC SYNDROME

ABSTRACT

of a dissertation for the award of scientific
and educational degree "Doctor"

Research supervisor:
Assoc. Prof. Dr. Ilina Dimitrova Micheva, Ph.D.

Official reviewers:
Prof. Dr. Valeria Kaleva, Ph.D.
Prof. Dr. Julian Raynov, Ph.D.

Varna, 2021

The dissertation is presented in a volume of 211 pages and contains 28 tables and 82 figures. References include 243 titles.

The dissertation has been discussed and proposed for defence by the Department Council of the Department of Internal Medicine II of the Medical University "Prof. Dr. P. Stoyanov" – Varna and is directed for public defence in front of a scientific jury in the following composition:

Prof. Dr. Valeria Kaleva, Ph.D.

Prof. Dr. Julian Raynov, Ph.D.

Prof. Dr. Lyudmila Angelova, MD

Prof. Dr. Georgi Balatsenko, Ph.D.

Assoc. Prof. Dr. Veselina Goranova-Marinova, Ph.D.

The defense of the dissertation will take place on ..... 2021 from ..... hours in hall ..... at an open meeting of the Scientific Jury.

The materials on the defence are available in the library of the Medical University "Prof. Dr. P. Stoyanov" – Varna and in electronic format on the website of MU – Varna.

Contents

ABBREVIATIONS

ACE-27	Adult co-morbidity evaluation-27
ALC	Absolute lymphocyte count
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
B2MG	Beta-2 microglobulin
BM	Bone marrow
CCI	Charlson comorbidity index
CFS	Clinical frailty scale
CMML	Chronic myelomonocytic leukemia
ECOG	Eastern Cooperative Oncology Group
FAB	French-American-British classification
FLT3	Fms like tyrosine kinase
FLT3-ITD	FLT3-internal tandem duplication
FLT3-TKD	FLT3-tyrosine kinase domain
IPSS	International prognostic scoring system
IPSS-R	Revised international prognostic scoring system
IWCG	International working cooperative group
IWG-PM	International working group for the prognosis of MDS
JAK2	Janus kinase 2
LDH	Lactatdehydrogenase
MDAPSS	MD Anderson prognostic scoring system
MDS	Myelodysplastic syndrome
MDS-CI	MDS comorbidity index
MDS-EB	MDS with excess of blasts
MDS-RS-SLD	MDS-ring sideroblast – single line dysplasia
MDS-RS-MLD	MDS-ring sideroblast multilineage dysplasia
MDS-SLD	MDS – single line dysplasia
MDS-U	MDS-unclassifiable
MPV	Mean platelet volume
NGS	Next generation sequencing

OS	Overall survival
PB	Peripheral blood
RA	Refractory anemia
RAEB-t	Reafractory anemia with excess of blasts in transformation
RARS	Refractory anemia with ring sideroblasts
RCC	Refractory cytopenias in child
RCMD	Refractory cytopenias with multilineage dysplasia
RCMD-RS	Refractory cytopenias with multilineage dysplasia with ring sideroblasts
RCUD	Refractory cytopenias with unilineage dysplasia
RN	Refractory neutropenia
RS	Ring sideroblast
RT	Refractory trombocytopenia
TNF	Tumor-necrosis factor
TSG	Tumor suppressor gene
WHO	World health organization
WPSS	WHO based prognostic scoring system

INTRODUCTION

Myelodysplastic syndrome (MDS) is a heterogeneous group of clonal diseases of the pluripotent hematopoietic stem cell. It is most commonly observed in elderly patients and is characterized by a variable clinical course. The incidence increases with age, and frequency in men is more often than in women. About a quarter of patients progress to acute myeloid leukemia (AML) (*Germing U et al, 2013*). For decades, MDS has posed a number of challenges in terms of diagnosis, risk stratification, clinical course, and treatment. The heterogeneity of the disease leads to the need to create classification systems and scales for risk stratification. In 1982, the first French-American-British (FAB) classification was approved *(Bennett J M et al, 1982)*, which was later replaced by the updated World Health Organization (WHO) classification. The risk of progression and survival in patients with MDS is determined using prognostic scoring systems. Risk stratification is performed through the approved scales of the International Prognostic Scoring System (IPSS) *(Greenberg P et al, 1997)*, its revised version (IPSS-R) *(Greenberg P et al, 2012)* and the World Health Organization-based scoring system (WPSS) *(Malcovati L et al, 2007)*. The scales are based on prognostic factors, including mainly disease characteristics – degree and number of cytopenias, percentage of bone marrow myeloblasts (BM) and cytogenetic profile. Other risk assessment models have been proposed, such as MD Andersen Prognostic Scoring System (MDAPSS), which include some patient-related factors, such as age and general condition. However, currently established risk assessment systems do not include prognostic factors related to the patient such as general health, the presence of comorbidities and their degree of manifestation. Comorbidities may precede MDS or occur during treatment without being an adverse event*(Feinstein A R et al, 1970)*. Three independent factors that are not included in risk stratification systems can be potentially decisive in determining the therapeutic approach and patient survival – "frailty" index, comorbidities and quality of life *(Piccirillo J F et al, 2004)*.

It is important to study and analyse the clinical and biological characteristics of the disease beyond those proven in the established classifications and scales for risk stratification and to evaluate their application in clinical practice in patients with MDS.

I. LITERATURE REVIEW

1. Myelodysplastic syndrome – general characteristics

MDS is a heterogeneous group of hematopoietic stem cell clonal diseases characterized by ineffective hematopoiesis, cytopenia, and an increased risk of transformation into AML. Progression in acute lymphoblastic leukemia (ALL) is less common *(Tefferi A et al, 2009; Arber D A et al, 2016; Sato N et al, 2004; Steensma D P 2015)*. The combination of peripheral cytopenia on the background of hypercellular BM is a major indication of MDS. *(Kerbauy D B & Deeg H J 2007; Emmanuel C Besa et al, 2020)*. MDS are divided into primary (de novo) and secondary.

MDS reports date back to the early 20th century *(Nageli O, 1913)*, with the first cases reported in the 1970s. *(Saarni M I et al, 1973)*. The terminology of the disease is extremely colorful in chronological order. MDS are referred to as "leukanemia" in 1900, "preleukemia" in 1953 to "oligoblastic leukemia", "dormant acute leukemia" in 1963. *(Hellström-Lindberg E et al, 2020)*.

MDS is a haematological neoplasia of the elderly. The average incidence is 4.8 / 100,000 people per year. The incidence varies from 0.2 / 100,000 people under the age of 40, increases to 29.6 / 100,000 people aged 70–79 and reaches 55.8 / 100,000 people over the age of 80. In men the incidence is more often than in women *(National cancer Institute SEER cancer statistics review, 2016)*.

2. Pathogenesis of MDS

The pathogenetic mechanisms remain unclear. The development of the disease is a multi-stage process. Biology is based on a number of cytogenetic and epigenetic violations, DNA methylation violations, apoptosis abnormalities and immune dysfunction. These factors lead to dysregulation of the pluripotent hematopoietic stem cell with the development of a pathological clones with signs of dysplasia and impaired function. As a result, the disease is demonstrated by ineffective hematopoiesis, distur-

bances in differentiation and genomic instability.

Apoptosis is a basic event in the early stages. An important pathogenetic mechanism in MDS is a premature intramedullary cell death resulting from increased apoptosis (*Kerbauy D B & Deeg H J,2007*). It explains the hypercellularity of BM and peripheral cytopenia. Secretion of apoptotic agents such as tumor necrosis factor (TNF), bone marrow stromal defect, and relative deficiency of hematopoietic growth factors lead to premature apoptosis in BM (*Fontenay M & Gyan E, 2008*). Disease progression is associated with a decreased immune response, loss of tumor suppressor activity, generation of cytogenetic mutations, and leukemic transformation. (*Mohammad A A, 2018; Deeg H J et al.,2000*).

In recent decades, our knowledge of cytogenetic disorders has gradually been enriched. Cytogenetic aberrations are found in 30–50% of newly diagnosed patients and 80% in secondary MDS *(Kawankar N et al, 2011).* Disturbances of the genome may involve regions containing tumor suppressor genes (TSGs) associated with the biology of the disease**,** and their discovery is extremely important *(Sole F et al, 2000; Le Beau M M et al, 1986*). Through the development of next generation sequencing (NGS) technology, our knowledge of the disease pathogenesis has significantly impoved. Complete mapping of the genome has been achieved. Recurrent mutations have the potential to participate in diagnosis, risk stratification, prognosis, and treatment response.

In the pathogenesis of the disease, the function of the immune system and the immunological imbalance is directed to T-lymphocytes. Authors have found that in low-risk patients there is an increased activity of cytotoxic T-lymphocytes, while in high-risk patients there is an increased function of regulatory T cells *(Kotsianidis I et al, 2009; Chamuleau M E et al, 2009).*

The microenvironment in BM in patients with MDS has abnormal morphological characteristics. Molecular disfunctions in stromal niche cells reveal changes involving abnormalities in stem cell differentiation and support functions *(Medyouf H et al, 2014).* It is not known whether changes in the bone marrow niche are initiating events or if they are induced by clonal cells. *(Kim Y W et al, 2008).*

3. Dysplastic changes and clinical manifestations of MDS

Dysplastic changes are the most characteristic diagnostic markers in MDS. In the analysed BM smears the presence of dysplastic changes is typical in over 10% of the analysed cells (*Parmentier S et al, 2012*). Important for the diagnosis is the presence of dysplastic changes in two or three lines of hematopoiesis, such as megaloblastoid erythropoiesis, asynchrony in the ratio of nucleus: cytoplasm in early myeloid and / or erythroid precursors and dysplastic megakaryocytes (*Kouides P A et al, 1996*).

The main characteristic changes in the erythroid line that are observed in peripheral blood (PB) smears are anisocytosis, poikilocytosis and the presence of basophilically punctured erythrocytes. In BM smears, dysplastic changes are manifested by the presence of ring sideroblasts, cytoplasmic inclusions, cytoplasmic connections, incomplete hemoglobinization, ciliated cytoplasm, vacuolation, the presence of multinucleated erythroblasts, irregular nuclear margins and megaloblastoidism. Ring sideroblasts are defined as erythroblasts with a minimum of 5 siderosomal granules covering at least 1/3 of the nucleus circumference (*Cazzola M et al, 2003; Mufti G J et al. 2008)*.

Regarding the myeloid line, the characteristic dysplastic changes in PB smears are the presence of neutrophils with hypolobular nuclei (pseudo Pelger-Huet), cytoplasmic hypogranulation and / or degranulation, hypergranular neutrophils and the presence of myeloblasts. The most common dysplastic changes in BM affecting the myeloid line are the presence of specific nuclear forms, nuclear hypersegmentation, hypolobulated nuclei, pseudo Chediak – Higashi granules, cytoplasmic hypogranulation / degranulation, and anisocytosis (*Goasguen J E et al, 2014*).

Typical dysplastic changes in the PB smears with respect to the megakaryocyte lineage are anisocytosis and giant platelets. Large monolobular megakaryocytes, small binuclear elements, scattered nuclei, micromegakaryocytes and degranulation are the characteristic changes observed in BM (*Invernizzi R et al, 2015*).

Dysplastic changes in BM affect the degree of cytopenias in PB.

The clinical course of the disease is non-specific and varies depending on the subtype and severity of cytopenias. A common manifestation of MDS is unexplained anemia and its subsequent symptoms of astheno – adynamia (*Foran J M, 2012*). Anemia in MDS is macrocytic with elevat-

ed values of erythrocyte distribution width (RDW) (Red Cell Distribution Width). They are most often followed by symptoms of thrombocytopenia with manifestations of mucocutaneous haemorrhagic diathesis. Fever, recurrent infections and shock are manifestations of neutropenia. A study by Moreno et al. found that 53% of newly diagnosed patients had anemic syndrome, 40% with thrombocytopenia and 20% with neutropenia with neutrophils below 0.8 x 10^9 / L (*Moreno Berggren D et al, 2018*).

4. MDS classifications

4.1. French-American-British (FAB) classification

In 1982, the FAB classification was established, which groups patients based on the percentage of myeloblasts in PB and BM, the presence or absence of ring sideroblasts (RS) and the percentage of monocytes *(Bennett J M et al, 1982).* According to FAB, AML is defined by the presence of blasts over 30% in BM.

According to the FAB classification, patients are divided into 5 groups:

- **Refractory anemia (RA)** – myeloblasts < 1% in PB, < 5% in BM and < 15% RS
- **Refractory anemia with ring sideroblasts (RARS) –** myeloblasts < 1% in PB, < 5% in BM and > 15% RS
- **Refaractory anemia with excess of blasts (RAEB) –** myeloblasts in PB < 5% and in BM 5–20%.
- **Refractory anemia with excess of blasts in transformation (RAEB-T) –** myeloblasts > 5% in PB and 21–29% in BM
- **Chronic myelomonocytic leukemia (CMML) –** < 5% myeloblasts and monocytes > 1 x 10^9/l in PB and < 20% myeloblasts in BM

4.2. World Health Organization (WHO) classifications

In 1999, the WHO classification was established. The enrichment of data on cytogenetic disturbances contributed to its update in 2001. Further modification followed in 2008 and 2016. An important prognostic factor for the WHO classification are cytogenetic abberations *(Bennett J M et al, 1985; Arber D A et al, 2016).*

The WHO 2016 classification differentiates the following subtypes of MDS:

- **MDS-single line dysplasia** (**MDS-SLD**) – characterized by mono – or bicitopenia in PB. BM – dysplasia in > 10% of cells per line, < 5% myeloblasts and < 15% RS.
- **MDS-multilineage dysplasia** (**MDS-MLD) –** it is characterized by cytopenias in PB, monocytes < 1 x 10^9 / l, BM – dysplasia in > 10% of the cells of two or more lines, ± 15% RS and < 5% blasts.
- **MDS with ring sideroblasts** (**MDS-RS**) – it is characterized by manifestations of anemic syndrome, with no myeloblasts in the PB. BM with ≥ 15% RS or > 5% RS in SF5B1 mutation and < 5% blasts.
- **MDS-ring sideroblasts with single line dysplasia (MDS-RS-SLD) –** anemia or bicitopenia, lack of blasts and BM with the presence of unilinear dysplasia.
- **MDS-ring sideroblasts with multilineage dysplasia (MDS-RS-MLD) –** PB – cytopenia(s), monocytes < 1 x 10^9 / l and lack of blasts, and in BM – dysplasia ≥ 10% of cells in ≥ 2 myeloid lines, < 5% blasts and ≥ 15% PC.
- **MDS-Refractory anemia with excess of blasts -1 (MDS-RAEB-1) –** characterized by PB with cytopenia(s), 2–4% blasts and monocytes < 1 x 10^9 / l, and in BM – uni – or multilinear dysplasia, 5–9% blasts, without Auer rods.
- **MDS – Refractory anemia with excess of blasts – 2 (MDS-RAEB-2) –** characterized by the following data from PB – cytopenia(s), 5–19% blasts and monocytes < 1 x 10^9 / l. Regarding changes in BM – uni – or multilinear dysplasia, 10–19% blasts and ± Auer rods.
- **MDS 5q-syndrome –** characterized with anemic syndrome with normal or elevated platelet count in the PB. BM with unilinear dysplasia, isolated del (5q) and < 5% blasts.
- **MDS-unclassifiable** (**MDS-U**) – determined with ± 1% myeloblasts at least twice in PB and BM, unilinear dysplasia or no dysplasia, but with specific MDS karyotype and < 5% myeloblasts.
- **Refractory cytopenia in children** (**RCC**) – it is characterized by the presence in the PB of cytopenia and myeloblasts < 2% and BM – dysplasia in 1–3 lines and myeloblasts < 5%.

5. Risk stratification scales in patients with MDS

Several prognostic scoring systems have been developed for risk strati-

fication in patients with MDS: the International Prognostic Scoring System (IPSS), its revised version (IPSS-R), the WHO Based Prognostic Scoring System (WPSS) and the MDAPSS (*Greenberg P et al, 1997; Malcovati L et al, 2007; Garcia-Manero G et al, 2008; Kantarjian H et al, 2008; Malcovati L et al, 2011; Greenberg P L et al, 2012*).

They are all based on 3 basic indicators:

- Cytogenetic findings
- Percentage of myeloblasts in BM (excluding WPSS)
- Cytopenias (WPSS reports transfusion dependence)

Although age is an important marker, only MDAPSS includes it as an independent prognostic factor.

IPSS and IPSS-R are the most commonly used prognostic scales in clinical practice.

6. Prognostic factors in MDS

Prognostic factors are a variable that is determined when diagnosing patients. Prognostic biomarkers are those that provide information about the disease and contribute to its diagnosis.

The prognostic factors in MDS can be divided into two main groups:

- ***Patient-related prognostic factors*** are age, general condition (ECOG, Karnofsky and "frailty" scale (CFS)), comorbidities and comorbid index score (CCI, HCT-CI, MDS-CI and ACE-27)
- ***The prognostic factors related to the disease*** can be divided into 3 main subgroups:
  1. Characteristics of the disease – WHO2016 classification.
  2. Clinical and laboratory parameters such as hemoglobin, absolute neutrophil count (ANC), platelets, ferritin, LDH, albumin and percentage of myeloblasts.
  3. Biological factors – cytogenetic and molecular profile, methylation status and microRNA profile

7. Conclusion of literature review

MDS is characterized by a remarkable diversity in clinical course, cytogenetic disturbances and outcome. Survival varies from months to years.

In some patients the disease progresses with an indolent course and long-term survival, while in others it progresses with an aggressive course similar to AML and a median survival of less than 6 months.

Prognostic factors studied in detail are the number and degree of cytopenias, the percentage of myeloblasts in BM, cytogenetic profile, the degree of anemic syndrome and transfusion dependence. The importance of some additional biomarkers such as albumin, bone marrow fibrosis, ferritin and LDH has also been established.

Patient-related factors such as age, ECOG and comorbidities have been shown to contribute to more accurate risk stratification and prognosis when added to risk stratification scales and classification systems.

II. AIM, TASKS AND HYPOTHESIS

1. Aim

The aim of this dissertation is to study and analyse the influence of factors related to the disease (clinical and biological) and the patient (age, comorbidities and ECOG) on the risk stratification, survival and risk of transformation of MDS into AML.

2. Tasks

The following main tasks were formulated:

1. To characterize patients with MDS according to:

1.1. demographic data;
1.2. classification systems;
1.3. risk assessment scales;
1.4. the clinical "frailty" scale and comorbid indices;
1.5 basic laboratory parameters;

2. To analyse survival according to**:**

2.1. demographic data;
2.2. classification systems and risk assessment scales;
2.3. laboratory parameters;
2.4. cytogenetic parameters;

3. To assess and analyse survival according to the comorbidity and "frailty" scales and to compare them with the classification systems and risk stratification scales for MDS.

4. To assess the relationship among risk stratification scales and comorbidity and "frailty" scales in patients with MDS.

5. To study and analyse the risk of transformation of MDS into AML and to assess patient survival before and after transformation.

6. To derive factors with favorable and unfavorable prognosis in terms of survival and transformation in AML.

3. Hypothesis

Analysis of some additional clinical and biological factors may be important for risk stratification. The inclusion of additional prognostic factors and comorbidities to established prognostic scales and classifications in MDS may play a key role in more accurate risk stratification, survival and risk of transformation into AML.

III. MATERIALS AND METHODS

1. Subject of the research

To conduct a retrospective analysis of demographic, clinical and biological indicators, classification and risk stratification systems, the "frailty" scale and the comorbidity scales in patients with MDS.

2. Object of research

The study included 219 patients with myelodysplastic syndrome over the age of 18 years. The patients were diagnosed and treated in the Clinic of Clinical Hematology at the University Hospital "St. Marina" – Varna for a period of 10 years (May 2010-May 2020).

The retrospective study was conducted after approval by the Commission for Ethics of Research of the Medical University "Prof. Dr. Paraskev Stoyanov" – Varna in accordance with the requirements of the Helsinki Declaration with decision № 98 / 26.11.2020.

2.1. Selection of patients

- Patients diagnosed with myelodysplastic syndrome according to the criteria for the diagnosis of IWCG are included. *(Valent P et al, 2007)*:
- dysplasia in more than 10% of cells on one or more hematopoietic lines or sideroblast ring ≥ 15% (or ≥ 5% in the presence of SF3B1 mutation)
- myeloblasts between 5–19% in BM (in the absence of AML-specific gene rearrangements) or 2–19% myeloblasts in PB
- cytogenetic mutation characteristic of MDS (del (5q), (-7), del (20q), (+8), complex karyotype)
- additional criteria are aberrant immunophenotyping and the presence of characteristic molecular markers

The retrospective study was conducted by analysing the available data from medical records including information from medical history, objec-

tive condition, concomitant diseases, laboratory tests and treatment.

An individual patient card has been prepared (Appendix 1).

2.2. Researched indicators. Methods of analysis

A retrospective analysis of:

- Demographic data – age and gender
- ECOG status
- Concomitant diseases – to determine the comorbid index (CFS, CCI, MDS-CI, HCT-CI, ACE-27)
- Classification according to FAB, WHO2008 and WHO2016 classifications
- Determination of risk group due to IPSS, IPSS-R and WPSS
- **Routine laboratory tests**
  1. Peripheral blood counts
  a. peripheral blood count – leukocytes, hemoglobin, platelets, ANC, ALC, MCV and reticulocytes
  b. Differential blood count – the diagnosis of MDS requires careful analysis of stained preparations of venous blood by light microscopy. Morphological exam of the blood smear analysis of at least 200 cells.

  2. *Biochemical parameters* – creatinine, bilirubin, liver parameters, LDH, albumin, B2MG, serum level of vitamin B12

  3. *Indicators of iron metabolism* – serum iron, ferritin, total iron binding capacity
- **Specialized laboratory tests**

  1. *BM aspiration with myelogramme.* All patients underwent morphological examination of BM aspirate by *May-Grunewald Giemsa (MGG) staining* and *iron staining (by Perls)* by analysis of at least 500 cells.

  Cellularity, number of dysplasia lines, percentage of myeloblasts and ring-sideroblasts in BM were analysed.

  2. *Cytogenetic analysis of the BM aspirate* – performed by the culture method for chromosome analysis by the method of *Moorhead et al. (1960)* with modifications.

  3. *Flowcytometry of BM aspirate* – A tricolor panel with combi-

nations of *Becton Dickinson monoclonal antibodies labeled with FITC, PE and PerCP fluorochromes* was used for staining. The markers are *CD2, CD3, CD4, CD5, CD7, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD22, CD33, CD34, CD38, CD41a, CD45, CD56, CD64, CD71, CD117,CD123, HLA-DR, GlycophorinA*. Samples were analysed on a *Becton Dickinson FACSort flow cytometer*. A standard CD45 / SSC gating strategy was applied in the analysis. Criteria for flow cytometric assessment of myeloid dysplasia were applied.

4. ***BM biopsy*** – for histological exam of BM by inclusion in a paraffin block, standard staining with *hematoxylin-eosin, Gomori staining* for bone marrow fibrosis. An *immunohistochemical protocol* was applied. *Immunohistochemical analysis* was performed by indirect immunoperoxidase method for immunohistochemical staining using a *mini KIT high Ph DAKO K8024.* The following antibodies were used: Monoclonal Mouse *Anti-Human CD34*, Class II, Clone: *QBEnd10*, Code-*GA632/IR632*, Polyclonal Rabbit *Anti-Human Myeloperoxidase* Clone; Polyclonal Code: *GA511/IR 511, pH-9.0*, Polyclonal Rabbit *Anti-Human CD117*, c-kit, *pH-6.0*, *6392, CA93013 USA.*
   Cellularity, fibrosis and IHC were analysed for the presence of myeloperoxidase (+), CD34 (+) and 117 (+) cells.
5. ***JAK^{V617F} mutation in peripheral blood*** **–** isolation of DNA from leukocytes by *Thermo Scientific TM Viral DNA / RNA Purification Kit from Thermo Scientific* according to the manufacturer‘s protocol.
6. ***FLT3-ITD(internal tandem duplication) mutation*** according to the methodology described by *Nakao et al.* in 1996 by isolating *total ribonucleic acid (tRNA)* from leukocytes using QIAGEN's QIAamp RNA Blood Mini Kit according to the manufacturer's protocol. Reverse transcription to *complementary deoxyribonucleic acid* was performed with *Maxima H MinusFirst Strand cDNA Synthesis Kit, catalog № K1652* from *Thermo Scientific according to the manufacturer's protocol*. *Polymerase chain reaction (PCR)* was performed with *Maxima Probe / ROX qPCR*

Master Mix (2X), catalog № K0231 from Thermo Scientific. Finally electrophoresis and analysis were performed**.** The fragments were visualized and documented with a *UV transilluminator*. The presence of additional fragments larger than the main one was considered a positive result for FLT3-ITD.

2.3. Statistical methods

Statistical analysis was performed with SPSS Statistics v.20.0 for Windows using the following analyses:

- **Dispersion analysis (ANOVA)**
- **Variation analysis**
- **Correlation analysis**
- **Regression analysis**
- **ROC curve**
- **Comparative analysis**
- **Graphic and tabular method**

In all analyzes performed, an acceptable level of significance $p < 0.05$ is assumed with a confidence interval of 95%.

IV. RESULTS

1. *To characterize patients with MDS according to:*

1.1. demographic data

93.6% (n = 205) of patients had de novo and 6.4% (n = 14) had secondary MDS.

The mean age was 70.7 ± 10.2 years. The results show a predominance of patients over 60 years old (84.9%, n = 186).

An analysis of the distribution of patients by sex revealed a predominance of males – 59.4% (n = 130) were men and 40.6% (n = 89) were women. The ratio between men: women is 1.5: 1. Although no significant difference was demonstrated, we found that men with MDS are younger than women (69.8 years of age for men and 72 years of age for women, respectively).

There was a significant difference in the distribution according to the type of MDS and gender, with men predominating in the group of patients with de novo MDS (61.0%), while women (64.3%) predominated in the group of secondary MDS (p = 0.048) (Fig. 1).

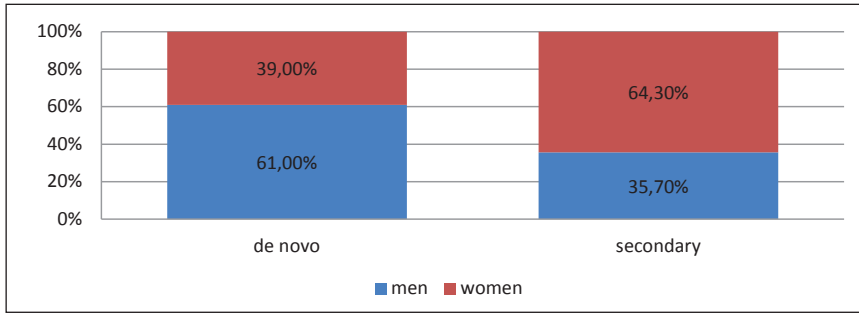


Figure 1. Comparative analysis of the distribution of patients according to the type of MDS and gender

1.2. classification systems

According to the FAB classification, patients were distributed as follows – RA (59%, n = 130), RAEB (37%, n = 81), RARS (2%, n = 5) and

RAEB-t (2%, n = 3). We found that RA patients predominated, followed by RAEB. Patients with CMML were excluded from the study.

According to the WHO2008 classification, patients with RCMD (45%, n = 98) predominate, followed by RAEB-2 (29%, n = 64).

According to the WHO2016 classification, patients with MDS-MLD (45%, n = 98) predominate, followed by RAEB2 (29%, n = 64), RAEB1 (9%, n = 20), MDS-5q (7%, n = 16), MDS-SLD (7%, n = 14), MDS-RS-SLD (1%, n = 3) and 2% all other subtypes (Fig. 2).

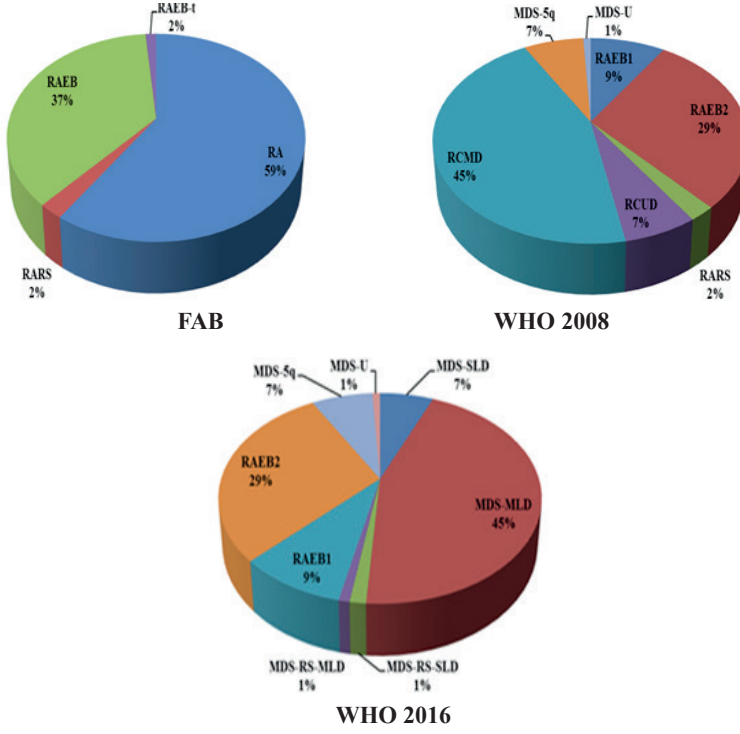


Figure 2. Distribution of patients according to FAB, WHO2008 and WHO2016 classifications

1.3. risk assessment scales

According to IPSS and IPSS-R, patients with intermediate risk predominate, and according to WPSS with high risk (Fig. 3).

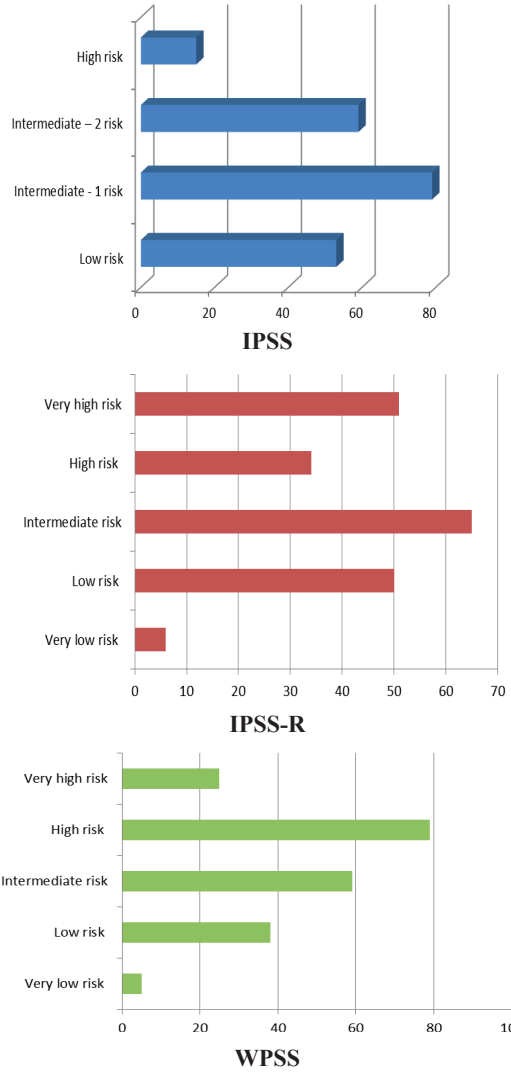


Figure 3. Distribution of patients according to IPSS, IPSS-R and WPSS

1.4. the clinical "frailty" scale and the comorbidity scales

In the analysis of the patients according to the ECOG status it was found

that the patients with ECOG = 0 (43.4%) prevail, with the smallest share of the patients with ECOG = 3 (3.2%).

We analysed the distribution of patients according to the Clinical "Frailty" Scale. We found that 47% (n = 103) of patients had a score of 1–3 points ("unfrail"). In the group of "frail" patients with a score of 4 points are 14.6% (n = 32), while in the group of very "frail" are 38.4% (n = 84) of patients.

The analysis according to the Charlson comorbidy index (CCI) showed that the prevailing group of patients with 0 points was 36.1% (n = 79) and the group with a score of 8 points 0.5% (n = 1) was the smallest. There is a tendency for a progressive decrease in the number of patients with an increase in CCI score.

In the analysis according to HCT-CI we found that low-risk patients (0 points) were 36.5% (n = 80) of the analysed patients. In the group of intermediate risk (1–2 points) were 40.7% (n = 89) and high risk (> 3 points) are 22.9% (n = 50) of patients. Patients with intermediate risk predominated.

In the analysis of the distribution of patients according to MDS-CI, we found that patients with low risk (0 points) were 43.8% (n = 96), intermediate risk (1–2 points) – 45.2% (n = 99) and high risk > 2 points) are 11% (n = 24). According to MDS-CI , patients with intermediate risk prevail.

Compared to ACE-27, we found that 32% (n = 70) of patients had no comorbidity, 26.5% (n = 58) had a mild form, 20.1% (n = 44) – a moderate form – and 21.5% (n = 47) had a severe form of comorbidity.

1.5. laboratory parameters

The main characteristics of the patients according to the clinical and laboratory parameters are presented in Table 1.

Table 1. Characteristics of patients according to the main laboratory parameters

Indicator		**Number /%**
Hemoglobin	**mean ± SD (range)**	**79.63 ± 18.79 (33–133)**
	< 80 g/l	115/52.5%
	80–100 g/l	74/33.8%
	> 100 g/l	30/13.7%

MCV	**mean ± SD (range)**	**101.14 ± 11.91 (67.7–155.1)**
	< 100 fl	102/46.6%
	> 100 fl	117/ 53.4%
Leukocyte	**mean ± SD (range)**	**4.5 ± 3.63 (0.42–23.20)**
	< 4 x 10^9/l	127/58.0%
	4–10 x 10^9/l	74/33.8%
	> 10 x 10^9/l	18/8.2%
Trombocyte	**mean ± SD (range)**	**174.93 ± 173.53 (2–1095)**
	< 50 x 10^9/l	49/22.4%
	50–100 x 10^9/l	51/23.3%
	> 100 x 10^9/l	119/54.3%
ALC	**mean ± SD (range)**	**1.55 ± 0.96 (0.11–9.75)**
	< 1.2 x 10^9/l	94/42.9%
	> 1.2 x 10^9/l	125/57.1%
ANC	**mean ± SD (range)**	**1.86 ± 1.96 (0.07–12.30)**
Segments	**mean ± SD (range)**	**49.86 ± 18.67 (0–95)**
Eosinophil	**mean ± SD (range)**	**1.42 ± 1.99 (0–13)**
Basophil	**mean ± SD (range)**	**0.19 ± 0.58 (0–5)**
Monocyte	**mean ± SD (range)**	**7.39 ± 5.92 (0–37)**
Lymphocyte	**mean ± SD (range)**	**37.46 ± 17.78 (0–84)**
Creatinine	**mean ± SD (range)**	**94.25 ± 45.79 (44–498)**
LDH	**mean ± SD (range)**	**483.42 ± 276.92 (200–2324)**
	< 380U/l	86/39.3%
	> 380U/l	133/60.7%
Albumin	**mean ± SD (range)**	**39.88 ± 5.29 (22–52)**
	< 35 g/l	39/17.8%
	35–40 g/l	61/27.9%
	> 40 g/l	119/54.3%
b2-MG	**mean ± SD (range)**	**3.57 ± 2.00 (0–11.60)**
	< 2 mg/l	38/17.4%
	> 2 mg/l	181/82.6%

Dysplasia(line)	**mean ± SD (range)**	**2.72 ± 0.64 (0–3)**
	0	1/0.5%
	1	20/9.1%
	2	19/8.7%
	3	179/81.7%
Myeloblasts in BM	**mean ± SD (range)**	**6.53 ± 5.63 (0–22)**
RBC transfusions (number)	**mean ± SD (range)**	**3.74 ± 3.25 (0–15)**
	< 4 единици	165/75.7%
	> 4 единици	53/24.3%
Feritin	**mean ± SD (range)**	**804.31 ± 1110.48 (2.30–8250)**
	< 500 mg/l	58/48.3%
	> 500 mg/l	62/51.7%
Serum iron	**mean ± SD (range)**	**21.79 ± 131.11 (0.90–55.50)**
TIBC	**mean ± SD (range)**	**48.33 ± 11.51 (27.2–107)**
VitaminB12	**mean ± SD (range)**	**416.74 ± 343.03 (78–1476)**

To analyse patients according to the hemoglobin level, we divided them into three groups. 1st group with Hb > 100 g / l (13.7%, n = 30), 2nd group with Hb-80–100 g / l (33.8%, n = 74) and 3rd group with Hb < 80 g / l (52.5%, n = 115). The majority of patients have anemic syndrome.

According to MCV, patients were divided into two groups. Group 1 with MCV > 100 fl (53.4%, n = 117) and group 2 with MCV < 100 fl (46.6%, n = 102).

To determine the significance of the leukocyte count, we divided the patients into three groups. Patients with leukocyte < 4 x 10^9 / l (58%, n = 127), 4–10 x 10^9 / l (33.8%, n = 74) and > 10 x 10^9 / l (8.2%, n = 18). It was found that the majority of patients have leukopenia.

To determine the role of platelet count, patients were divided into three groups. 1st group with platelets > 100 x 10^9 / l (54.3%, n = 119), 2nd group – 50–100 x 10^9 / l (23.3%, n = 51) and 3rd group with platelets < 50 x 10^9 / l (22.4%, n = 49). Patients with a platelet count > 100 x 10^9 / l predominate.

To review and analyse LDH values, patients were divided into two

groups. 1st group of patients with LDH < 380U / l (39.3%, n = 86) and 2nd group of LDH > 380U / l (60.7%, n = 133).

An analysis of the degree of BM fibrosis was performed in 68 patients. We found a predominance of patients without fibrosis in BM – 83.8% (n = 57). Fibrosis – grade 1 was detected in 10.3% (n = 7) and grade 2 in 5.9% (n = 4).

In an analysis of cytogenetic findings, we found a predominance of patients with normal karyotype – 59.4% (n = 130), followed by the group with complex karyotype – 10.5% (n = 23) and 7.3% with isolated del (5q) (n = 16). We found a predominance of patients with good IPSS-R cytogenetic risk (n = 148) followed by a very high IPSS-R cytogenetic risk (n = 23).

FLT3 mutation status was studied in 29 patients. The results showed the presence of FLT3-ITD mutation in 7% (n = 2) of patients. The remaining 93% (n = 27) had FLT3-ITD (-) status.

JAK2V617F status was studied in 17 patients. The results revealed the presence of the JAK2V617F mutation in 6% (n = 1) of the patients. The remaining 94% (n = 16) patients had JAK2V617F (-) status.

We proved the importance of the following laboratory parameters – hemoglobin, leukocytes, platelets, ANC, LDH and the percentage of myeloblasts in BM in the analysis of the classification and risk stratification systems.

No difference was found in the distribution according to the number of eosinophils, basophils, creatinine, the number of blood transfusions, albumin, ferritin and B2MG according to the classifications considered.

2. *To assess and analyse survival according to demographic, classification and risk stratification systems, clinical-biological and cytogenetic parameters*

2.1. To analyse the survival of patients according to demographic data

We found that the mean overall survival of the patients studied was *18.4 ± 21.9 months* (1–132 months) (Figure 4).

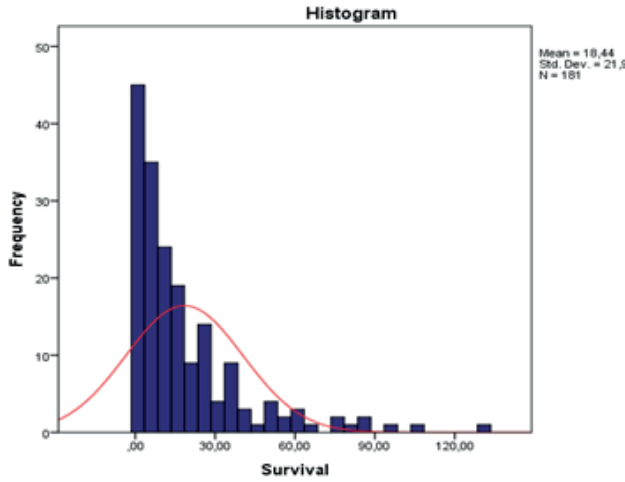


Figure 4. Analysis of variance of mean overall survival in patients with MDS

When comparing survival **by gender**, a statistically significant difference was found (p = 0.042). Women have a longer survival than men (respectively *22.5 ± 26.3 months* for women and *15.7 ± 18.4 months* for men) (Fig. 5).

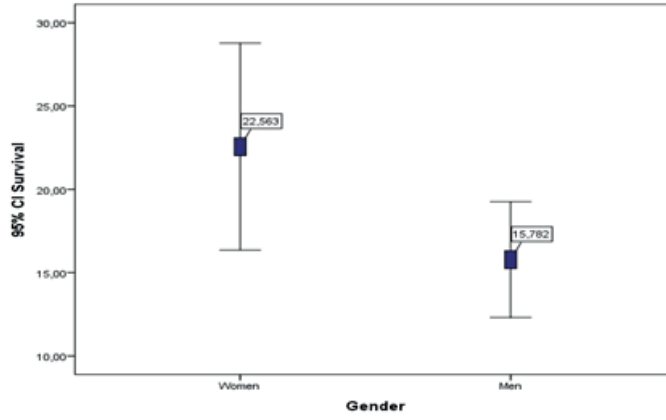


Figure 5. Comparative analysis of survival by gender (p = 0.042)

With age, an inverse relationship was found (r = -0.204; p = 0.006), which shows that with increasing age, survival decreases (Fig. 6). The results of the analysis show that 4.10% of the duration of overall survival is caused by age.

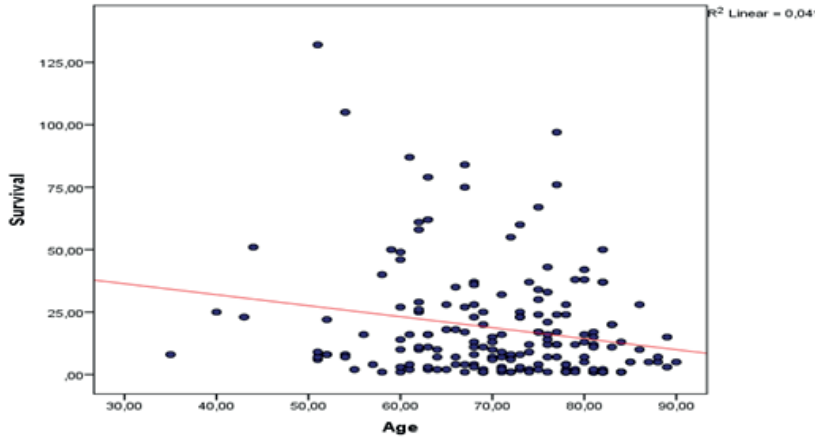


Figure 6. Correlation analysis between age and survival (r = -0.204; p = 0.006)

Although no statistical difference was found, the results of the analysis according to **age groups** showed that the patients with the shortest survival were over 71 years old, as well as those in the age group 31–40 years old (16.5 months). We found the longest survival in patients in the age group 41–50 years old (37.0 months) (Fig. 7).

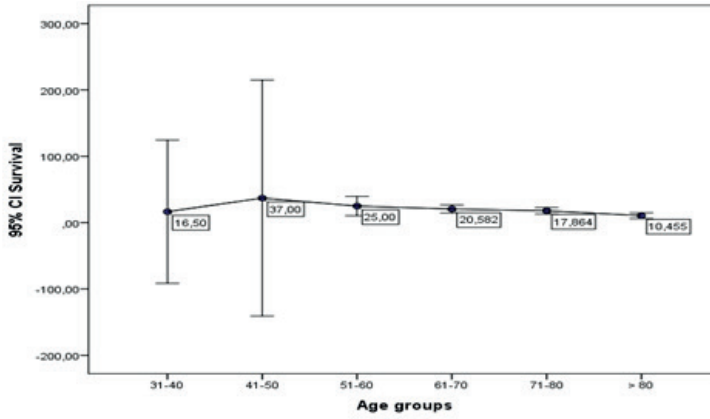


Figure 7. Analysis of variance of average survival by age group

No statistically significant difference in survival was found by **type of MDS**. The survival of patients with de novo MDS was on average *18.5 ± 21.9 months* (1–132 months), and those with secondary MDS was on average *17.5 ± 23.9 months* (1–87 months).

2.2. To assess and analyse survival according to classification systems and risk assessment scales

We found a statistically significant difference in the analysis of survival according to the **FAB classification** ($p < 0.001$). The shortest life expectancy was found in patients with RAEB (*10.6 ± 10.8 months*) and RAEB-t (*10.3 ± 4.9 months*).

Analysis according to the WHO2016 classification showed that survival was the longest in patients with MDS-5q syndrome (*44.8 ± 38.5 months*), followed by MDS-RS-SLD (*43.0 ± 49.1 months*) and MDS-SLD (*39.4 ± 34.4 months*). The shortest survival was in the group of MDS-U (9.0 ± 11.3 months) and RAEB-2 (9.9 ± 9.9 months) ($p < 0.001$) (Table 2).

No relationship was found between age, gender, distribution according to the WHO 2016 classification and survival.

Table 2. Comparative analysis of the distribution of patients according to classifications and survival ($p < 0.001$)

Classification		Number/%	Survival (month) (mean ± SD)
FAB	RA	130/59.4%	31.0 ± 29.4
	RARS	5/2.3%	32.0 ± 39.0
	RAEB	81/37.0%	10.6 ± 10.8
	RAEB-t	3/1.4%	10.3 ± 4.9
WHO2016	MDS-SLD	14/6.4%	39.4 ± 34.4
	MDS-MLD	98/44.7%	28.0 ± 26.5
	MDS-RS-SLD	3/1.4%	43.0 ± 49.1
	MDS-RS-MLD	2/0.9%	15.5 ± 19.1
	RAEB1	20/9.1%	13.2 ± 13.1
	RAEB2	64/29.2%	9.9 ± 9.9
	MDS-5q	16/7.3%	44.8 ± 38.5
	MDS-U	2/0.9%	9.0 ± 11.3

We also performed a survival analysis according to the **risk scales**. The distribution of patients is presented in Table 3.

Table 3. Comparative analysis of the distribution of patients according to the risk and survival assessment scales ($p < 0.001$).

Prognostic Scoring Systems		Number/%	Survival (month) (mean ± SD)
IPSS	Low risk	53/25.7%	37.9 ± 30.5
	Intermediate-1 risk	79/38.3%	26.8 ± 27.5
	Intermediate-2 risk	59/28.6%	11.8 ± 15.5
	High risk	15/7.3%	9.3 ± 7.8
IPSS-R	Very low risk	6/2.9%	62.0 ± 42.7
	Low risk	50/24.3%	40.6 ± 33.5
	Intermediate risk	65/31.6%	26.2 ± 22.8
	High risk	34/16.5%	12.0 ± 8.8
	Very high risk	51/24.8%	8.9 ± 10.8

WPSS	Very low risk	5/2.4%	44.2 ± 32.1
	Low risk	38/18.4%	45.7 ± 37.7
	Intermediate risk	59/28.6%	28.9 ± 24.3
	High risk	79/38.3%	13.5 ± 13.8
	Very high risk	25/12.1%	9.4 ± 12.5

An IPSS survival analysis found that high-risk MDS patients had the shortest survival (9.3 months) ($p < 0.001$).

Survival analysis according to IPSS-R found that patients with MDS who were at very high risk had the lowest survival (8.9 months) ($p < 0.001$).

Survival analysis according to WPSS showed that patients with MDS who were at very high risk had the shortest survival (9.4 months) ($p < 0.001$).

2.3. To analyse the survival of patients according to laboratory parameters

There was no statistical relationship between **hemoglobin** levels and survival of patients with MDS, but it can be said that patients with hemoglobin < 80 g / l have a shorter survival than others ($p < 0.05$) (Fig. 8).

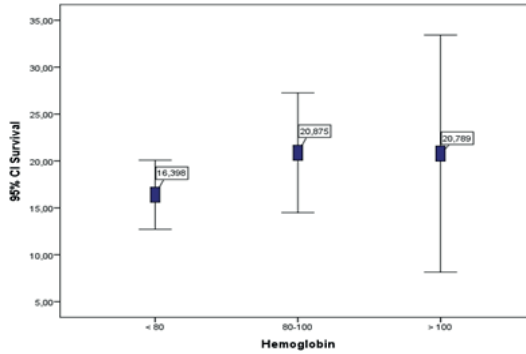


Figure 8. Dispersion analysis of mean survival according to hemoglobin levels ($p < 0.05$)

Statistical dependence in overall survival was also not found with **MCV**, although patients with MCV < 100 fl had a shorter survival than other patients (*16.4 ± 17.6 months* and *20.4 ± 25.5 months*, respectively).

In the analysis of the overall survival according to the **leukocyte** count, it was found that there is a significant difference between the groups (p = 0.05). Survival is shorter in patients with leukocytes < 4 x 10^9 / l and > 10 x 10^9 / l (Fig. 9).

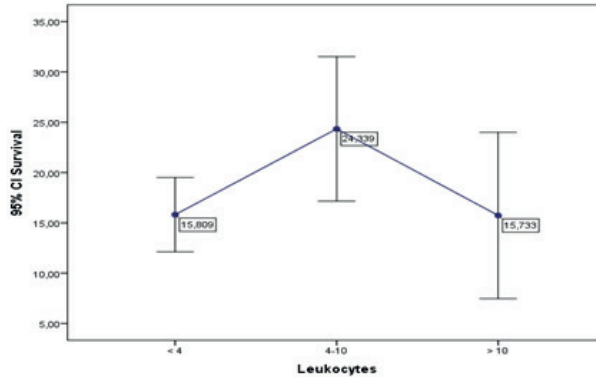


Figure 9. Dispersion analysis of mean survival due to leukocyte count (p = 0.05)

In the analysis of the relationship between platelet count and survival, a weak positive relationship was found, which shows that with increasing platelet count, survival also increases (r = 0.253; p = 0.001) (Fig. 10).

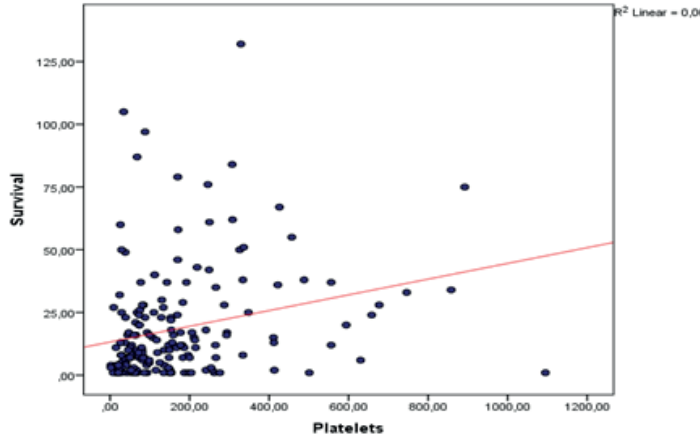


Figure 10. Correlation analysis between survival and platelets (r = 0.253; p = 0.001)

It can be said that 6.4% of the survival time is due to the higher platelet count. Patients with platelet < 50 x 10^9 / l had the shortest survival (*12.7 ± 19.8 months*), followed by patients with platelets-50–100 x 10^9 / l (*14.9 ± 19.1 months*). The longest survival was in patients with a platelet count > 100 x 10^9 / l (*22.8 ± 23.5 months*) (p = 0.019).

A weak positive relationship was found between **neutrophil count** and survival (r = 0.167; p = 0.024) (Fig. 11). Survival increases with increasing of neutrophil count.

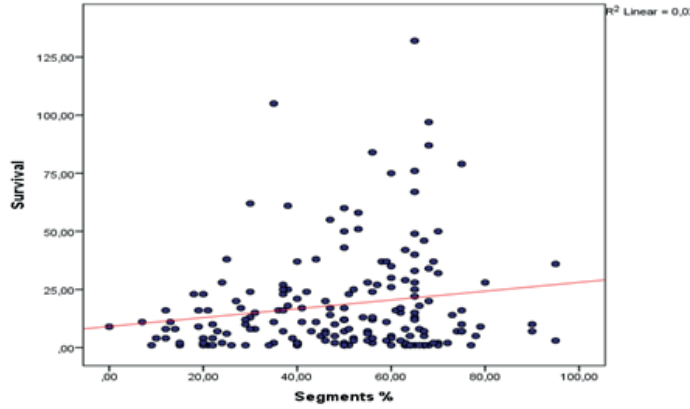


Figure 11. Correlation analysis between survival and neutrophil count (r = 0.167; p = 0.024)

With regard to the analysis of LDH values, a weak negative dependence was found, which shows that with increasing LDH levels, survival decreases (r = -0.157; p = 0.035) (Fig. 12). Survival was longer in patients with LDH < 380U / l compared to patients with LDH > 380U / l (*21.5 ± 25.1 months* and *16.6 ± 19.7 months*, respectively).

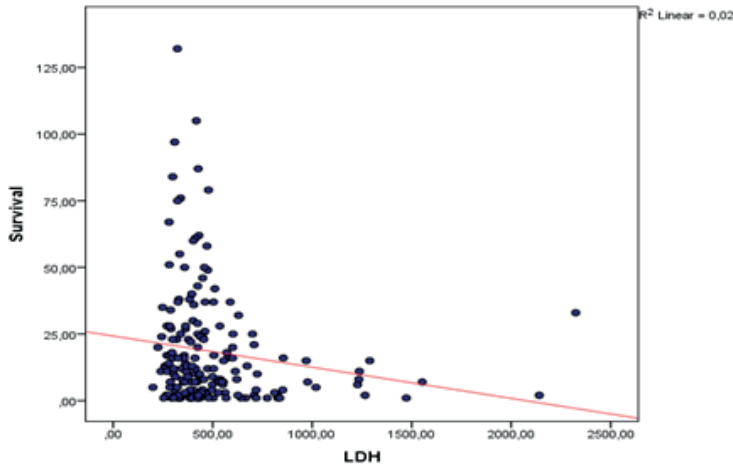


Figure 12. Correlation analysis between survival and LDH (r = -0.157; p = 0.035)

An analysis was performed with regard to the **number of dysplasias in BM** and it was found that it correlates slightly negatively with survival, as the increase in their number decreases the survival (r = -0.143; p = 0.05) (Fig. 13).

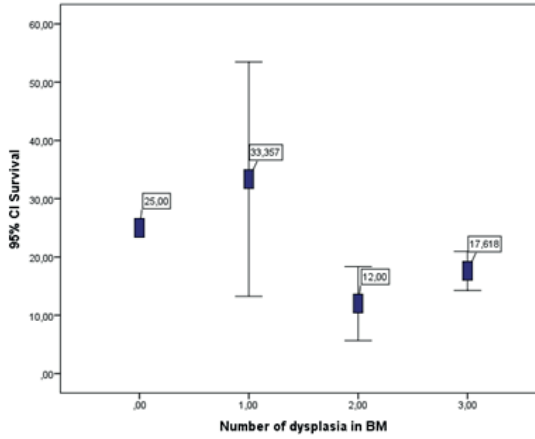


Figure 13. Dispersion analysis of survival according to the number of dysplasias in BM (r = -0.143; p = 0.05)

The analysis of the relationship between the **percentage of myeloblasts in BM** and survival revealed a moderate negative relationship. They affect survival by about 10.3% (r = -0.322; p < 0.001) (Fig. 14).

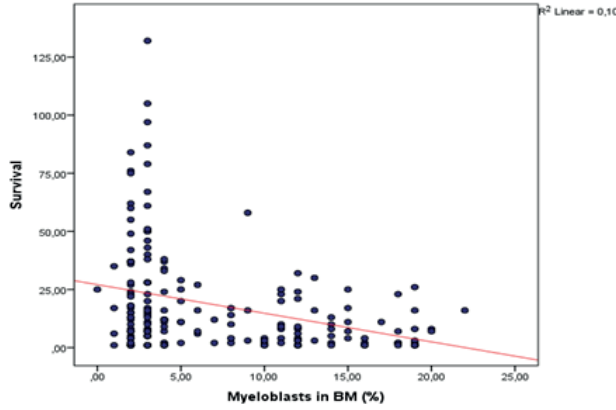


Figure 14. Correlation analysis between survival and myeloblasts in BM (r = -0.322; p < 0.001)

We found that serum iron levels correlated slightly positively with survival (r = 0.234; p = 0.025) (Fig. 15). Elevated serum iron levels lead to prolonged survival in patients with MDS.

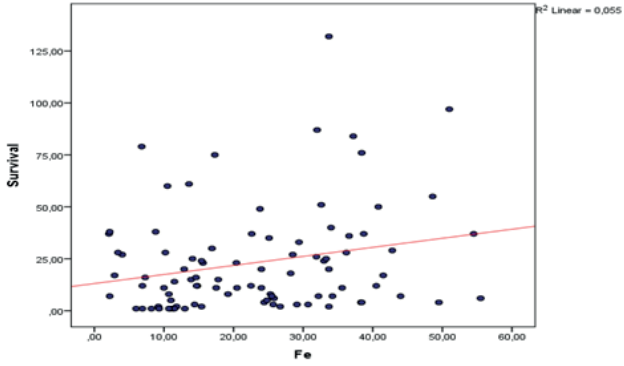


Figure 15. Correlation analysis between survival and serum iron levels (r = 0.234; p = 0.025)

2.4. To analyse the survival of patients according to cytogenetic parameters

In the analysis of survival according to cytogenetics, the presence of a significant difference was found (p = 0.008) (Fig. 16). The shortest survival was in patients with del (9q) (*2 months*) and the longest in del (5q) (*39.6 months*).

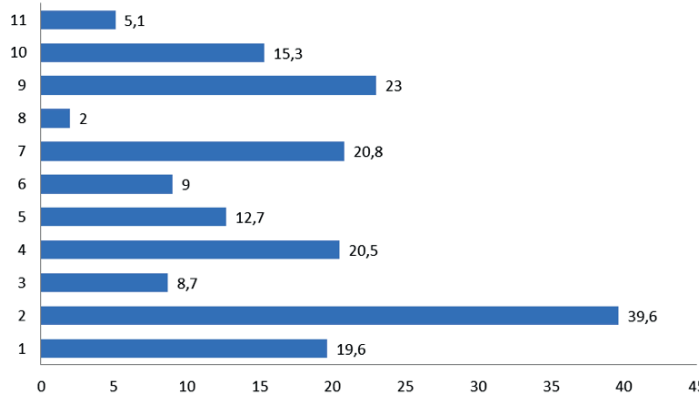


Figure 16. Mean overall survival by cytogenetic group (p = 0.008)
1gr-46,XX; 46,XY; **2gr**-isolated del(5q); **3gr**- complex karyotype (>3 abberations); **4gr**-del (20)(q11); **5gr**-del (7)(q31); **6gr**- (-Y); **7gr**- (+8); **8gr** – del (9q); **9gr**-del (5) with +21; **10gr**- others- +11; del (11)(q23); del(16)(q22); add(18)(q23); del(16)(9q22); del(12); del(9q); add(17)(p13); inv(12)(p13;p15); add(2)(p25); del(15)(q22); -21; der ;t(1;3)(q42;q21); i(17)(q10); del(11)(q22;q23) +14; ider(20)(q10); del(20)(q11;q13); **11gr**-without methaphases

When conducting a multi-regression step analysis to determine the main factors that affect the survival of patients with MDS it was found that the most important ones are myeloblasts in BM, age, platelets and serum iron levels that affect 26.2% of survival overall (Table 4).

Table 4. Multi-regression step analysis to determine the main factors influencing overall survival in patients with MDS

Coefficients[a]						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	32,569	3,580		9,098	,000
	Myeloblats in BM	-1,659	,457	-,357	-3,629	,000
2	(Constant)	79,733	18,506		4,309	,000
	Myeloblasts in BM	-1,949	,457	-,420	-4,264	,000
	Age	-,643	,248	-,255	-2,595	,011
3	(Constant)	78,558	18,203		4,316	,000
	Myeloblasts in BM	-1,798	,456	-,387	-3,947	,000
	Age	-,705	,245	-,280	-2,873	,005
	Platelets	,023	,012	,195	2,020	,046
4	(Constant)	64,655	19,048		3,394	,001
	Myeloblasts in BM	-1,660	,452	-,357	-3,673	,000
	Age	-,648	,242	-,257	-2,671	,009
	Platelets	,026	,011	,219	2,297	,024
	Fe	,368	,175	,197	2,100	,039

a. Dependent Variable: Overall Survival

3. *To assess and analyse survival according to the comorbidity indices and "frailty" scale and to compare them with the classification systems and the risk stratification scales for MDS*

3.1. To analyse and assess survival due to ECOG, comorbidity indexes and clinical "frailty" scale

3.1.1. In the analysis of survival according to the ECOG status, we found the highest percentage of patients in the group with ECOG = 0 (43.4%, n = 95). They did not have a statistically significant longer survival (*20.8 ± 23.3 months*) compared to patients with ECOG = 3 (*18.2 ± 13.3 months*) (Fig. 17). No significant difference in survival was found between groups according to their ECOG status.

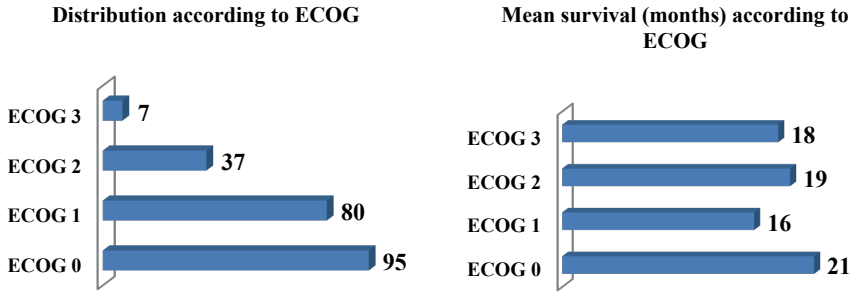


Figure 17. Distribution and mean patient survival according to ECOG

3.1.2. In an analysis of the clinical "frailty" scale, we found that the predominant group was "unfrail" patients (47%, n = 103, CFS = 1–3), with whom the median survival did not differ significantly from the "frail" group (14.6%, n = 32, CFS = 4) and very "frail" patients (38.4%, n = 84, CFS = 5–9) (respectively *28.6 ± 29.2 months*; *13.6 ± 15.7 months*; *18.0 ± 11.2 months*) (Fig.18).

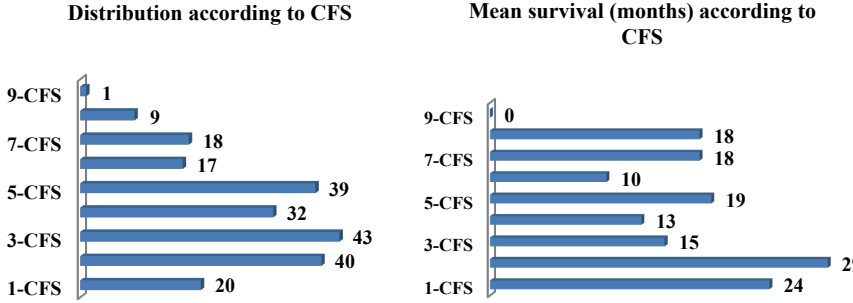


Figure 18. Distribution and mean survival of patients according to CFS

3.1.3. In the CCI analysis, we found that patients without comorbidities predominated (n = 79). There was no significant difference in survival between the different groups according to CCI (Fig. 19).

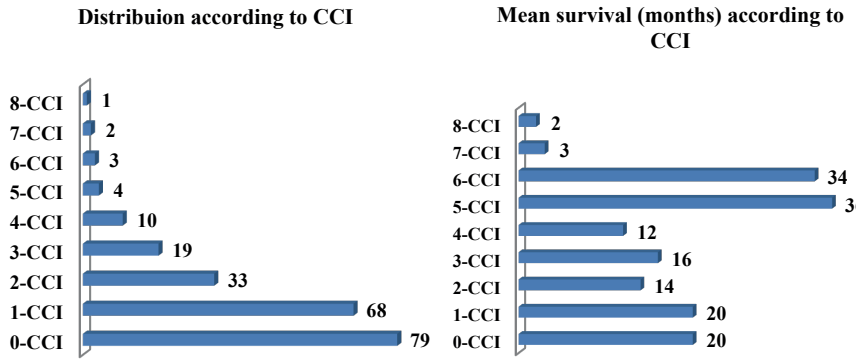


Figure 19. Distribution and mean survival of patients according to CCI

3.1.4. According to HCT-CI, patients with intermediate risk prevailed (n = 89), and no significant difference in survival was demonstrated between the different groups (Fig. 20).

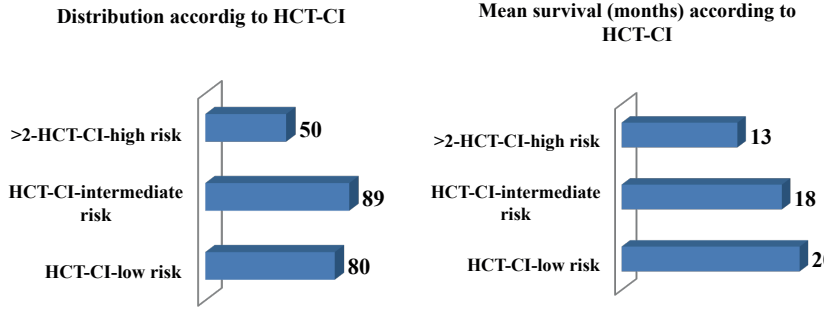


Figure 20. Distribution and mean survival of patients according to HCT-CI

3.1.5. Survival analysis for MDS-CI showed a predominance of patients at intermediate risk (n = 99). Low-risk patients (43.8%, n = 96, MDS-CI = 0) had a longer survival (*21.8 ± 25.9 months*) compared to the high-risk group (MDS-CI > 2) (*15.0 months*) (Fig. 21).

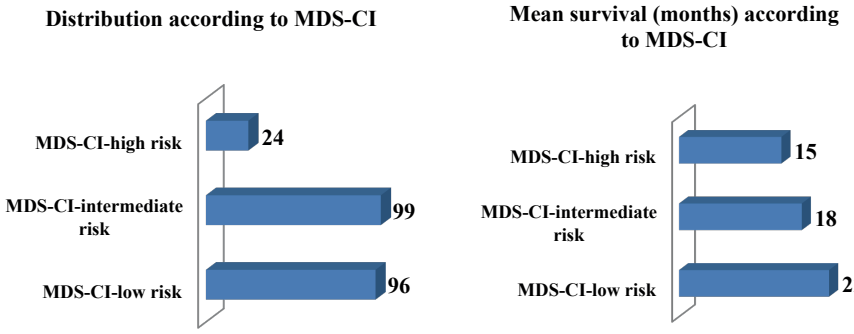


Figure 21. Distribution and mean survival of patients according to MDS-CI

3.1.6. The results of the analysis of the ACE-27 system are similar. Patients without comorbidities (ACE-27 = 0) (n = 70) had a longer survival (*22.7 ± 27.5 months*) than patients with mild (ACE-27 = 1) (n = 58) (*17.2 ± 18.2*), medium (ACE-27 = 2) (n = 44) (*11.2 ± 13.7 months*) or severe (ACE-27 = 3) (n = 47) comorbidity (*19.8 ± 22.0 months*) (Fig. 22)

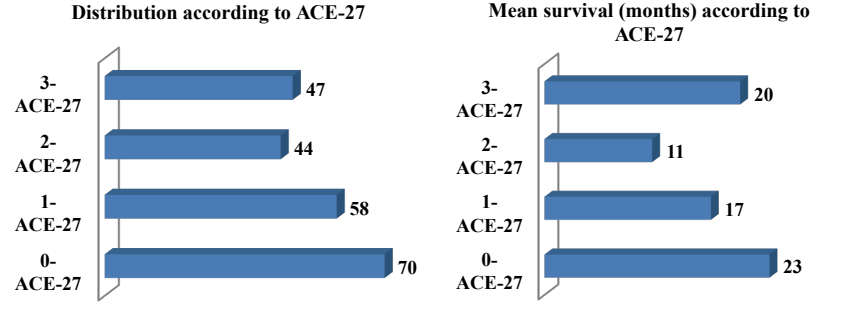


Figure 22. Distribution and mean survival of patient according to ACE-27

No significant difference was found in survival according to the comorbidity and 'frailty' scales.

3.2. To analyse and evaluate survival according to ECOG, CFS, the comorbidity indices (CCI, HCT-CI, MDS-CI and ACE-27) and the FAB subgroups

There was a statistically significant difference in overall survival according to the FAB classification and the scales for comorbidity and "frailty" – ECOG ($p < 0.001$), CFS ($p < 0.001$), CCI ($p < 0.001$), HCT-Cl ($p < 0.001$), MDS-Cl ($p < 0.001$) and ACE-27 ($p < 0.001$).

There was a negative moderate correlation between the ECOG status and overall survival according to FAB ($r = -0.336$; $p < 0.001$) (Table 5). The longest survival was in patients with ECOG = 0 and RARS (*63.0 ± 48.1 months*), followed by ECOG = 0 and RA (*25.6 ± 26.7 months*). At ECOG = 1–3, the longest survival is in RA (*26.7 ± 28.5 months*). The survival of patients with RA and ECOG = 3 (*18.6 ± 14.8 months*) was comparable to patients with RAEB-t and ECOG = 0 (*16.0 months*). We found a decrease in the survival of patients with the increase of the ECOG score in the groups of the FAB classification.

In the analysis of CFS and FAB, we found a longer average survival in "unfrail" patients (CFS = 1–3) compared to the groups of "frail" (CFS = 4) and very "frail" patients (CFS > 5). Survival in all FAB groups decreased with increasing CFS score. The survival of RA patients with CFS > 5 (*17.7 ± 12.3 months*) was comparable to that of RAEB-t patients with CFS = 1–3 (*16.0 months*).

Compared to CCI and FAB, we found a decrease in survival in all FAB groups with an increase in the CCI score. The survival of patients with RA and CCI = 4 (*15.2 ± 13.5 months*) is comparable to that of patients with RAEB-t and CCI = 0 (*12.0 ± 5.6 months*).

In the analysis of HCT-CI, MDS-CI and FAB, we found a reduction in mean survival with increasing comorbidity risk. The survival of patients with RA with high HCT-CI risk (*11.0 ± 5.6 months*) is comparable to that of patients with RAEB-t with low HCT-CI risk (*12.0 ± 5.6 months)*.

According to the severity of comorbidities (ACE-27) and FAB, we found that in all 4 FAB groups, patients without comorbidities had the longest survival.

Table 5. Comparative analysis of the distribution on survival of patients with MDS according to the ECOG, comorbidity and "frailty" scales and FAB classification ($p < 0.001$)

Scales		Survival (mean ± SD)			
		RA	RARS	RAEB	RAEB-t
ECOG	0	25.6 ± 26.7	63.0 ± 48.1	12.0 ± 9.0	16.0
	1	24.2 ± 23.9	1.5 ± 0.7	9.1 ± 11.5	7.5 ± 0.7
	2	26.7 ± 28.5	-	6.0 ± 8.5	-
	3	18.6 ± 14.8	-	16.0	-
CFS	1	27.3 ± 34.8	97.0	7.5 ± 9.0	-
	2	40.1 ± 33.8	1.0	14.5 ± 8.6	-
	3	18.3 ± 20.3	29.0	10.5 ± 9.3	16.0
	4	17.4 ± 18.4	-	7.3 ± 6.7	-
	5	34.5 ± 30.4	2.0	10.8 ± 13.6	7.5 ± 0.7
	6	17.7 ± 12.3	-	2.4 ± 0.7	-
	7	22.6 ± 16.7	-	11.0 ± 11.8	-
	8	18.4 ± 15.6	-	11.0	-
CCI	0	29.2 ± 30.6	97.0	8.4 ± 8.3	12.0 ± 5.6
	1	26.8 ± 23.9	29.0	12.4 ± 12.0	7.0
	2	17.7 ± 20.9	1.0	5.7 ± 7.0	-
	3	19.7 ± 10.9	-	13.6 ± 12.0	-
	4	15.2 ± 13.5	-	2.5 ± 0.7	-
	5	36.3 ± 44.1	-	-	-
	6	34.3 ± 36.1	-	-	-
	7	-	2.0	4.0	-
	8	-	-	2.0	-

HCT-CI	**0**	29.2 ± 30.6	49.0 ± 67.9	8.7 ± 8.3	12.0 ± 5.6
	1	23.8 ± 22.7	29.0	12.3 ± 12.6	-
	2	23.5 ± 19.9	-	10.5 ± 10.5	-
	3	21.6 ± 24.5	-	11.5 ± 12.9	7.0
	4	11.0 ± 5.6	-	7.7 ± 9.2	-
	5	15.7 ± 15.5	-	2.0	-
	6	13.0	-	-	-
	7	87.0	-	-	-
	8	-	2.0	-	-
MDS-CI	**0**	32.6 ± 29.9	97.0	10.2 ± 11.1	12.0 ± 5.6
	1	27.5 ± 27.9	1.0	10.6 ± 13.9	7.0
	2	18.7 ± 18.6	29.0	9.1 ± 7.2	-
	3	19.6 ± 24.5	-	9.1 ± 10.9	-
	4	-	2.0	-	-
ACE-27	**0**	33.4 ± 30.6	49.0 ± 67.9	8.6 ± 9.0	12.0 ± 5.6
	1	22.5 ± 23.6	29.0	11.3 ± 7.3	-
	2	16.2 ± 16.4	-	6.2 ± 8.1	7.0
	3	23.7 ± 24.3	2.0	14.1 ± 16.3	-

3.3. To analyse and evaluate patient survival according to ECOG, CFS, the comorbidity indices (CCI, HCT-CI, MDS-CI and ACE-27) and the WHO2008 subgroups

The analysis of the results showed a statistically significant difference in overall survival according to the WHO 2008 classification and ECOG ($p < 0.001$), CFS ($p < 0.001$), CCI ($p < 0.001$), HCT-Cl ($p < 0.001$), MDS-Cl ($p < 0.001$) and ACE-27 ($p < 0.001$).

In patients with RAEB-2, RARS and MDS-5q, survival decreased significantly with increasing the ECOG score. The survival of patients with MDS-5q syndrome and ECOG = 3 (*20 months*) is comparable to that of patients with RAEB-1 and ECOG = 1 (*15.6 ± 18.9 months*).

When comparing survival according to the WHO2008 classification and CFS, a shorter life expectancy was found in the group of very "frail"

patients compared to “unfrail” and “frail” patients. The survival of very “frail” patients (CFS = 5) with RAEB-1 (*24.3 ± 24.4 months*) is comparable to that of very “frail” patients with MDS-5q syndrome (*25.0 ± 10.4 months*).

An analysis according to the WHO2008 classification and CCI showed a decrease in survival with increasing the CCI score. The survival of patients with MDS-5q and CCI = 5 (*15.0 months*) was comparable to that of patients with RAEB-1 and CCI = 3 (*14.3 ± 11.6 months*).

In patients with RCMD, MDS-5q and MDS-U, a decrease in survival was observed with an increase in the risk of MDS-Cl. The median survival of patients with MDS-5q and MDS-Cl = 3 (high risk) (*15.0 months*) was comparable to RAEB-1 and low MDS-Cl risk (*13.8 ± 13.8 months*).

In the analysis according to the WHO2008 classification and HCT-CI, we found shorter survival in high-risk patients compared to patients with low and intermediate HCT-CI risk. The survival of patients with high-risk MDS-5q and HCT-CI (*15.0 months*) was comparable to RAEB-1 and HCT-CI intermediate risk (*14.0 ± 2.8 months*).

In patients with RARS and RCUD, there is a tendency to decrease survival with increasing ACE 27 (Table 6).

Table 6. Comparative analysis of the distribution on survival of patients with MDS according to the ECOG, comorbidity and “frailty” scales and WHO2008 groups ($p < 0.001$)

Scales		Survival (mean ± SD)						
		RAEB1	**RAEB2**	**RARS**	**RCUD**	**RCMD**	**MDS-5q**	**MDS-U**
ECOG	**0**	13.4 ± 7.9	11.7 ± 9.3	63.0 ± 48.1	32.2 ± 24.7	20.9 ± 19.8	58.0 ± 52.9	9.0 ± 11.3
	1	15.6 ± 18.9	7.0 ± 6.9	1.5 ± 0.7	34.3 ± 39.0	19.6 ± 18.7	46.5 ± 35.3	-
	2	5.0 ± 5.2	6.3 ± 9.5	-	44.0 ± 60.8	25.4 ± 29.6	23.4 ± 11.2	-
	3	16.0	-	-	-	18.3 ± 17.0	20.0	-

CFS	**1**	6.0	7.8 ± 10.0	97.0	1.0	31.0 ± 35.8	-	-
	2	12.0 ± 9.2	15.5 ± 8.8	1.0	52.0 ± 14.1	28.5 ± 25.6	74.7 ± 50.5	-
	3	16.3 ± 7.8	9.1 ± 9.1	29.0	34.0 ± 34.8	15.5 ± 14.4	8.0	9.0
	4	8.7 ± 6.6	6.7 ± 7.0	-	17.0	17.4 ± 19.0	-	-
	5	24.3 ± 24.4	7.5 ± 7.1	2.0	87.0	21.1 ± 19.5	48.0 ± 34.3	-
	6	2.0	2.7 ± 0.9	-	7.0	18.7 ± 16.3	21.5 ± 9.2	-
	7	13.5 ± 3.5	9.7 ± 14.9	-	-	21.3 ± 20.0	25.0 ± 10.4	-
	8	-	11.0	-	-	19.1 ± 16.7	13.0	-
CCI	**0**	10.2 ± 7.8	7.6 ± 8.4	97.0	43.0	24.5 ± 24.7	55.0 ± 54.5	-
	1	28.7 ± 25.5	10.3 ± 8.0	29.0	33.7 ± 32.6	23.6 ± 20.5	40.7 ± 24.7	1.0
	2	-	5.8 ± 6.5	1.0	15.6 ± 16.2	15.6 ± 16.2	33.0 ± 44.2	17.0
	3	14.3 ± 11.6	13.2 ± 13.3	-	15.2 ± 9.3	15.2 ± 9.3	31.0 ± 4.2	-
	4	-	2.5 ± 0.7	-	8.7 ± 7.6	8.7 ± 7.6	37.0	-
	5	-	-	-	7.0	7.0	15.0	-
	6	-	-	-	-	-	-	-
	7	-	4.0	2.0	-	-	-	-
	8	2.0	-	-	-	-	-	-

HCT-CI	**0**	10.2 ± 7.8	8.1 ± 8.4	49.0 ± 67.9	43.0	24.5 ± 24.7	55.0 ± 54.5	-
	1	37.0 ± 29.7	9.5 ± 6.7	29.0	32.0 ± 36.3	20.6 ± 17.9	39.7 ± 24.9	1.0
	2	14.0 ± 2.8	9.6 ± 11.7	-	-	26.2 ± 22.3	17.3 ± 17.0	17.0
	3	25.0	8.5 ± 11.1	-	24.5 ± 24.7	15.8 ± 19.6	56.0 ± 39.6	-
	4	2.0	8.6 ± 9.6	-	-	7.0	15.0	-
	5	2.0	2.0	-	-	8.7 ± 7.6	37.0	-
	6	-	-	-	13.0	-	-	-
	7	-	-	-	-	-	-	-
	8	-	-	2.0	-	-	-	-
MDS-CI	**0**	13.8 ± 13.8	8.0 ± 7.9	97.0	61.0 ± 25.5	26.8 ± 24.4	57.4 ± 47.5	17.0
	1	-	10.1 ± 13.0	1.0	-	27.7 ± 32.5	37.0	1.0
	2	7.0 ± 7.1	9.3 ± 7.4	29.0	21.7 ± 25.0	15.6 ± 13.7	30.8 ± 26.3	-
	3	13.5 ± 16.3	7.4 ± 10.1	-	50.0 ± 52.3	11.6 ± 7.7	15.0	-
	4	-	-	2.0	-	-	-	-
ACE-27	**0**	9.3 ± 8.7	8.6 ± 9.1	49.0 ± 67.9	-	29.9 ± 25.1	55.0 ± 54.5	-
	1	12.7 ± 4.6	10.7 ± 8.1	29.0	41.3 ± 31.8	17.0 ± 17.7	67.0	1.0
	2	-	6.2 ± 8.1	-	39.5 ± 31.8	11.5 ± 11.6	17.5 ± 13.9	-
	3	20.6 ± 23.1	9.7 ± 9.6	2.0	27.0 ± 40.3	18.7 ± 19.4	39.6 ± 26.2	17.0

3.4. To analyse and evaluate patient survival according to ECOG, CFS, the comorbidity indices (CCI, HCT-CI, MDS-CI and ACE-27) and the WHO2016 subgroups

The analysis of the results showed a statistically significant difference in overall survival according to the WHO 2016 classification and ECOG ($p < 0.001$), CFS ($p < 0.001$), CCI ($p < 0.001$), HCT-Cl ($p < 0.001$), MDS-Cl ($p < 0.001$) and ACE-27 ($p < 0.001$) (Table 7).

In patients with RAEB2, MDS-RS-SLD, MDS-RS-MLD and MDS-5q, a decrease in survival was observed with an increase in the ECOG score..

Survival analysis according to the WHO2016 classification and CFS found that "unfrail" patients in all groups had better survival than "frail" and very "frail" patients in the same group. The survival of "unfrail" (CFS = 3) and RAEB-1 patients (*16.3 ± 7.8 months*) was comparable to that of "frail" (CFS = 4) and MDS-SLD patients (*17.0 months*).

The analysis according to the WHO2016 classification and CCI revealed a decrease in survival with increasing CCI score in patients with MDS-RS-SLD, MDS-SLD, MDS-MLD and MDS-5q. The survival of patients with RAEB-2 and CCI = 0 (*7.6 ± 8.4 months*) is comparable to that of patients with MDS-MLD and CCI = 4 (*8.7 ± 7.6 months*).

In the analysis according to the WHO2016 classification and HCT-CI, we found shorter survival in high-risk patients compared to patients with low and intermediate HCT-CI risk. The survival of patients with high-risk HCT-CI and RAEB-2 (*8.6 ± 9.6 months*) was comparable to patients with high-risk HCT-CI and MDS-MLD (*8.6 ± 7.6 months*).

In patients with MDS-5q, MDS-RS-SLD and MDS-MLD, a decrease in survival was observed with an increase in MDS-Cl risk. The survival of low-risk MDS-CI and RAEB-1 patients (*13.8 ± 13.8 months*) was comparable to that of a patient with intermediate MDS-CI risk and MDS-MLD (*15.6 ± 13.7 months*). There is a tendency to decrease survival with increasing ACE value 27.

Table 7. Comparative analysis of the distribution on survival of patients with MDS according to the ECOG, comorbidity and "frailty" scales and WHO2016 groups ($p < 0.001$)

Scales		Survival (mean ± SD)							
		RAEB1	RAEB2	MDS-RS-SLD	MDS-SLD	MDS-5q	MDS-MLD	MDS-U	MDS-RS-MLD
ECOG	0	13.4 ± 7.9	11.7 ± 9.3	97.0	32.2 ± 24.7	58.0 ± 52.9	20.9 ± 19.8	9.0 ± 11.3	29.0
	1	15.6 ± 18.9	7.0 ± 6.9	1.0	34.3 ± 39.0	46.5 ± 35.3	19.6 ± 18.7	-	2.0
	2	5.0 ± 5.2	6.3 ± 9.5	-	44.0 ± 60.8	23.4 ± 11.2	25.4 ± 29.6	-	-
	3	16.0	-	-	-	20.0	18.3 ± 17.0	-	-

CFS	**1**	6.0	7.8 ± 10.0	97.0	1.0	-	31.0 ± 35.8	-	-
	2	12.0 ± 9.2	15.5 ± 8.8	1.0	52.0 ± 14.1	74.7 ± 50.5	28.5 ± 25.5	-	-
	3	16.3 ± 7.8	9.1 ± 9.1	-	34.0 ± 34.8	8.0	15.5 ± 14.4	9.0 ± 11.3	29.0
	4	8.7 ± 6.6	6.7 ± 7.0	-	17.0	-	17.4 ± 19.0	-	-
	5	24.3 ± 24.3	7.5 ± 7.1	-	87.0	48.0 ± 34.3	21.1 ± 19.5	-	2.0
	6	2.0	2.7 ± 0.9	-	7.0	21.5 ± 9.2	18.7 ± 16.3	-	-
	7	13.5 ± 3.5	9.7 ± 14.9	-	-	25.0 ± 10.4	21.3 ± 20.1	-	-
	8	-	11.0	-	-	13.0	19.1 ± 16.7	-	-
CCI	**0**	10.2 ± 7.7	7.6 ± 8.4	97.0	43.0	55.0 ± 54.5	24.5 ± 24.7	-	-
	1	28.7 ± 25.5	10.3 ± 8.0	-	33.7 ± 32.7	40.6 ± 24.7	23.6 ± 20.5	1.0	29.0
	2	-	5.8 ± 6.5	1.0	7.0	33.0 ± 44.2	15.6 ± 16.2	17.0	-
	3	14.3 ± 11.6	13.2 ± 13.3	-	-	31.0 ± 4.2	15.2 ± 9.3	-	-
	4	-	2.5 ± 0.7	-	13.0	37.0	8.7 ± 7.6	-	-
	5	-	-	-	-	15.0	7.0	-	-
	6	-	-	-	-	-	-	-	-
	7	-	4.0	-	-	-	-	-	2.0
	8	2.0	-	-	-	-	-	-	-

HCT-CI	**0**	10.2 ± 7.8	8.1 ± 8.4	49.0 ± 67.9	43.0	55.0 ± 54.5	24.5 ± 24.7	-	-
	1	37.0 ± 29.7	9.5 ± 6.6	-	32.0 ± 36.2	39.7 ± 24.9	20.6 ± 17.9	1.0	29.0
	2	14.0 ± 2.8	9.6 ± 11.7	-	-	17.3 ± 17.0	26.2 ± 22.3	17.0	-
	3	25.0	8.5 ± 11.1	-	24.5 ± 24.7	56.0 ± 39.6	15.8 ± 19.6	-	-
	4	2.0	8.6 ± 9.6	-	-	15.0	7.0	-	-
	5	2.0	2.0	-	-	37.0	8.6 ± 7.6	-	-
	6	-	-	-	13.0	-	-	-	-
	7	-	-	-	87.0	-	-	-	-
	8	-	-	-	-	-	-	-	2.0
MDS-CI	**0**	13.8 ± 13.8	8.0 ± 7.9	97.0	61.0 ± 25.5	57.4 ± 47.5	26.8 ± 24.4	-	-
	1	-	10.1 ± 13.0	1.0	-	37.0	27.7 ± 32.5	17.0	29.0
	2	7.0 ± 7.1	9.3 ± 7.4	-	21.7 ± 25.0	30.8 ± 26.3	15.6 ± 13.7	1.0	-
	3	13.5 ± 16.3	7.4 ± 10.1	-	50.0 ± 52.3	15.0	11.6 ± 7.7	-	-
	4	-	-	-	-	-	-	-	2.0
ACE-27	**0**	9.3 ± 8.7	8.6 ± 9.1	49.0 ± 67.9	-	55.0 ± 54.5	29.9 ± 25.1	-	-
	1	12.7 ± 4.6	10.7 ± 8.1	-	41.3 ± 31.8	67.0	17.0 ± 17.7	1.0	29.0
	2	-	6.2 ± 8.1	-	39.5 ± 31.8	17.5 ± 13.9	11.5 ± 11.6	-	-
	3	20.6 ± 23.1	9.7 ± 9.6	-	27.0 ± 40.3	39.6 ± 26.2	18.7 ± 19.4	17.0	2.0

3.5. To analyse and assess survival according to ECOG, comorbidity indices and the "frailty" scale and WPSS risk groups

A statistically significant difference in survival was also found in an analysis between the comorbidity indices and "frailty" scale and the risk assessment scales. A statistically significant difference was also found for WPSS and CFS ($p < 0.001$), CCI ($p < 0.001$), HCT-Cl ($p < 0.001$), MDS-Cl ($p < 0.001$) and ACE-27 ($p < 0.001$)) (Table 8).

Longer survival was observed with very low and low risk group according to WPSS at ECOG = 0 ($p < 0.001$).

In the analysis with WPSS and CFS, we found a decrease in survival with an increasing CFS score. In patients with CFS = 1 and very low WPSS risk, survival reached *97.0 months*, while in CFS = 1 and very high risk the risk was only *2.5 ± 2.1 months*. In very "frail" patients (CFS > 5) with a very low WPSS risk, the survival rate reached *20.0 months*, in contrast to CFS > 5 and a very high WPSS risk, where it reached only *1.0 month*. The mean survival of patients with low WPSS risk and CFS > 5 (very "frail") (*13 months*) was comparable to that of very high WPSS risk and CFS = 2 ("unfrail") (*11.7 ± 9.7 months*).

Table 8. Comparative analysis on survival distribution according to CFS, ECOG and WPSS ($p < 0.001$)

Scales		Survival (mean ± SD)				
		Very low risk	Low risk	Intermediate risk	High risk	Very high risk
ECOG	0	70.0 ± 38.2	40.8 ± 40.3	23.8 ± 21.9	13.9 ± 10.3	9.8 ± 6.6
	1	-	39.0 ± 34.2	19.4 ± 13.9	8.9 ± 7.1	12.3 ± 17.4
	2	-	29.3 ± 28.3	31.4 ± 34.2	12.1 ± 15.6	3.3 ± 3.8
	3	20.0	-	22.0 ± 21.2	15.0 ± 13.5	-

CFS	**1**	97.0	1.0	34.4 ± 41.4	14.2 ± 15.4	2.5 ± 2.1
	2	-	54.8 ± 43.3	34.3 ± 27.4	16.2 ± 8.5	11.7 ± 9.7
	3	43.0	23.6 ± 31.5	18.9 ± 16.1	10.8 ± 9.8	10.0 ± 4.9
	4	-	46.5 ± 41.7	16.7 ± 15.1	11.7 ± 5.3	2.2 ± 1.3
	5	-	48.8 ± 34.9	16.5 ± 14.6	8.5 ± 7.8	20.8 ± 20.3
	6	-	16.7 ± 10.6	18.7 ± 16.3	2.8 ± 0.9	2.0
	7	20.0	27.5 ± 13.4	31.7 ± 21.5	12.3 ± 12.1	1.0
	8	-	13.0	19.7 ± 11.2	23.7 ± 23.8	11.0

Analysis of WPSS and CCI has shown that increasing the CCI score reduces survival. In patients with low WPSS risk but different CCI scores, survival differed – CCI = 0 (*66 months*), CCI = 1 (*26 months*) and CCI > 2 (*25 months*). There was an impressive decrease in survival with increasing CCI score in the same group (Fig. 23).

In the analysis against WPSS and MDS-CI it was found that patients with low WPSS risk, but with different MDS-CI score differ significantly in survival-MDS-CI = 0 (*69 months*), MDS-CI = 1 (*26 months*) and MDS-CI > 2 (*2 months*). Reduction in survival with an increasing MDS-CI score in WPSS risk groups is impressive (Fig. 23).

An analysis of WPSS and HCT-CI revealed a significant difference in the survival of the individual groups with an increasing HCT-CI score. In patients with low WPSS risk but with different HCT-CI scores, survival differed significantly – HCT-CI = 0 (*50 months*), HCT-CI = 1 (*34 months*) and HCT-CI > 2 (*13 months*). There was a decrease in survival with increasing HCT-CI score in the risk groups of WPSS (Fig. 23).

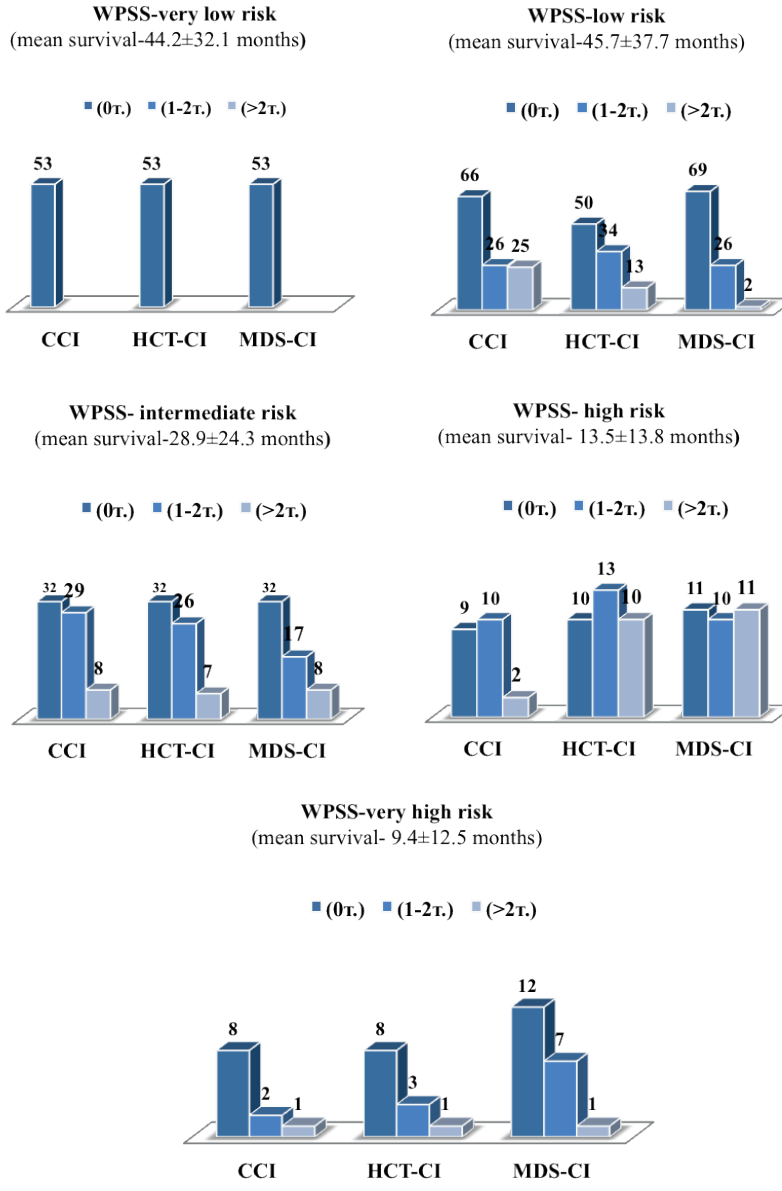


Figure 23. Comparative analysis of mean survival according to the score on the comorbidity scales and WPSS ($p < 0.001$)

The analysis with WPSS and ACE-27 showed a decrease in survival in the very low, low and intermediate risk groups with increasing comorbidity severity. The tendency is maintained with high and very high risk groups.

In an analysis of all available scales, we found a decrease in survival with an increase in the degree of risk compared to WPSS and with an increase in the score on each system.

3.6. To analyse and assess survival according to ECOG, comorbidity and "frailty" scales and IPSS risk groups

A statistically significant difference in overall survival was also found in the risk assessment against IPSS and ECOG ($p < 0.001$), CFS ($p < 0.001$), CCI ($p < 0.001$), HCT-Cl ($p < 0.001$), MDS-Cl $p < 0.001$) and ACE-27 ($p < 0.001$) (Table 9).

In the analysis according to ECOG and IPSS, we found that with increasing the IPSS risk and increasing ECOG, survival decreases. The most reliable data are from a group with ECOG = 0. In patients with low IPSS risk and ECOG = 0, the survival was *42.3 ± 35.3 months*, differing from that in patients with low IPSS risk and ECOG = 3–*28.5 ± 12.0 months*. Similar results are found in other IPSS risk groups. The survival of patients with intermediate-1 IPSS risk and ECOG = 3 (*13.0 ± 11.7 months*) was comparable to high IPSS risk and ECOG = 1 (*11.5 ± 10.1 months*). Survival analysis according to CFS and IPSS shows that with increasing CFS, survival decreases.

Table 9. Comparative analysis of the distribution according to the "frailty" scale and ECOG and IPSS ($p < 0.001$)

Scales		Survival (mean ± SD)			
		Low risk	Intermediate 1 risk	Intermediate 2 risk	High risk
ECOG	0	42.3 ± 35.3	21.4 ± 21.1	12.5 ± 9.8	8.4 ± 6.3
	1	26.3 ± 28.7	21.3 ± 20.4	8.0 ± 12.3	11.5 ± 10.1
	2	19.7 ± 15.2	34.9 ± 34.7	6.3 ± 8.7	6.0 ± 7.1
	3	28.5 ± 12.0	13.0 ± 11.7	-	-

CFS	**1**	26.0 ± 16.9	38.3 ± 44.7	8.8 ± 11.3	2.5 ± 2.1
	2	61.2 ± 42.9	27.0 ± 20.7	14.7 ± 8.7	16.0
	3	23.8 ± 18.7	18.4 ± 19.2	8.0 ± 10.3	9.5 ± 5.8
	4	25.8 ± 31.7	15.1 ± 9.8	7.6 ± 7.2	2.5 ± 2.1
	5	26.6 ± 28.8	37.0 ± 30.3	8.7 ± 14.7	16.0 ± 9.4
	6	17.0 ± 22.6	12.7 ± 10.8	2.6 ± 0.9	-
	7	28.0 ± 10.4	16.8 ± 17.1	12.7 ± 16.8	1.0
	8	13.0	27.5 ± 18.1	10.0 ± 4.2	11.0

In an analysis of IPSS and CCI, we found that survival decreased with increasing the CCI score in each individual IPSS group. We found that in the group with low IPSS risk and different CCI score, survival differed significantly (CCI = 0 reaches *45 months*, CCI = 1 is *32 months* and in CCI > 2 only *16 months*) (Fig. 24).

In an analysis of IPSS and HCT-CI, we found that survival also decreased with increasing the HCT-CI score in a given IPSS group. We found that between the different risk groups for HCT-CI survival differed (in the group with low IPSS risk and different HCT-CI = 0 it reached *41 months*, HCT-CI = 1 was *26 months* and in HCT-CI > 2 only *17 months* at low IPSS risk) (Fig. 24).

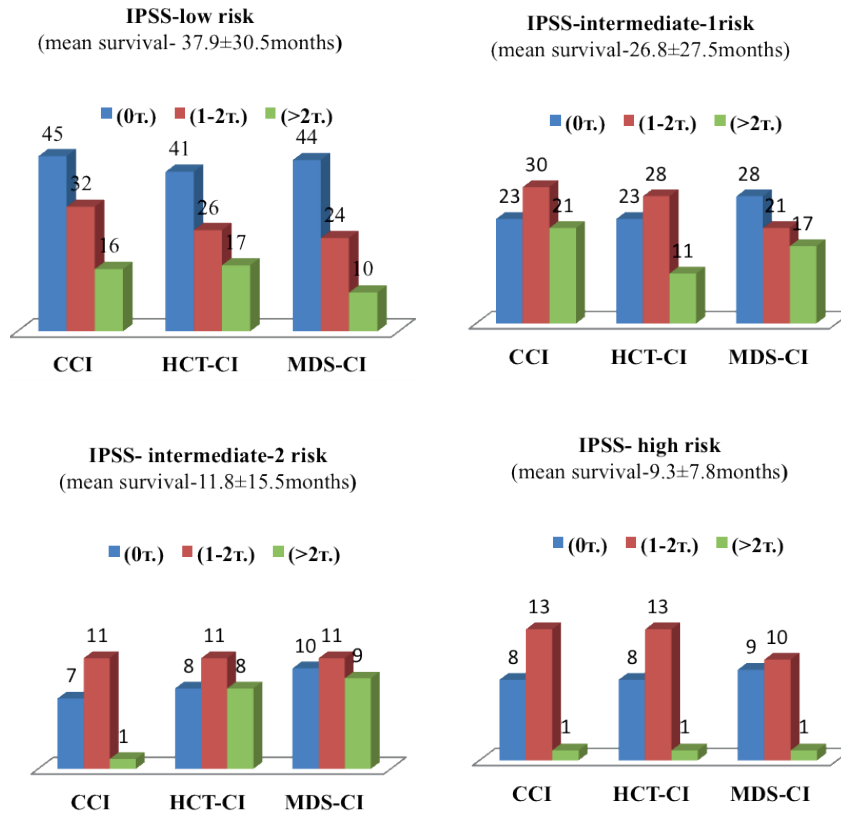


Figure 24. Comparative analysis of survival according to the score of the comorbidity scales and IPSS risk groups ($p < 0.001$)

In an analysis of IPSS and MDS-CI, we found that survival also decreased with an increasing MDS-CI score in certain IPSS groups. We found that between the individual MDS-CI scores survival differed (MDS-CI = 0 reaches *44 months*, MDS-CI = 1 is *24 months* and in MDS-CI > 2 only *10 months* with low IPSS risk) (Fig.24). The results are similar for the other IPSS risk groups.

An analysis of ACE-27 and IPSS found that the degree of comorbidities affected survival. With the appearance and deepening of the severity of comorbidity, survival is reduced, with the most indicative data from the

high-risk group (*8.3 ± 8.9 months* for ACE-27 = 0 compared to *1.0 month* for ACE-27 = 3) (Table 10).

Table 10. Comparative analysis of the distribution of survival according to ACE-27 and IPSS ($p < 0.001$)

Scale		Survival (mean ± SD)			
		Low risk	Intermediate 1 risk	Intermediate 2 risk	High risk
ACE-27	0	41.5 ± 36.5	30.7 ± 28.8	7.7 ± 8.4	8.3 ± 8.9
	1	26.6 ± 19.0	23.7 ± 23.0	9.3 ± 9.3	13.3 ± 5.3
	2	24.4 ± 24.2	12.5 ± 11.7	6.8 ± 8.5	-
	3	26.7 ± 28.1	20.0 ± 20.2	14.8 ± 16.5	1.0

3.7. To analyse and assess patient survival according to ECOG, comorbidity and "frailty" scales and IPSS-R risk groups

A statistically significant difference in overall survival was also found in the analysis of IPSS-R and ECOG ($p < 0.001$), CFS ($p < 0.001$), CCI ($p < 0.001$), HCT-Cl ($p < 0.001$), MDS-Cl $p < 0.001$) and ACE-27 ($p < 0.001$).

There are 6 patients in the group of very low IPSS-R risk with a survival curve that has not yet been reached.

In the analysis according to ECOG and IPSS-R, we found that with increasing ECOG, survival decreases in the groups with low, intermediate and very high risk. Patients with ECOG = 1 and low IPSS-R risk had survival (*56.1 ± 24.9 month*s) in contrast to patients with ECOG = 1 and very high IPSS-R risk (*7.6 ± 12.3 months*). The mean survival of patients with low IPSS-R risk and ECOG = 3 (*10.5 ± 13.4 months*) is comparable to that of patients with very high IPSS-R risk and ECOG = 0 (*9.4 ± 7.8 months*) (Table 11).

Survival analysis according to CFS and IPSS-R showed that in very "frail" (CFS = 8) patients at low risk (*21.7 ± 7.7 months*), the average survival could be compared with that of "frail" (CFS = 2) high-risk patients (*20.0 ± 4.1 months*) (Table 11).

The tendency is maintained when comparing survival to CCI, HCT-Cl, MDS-Cl and IPSS-R (Fig.25).

Table 11. Comparative analysis of survival distribution according to ECOG, CFS and IPSS-R ($p < 0.001$)

Scale		Survival (mean ± SD)			
		Low risk	Intermediate risk	High risk	Very high risk
ECOG	0	31.9 ± 34.6	27.4 ± 23.3	14.2 ± 9.3	9.4 ± 7.8
	1	56.1 ± 24.9	16.9 ± 15.4	10.8 ± 7.3	7.6 ± 12.3
	2	16.4 ± 12.7	43.0 ± 34.7	2.8 ± 1.3	7.7 ± 9.5
	3	10.5 ± 13.4	22.0 ± 21.2	22.0 ± 21.2	-
CFS	1	7.5 ± 5.4	52.5 ± 39.2	4.5 ± 4.9	2.0 ± 1.07
	2	52.7 ± 39.8	26.8 ± 22.9	20.0 ± 4.1	11.8 ± 8.7
	3	25.6 ± 26.7	16.7 ± 13.4	14.0 ± 10.8	7.3 ± 7.1
	4	39.0 ± 52.3	16.9 ± 12.5	14.8 ± 3.8	3.5 ± 2.3
	5	60.3 ± 24.0	26.3 ± 27.4	9.2 ± 7.7	11.1 ± 15.6
	6	22.0	16.8 ± 13.6	2.8 ± 0.9	2.0
	7	16.8 ± 13.7	32.7 ± 19.8	12.3 ± 12.3	12.7 ± 16.8
	8	21.7 ± 7.7	29.0 ± 31.1	-	10.3 ± 3.1

Survival analysis according to MDS-Cl and IPSS-R revealed a shorter survival in the high-risk MDS-Cl group compared to low-risk patients. We found that the mean survival in the low IPSS-R risk group was *40 months*. When comparing the survival of patients in this group, differing in MDS-Cl risk, we found differences in survival according to MDS-Cl – MDS-Cl = 0 (*36 months*), MDS-Cl = 1–2 (*28 months*) and MDS-Cl > 2 (*7 months*). With an increasing MDS-Cl score, survival in all IPSS-R risk groups is reduced. The mean survival in patients with low IPSS-R risk and MDS-Cl = 3 was *7.0 ± 5.6 months* and was comparable to that in patients with very high IPSS-R risk and MDS-Cl = 1–2 – *7.3 ± 5.2 months* (Fig.25)

In HCT-CI and IPSS-R survival analysis, we found that patient survival differed in the HCT-CI score. We found significant differences in survival according to the score – HCT-CI = 0 (*34 months*), HCT-CI = 1–2 (*27 months*) and HCT-CI > 2 (*13 months*) at low IPSS-R risk. There is a similar tendency in the other IPSS-R risk groups (Fig. 25).

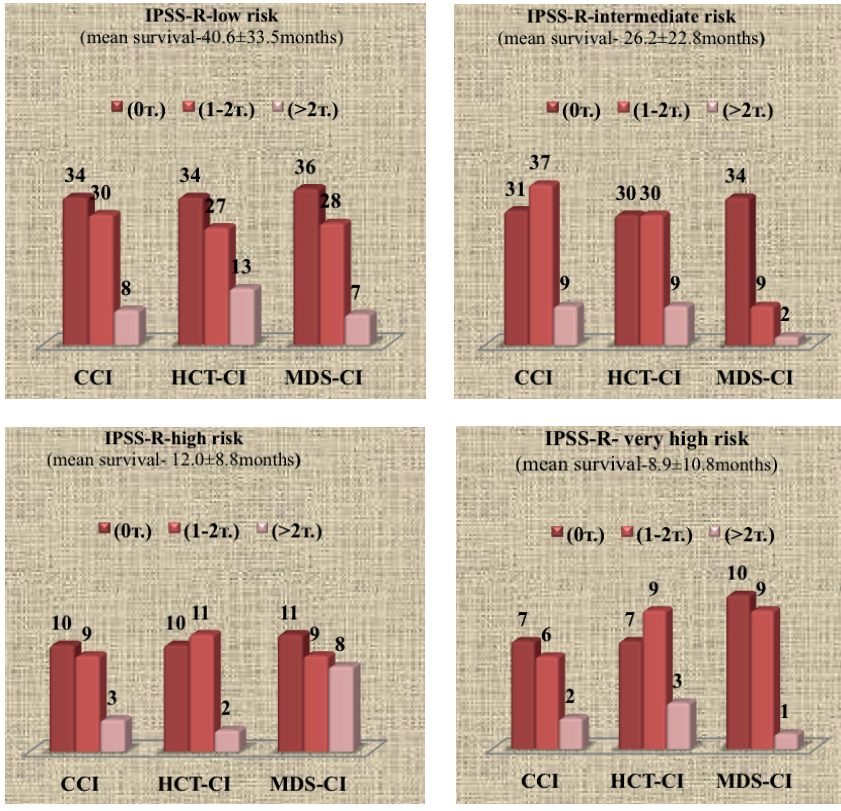


Figure 25. Comparative analysis of mean survival according to the score of the comorbidity scales and IPSS-R risk groups ($p < 0.001$)

Based on the analysis of ACE-27 and IPSS-R, it was found that increasing the risk group reduces survival at all stages of ACE-27. At ACE-27 = 0 at low risk it is *37.1 ± 35.9 months* compared to ACE-27 = 0 at very high risk is only *7.2 ± 8.4 months* (Table 12).

Table 12. Comparative analysis of survival distribution according to ACE-27 and IPSS-R risk groups ($p < 0.001$)

Scale		Survival (mean ± SD)			
		Low risk	Intermediate risk	High risk	Very high risk
ACE-27	0	37.1 ± 35.9	35.8 ± 30.5	11.7 ± 8.2	7.2 ± 8.4
	1	32.1 ± 27.2	23.7 ± 22.2	13.4 ± 8.8	10.0 ± 8.3
	2	21.8 ± 22.4	14.6 ± 11.1	10.1 ± 10.8	3.9 ± 2.7
	3	32.4 ± 34.7	21.4 ± 19.8	11.7 ± 9.3	12.0 ± 18.4

4. *To assess the relationship between risk assessment scales and comorbidity and "frailty" scales in patients with MDS*

When assessing the relationship between IPSS and the comorbidity and 'frailty' scales in patients with MDS, no dependence or difference in comorbidity risk was found.

A positive weak correlation was found between IPSS-R and CFS, which shows that as the degree of CFS increases, so does the risk according to IPSS-R ($r = 0.148$; $p = 0.033$) (Fig. 26).

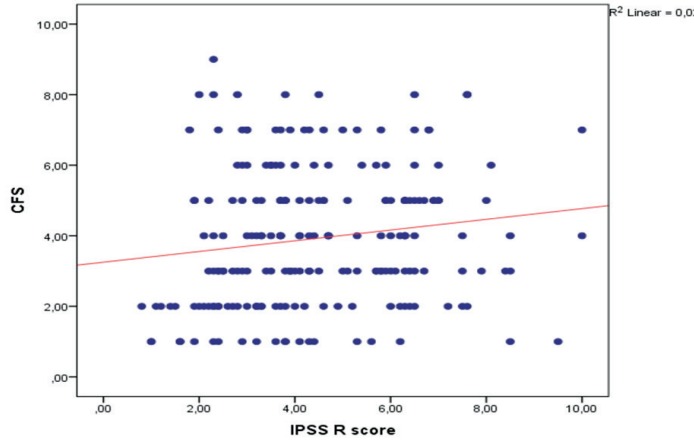


Figure 26. Correlation analysis between IPSS-R and CFS ($r = 0.148$; $p = 0.033$)

A difference was also found in the risk analysis according to WPSS and

MDS-Cl (p = 0.05) (Table 13). All patients at very low risk fall into the group of MDS-Cl = 0. It is observed that with an increase in risk according to WPSS, there is an increase in the number of patients in groups 1 to 3 on MDS-Cl .

Table 13. Distribution of patients according to WPSS and MDS Cl (p = 0.05)

WPSS	MDS Cl			
	0	1	2	3
Very low risk	5	-	-	-
Low risk	12	5	17	5
Intermediate risk	24	2	26	7
High risk	36	10	21	12
Very high risk	13	-	11	1

5. To *study and analyse the transformation of MDS into AML and to assess patient survival before and after the transformation*

From the analysis we found that *22.4%* (n = 49) of the patients transformed into AML. The time from diagnosis to transformation averaged *16.3 ± 19.8 months* (1–100 months).

After transformation into AML, patients with MDS survive significantly less than *3.1 ± 5.1 months* (0–34 months), which is 6 times less than their survival before the transformation (*18.0 ± 20.1 months*) (p < 0.001).

In the analysis of the patients who transformed into AML, it was found that they were younger compared to those who did not undergo transformation (64.7 years to 72.8 years, respectively; p < 0.001). From the point of view of gender, it can be said that men predominate (63.3%), which is the general trend in the studied group of patients with MDS.

The analysis of the patients according to the classifications revealed a statistical difference between the patients who transformed into AML and those who did not undergo transformation.

According to the FAB classification, the majority of patients who transformed into AML had RAEB (63.3%), while the majority of patients with MDS who did not transform were from the group of RA (62.2%) (p = 0.001) (Fig.27).

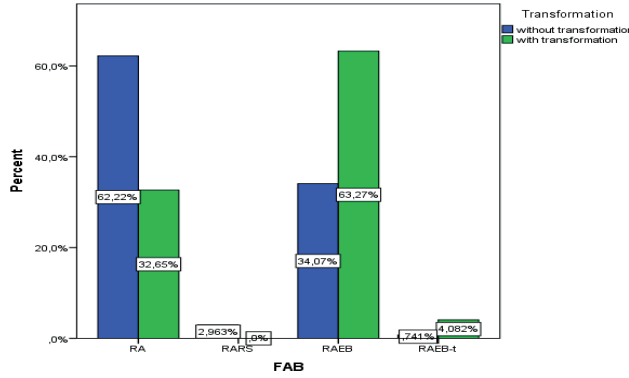


Figure 27. Comparative analysis of the distribution of patients with / without transformation according to the FAB classification (p = 0.001)

A statistically significant difference was observed according to the WHO2008 classification, where 55.1% of patients who transformed into AML were again from the group of RAEB-2, while 45.9% of patients who did not transform belonged to the group RCMD (p = 0.005) (Fig.28)

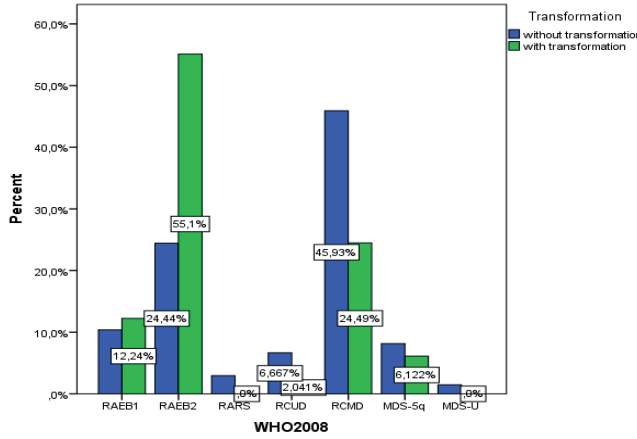


Figure 28. Comparative analysis of the distribution of patients with / without transformation according to the WHO2008 classification (p = 0.005)

The analysis of transformed patients according to the WHO2016 clas-

sification also revealed a significant difference, as 55.1% of patients who transformed into AML were again in the group RAEB-2 and 45.9% of patients with MDS who did not transform belonged to the group MDS-MLD (p = 0.01) (Fig.29).

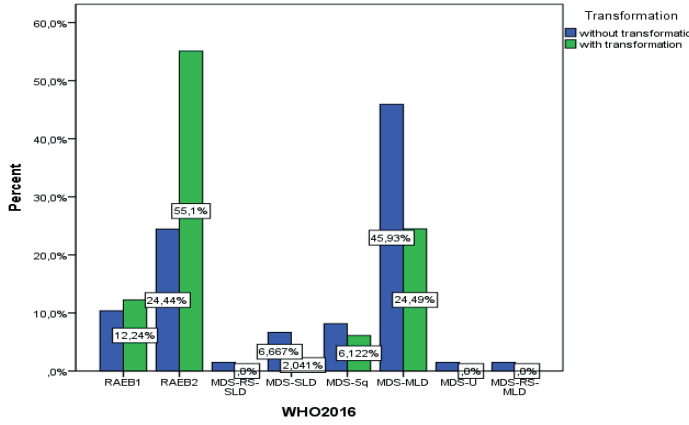


Figure 29. Comparative analysis of the distribution of patients with / without transformation according to the WHO2016 classification (p = 0.01)

According to the risk assessment scales, it can be said that 51.1% of the patients who transformed into AML according to WPSS are at high risk (p = 0.011), according to IPSS 48.9% are at intermediate-2 risk (p = 0.001) and according to IPSS-R – 42.6% are at very high risk (p = 0.013) (Fig.30, Fig.31 and Fig.32).

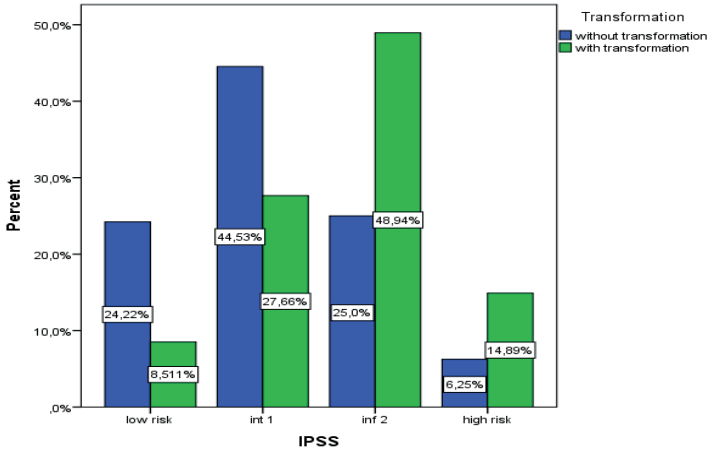


Figure 30. Comparative analysis of the distribution of patients with / without transformation according to IPSS (p = 0.001)

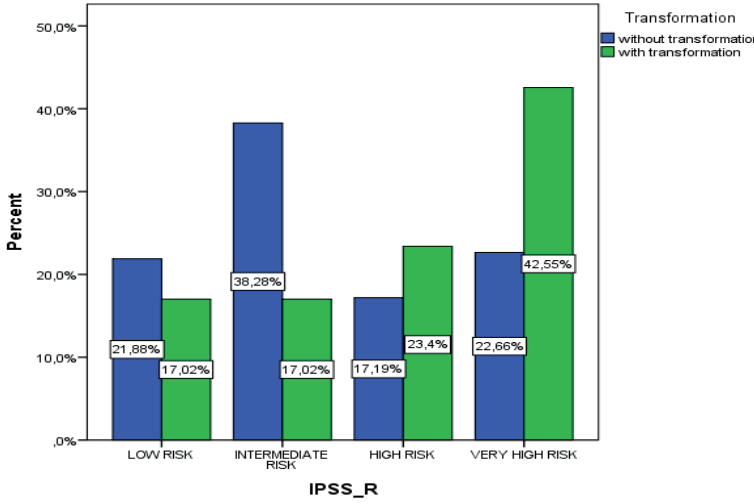


Figure 31. Comparative analysis of the distribution of patients with / without transformation according to IPSS-R (p = 0.013)

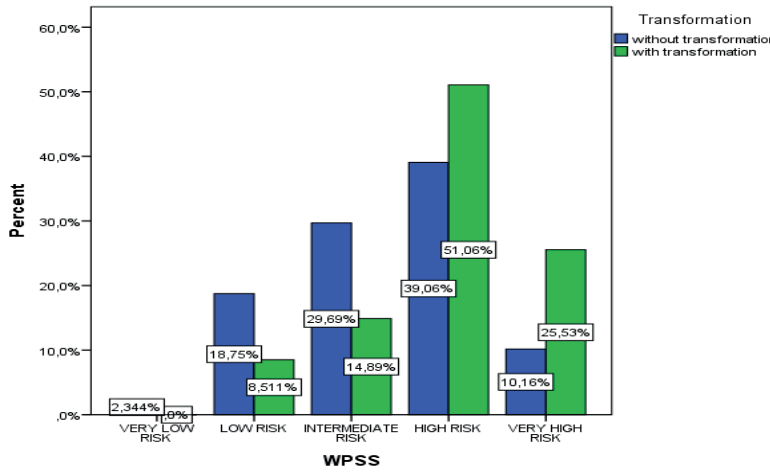


Figure 32. Comparative analysis of the distribution of patients with / without transformation according to WPSS (p = 0.011)

The assessment of patients who transformed into AML did not reveal a significant difference between them and those who did not transform according to the comorbidity and "frailty" scales.

In the analysis of laboratory parameters, a significant difference was found with respect to the level of leukocytes, which is lower in transformed patients (respectively 3.4 to 4.8; p = 0.025), as well as with respect to ANC, where again the values are lower in transformed patients (1.2 to 1.9, respectively; p = 0.011).

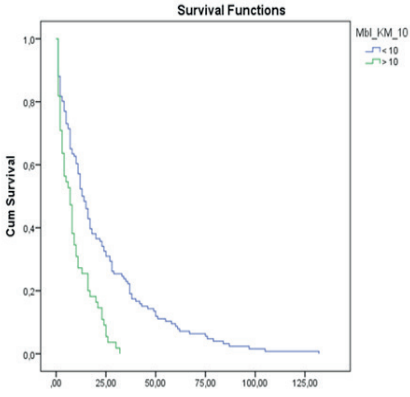
Patients who transformed into AML had a significantly lower percentage of segmental neutrophils (42.6 to 51.7; p = 0.004), a higher percentage of lymphocytes (43.3 to 35.9; p = 0.014), and a lower creatinine (80.6 to 97.5 ; p = 0.006), lower value of beta2 microglobulin (2.9 to 3.9; p = 0.007).

6. *To derive the prognostic factors for survival in patients with MDS*

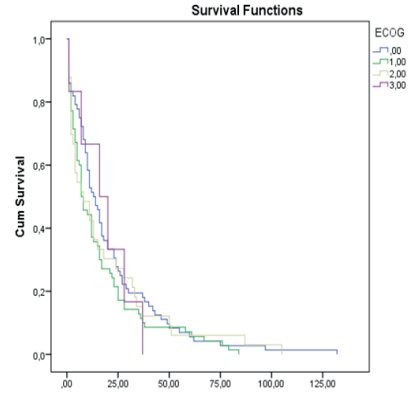
After analyzing the results of the conducted examinations, the following main profiles of patients with MDS can be deduced (Table 14).

Table 14. Prognostic indicators for survival in patients with MDS

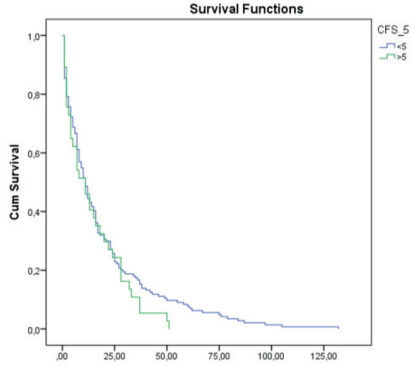
Factors with unfavorable prognosis	Factors with favorable prognosis
• Age (31–40 г.) and > 80 years • Men • Secondary MDS • Hemoglobin < 80 g/l • Leukocyte < 4 x 10^9/l и > 10 x 10^9/l • LDH > 380IU • Albumin < 35 g/l • Dysplasia – 3 • Myeloblasts in BM > 10% • RBC transfusions > 4E/monthly • IPSS – high risk • IPSS-R – high and very high risk • WPSS – very high risk • FAB – RAEB • WHO2008, WHO2016 – RAEB1, RAEB2 • del(20q), abnormalities in 7 and complex karyotype • high-risk cytogenetic abnormalities • transformation up to 8 months. • ECOG – 3 • CFS > 5	• Age 51–60 years • Female • Hemoglobin > 100 g/l • Dysplasia – 1 • Normal karyotype or del(5q) • low-risk cytogenetic abnormalities • IPSS – low risk • IPSS-R – very low and low risk • WPSS – low risk • WHO2008, WHO2016 – MDS-5q • ECOG – 0–1 • ACE 27 score – 0–1



Overall survival and myeloblasts in BM



Overall survival and ECOG



Overall survival and CFS

Figure 33. Overall survival according to some risk factors (BM blasts, ECOG and CFS)

V. DISCUSSION

MDS is a heterogeneous group of clonal diseases of the pluripotent hematopoietic stem cell with significant morbidity in elderly patients and high mortality. The heterogeneous course of the disease can be explained by examining and analyzing the potential risk and prognostic factors associated with the patient and the disease.

Age is one of the main risk factors for its development**.** In 2007, Xiaomei Ma and colleagues found that about 86% of patients were over the age of 60 at the time of diagnosis. They report a mean patient age of -71 years *(Ma X et al, 2007)*. Our analysis found that the mean age of patients diagnosed with the disease was 70.7 ± 10.2 years (35–93 years), with patients over 60 years of age predominating (84.9%). Our results are comparable with data from a study by Sekeres and colleagues who reported a mean age of male patients of 71 years with a predominance of males. (*Sekeres MA et al, 2008).* Similar data were reported in the study by Alicia Marsà and co-authors, who analyzed an impressive number of 33,091 patients with MDS for the period 2008–2015 establishing an average incidence age of 81 years *(Alicia Marsà et al, 2020).* The results of another Xiaomei Ma study conducted in 2012 show a sharp increase in the incidence of morbidity after the sixth decade of life, as well as a re-prevalence of men over women. (*Ma X. 2012).* In our analysis we also found a predominance of males. Lee and colleagues conducted a retrospective analysis of 227 patients with MDS to determine prognostic factors for survival and risk of AML transformation. They report the prevalence of men (63%) over women (37%). In contrast to our data, this study by Lee and colleagues showed that the average age of patients in the Korean group was 57. There is a significant difference in age compared to patients in Western countries *(Lee J H et al, 2003).* Another retrospective study by Müller-Berndorff and colleagues analyzed 89 patients with primary MDS also in order to identify the prognostic factors determining overall survival which proves the average age of patients is 63 (26–85 years) *(Müller-Berndorff H et al, 2006).* Based on a large number of studies, it is proven that age is one of the main risks and prognostic factors in MDS.

In our analysis, we found that the majority of patients had de novo

(93.6%) and only 6.4% had secondary MDS. Aul and colleagues analyzed 584 patients with MDS and found that only 5.3% of patients had secondary MDS and a history of previous cytotoxic chemotherapy and / or radiation therapy. (*Aul C et al, 1992).* Our analysis showed that 78.5% (n = 11) of patients with secondary MDS (n = 14) had a history of previous chemotherapy / radiation therapy. Exposure to toxic agents is a risk factor for the development of secondary MDS.

The heterogeneous course of the disease leads to the need to group patients according to established classification systems in order to determine the prognosis and risk. In an analysis of the FAB classification, we found a predominance of RA patients followed by RAEB. Similar results were presented in 1997 by Elizabeth Souto and team, who conducted a study with 59 patients with MDS in order to establish indicators with a prognostic value. When determining the type of MDS according to the FAB classification, the researchers found that 33.9% had RA, 20.3% – RARS, 27.1% – RAEB, 3.4% – RAEB-t, 8.5% – CMML and 6.8% had an indeterminate . They found significant differences in life expectancy between the different subtypes. They reported a mean survival of 88.7 months for for RA, RARS-57.4 months, for RAEB-24.2 months, for RAEB-t-3.4 months, and for CMML-31.1 months. The study by Elizabeth Souto and team was dominated by RA patients followed by RAEB. When comparing survival, it was found to be the longest in RA (*Elizabeth Xisto Souto et al, 1997).* In contrast, our results showed the longest survival in RARS (32.0 ± 39.0 months), but maintained the trend for the shortest survival in RAEB-t (10.3 ± 4.9 months). Similar are the results of a 2012 study by Irina Triantafyllidis and colleagues who analyzed 119 patients with MDS. The researchers reported the distribution in the FAB subgroups to be as follows: RA (42%), RARS (17.6%), RAEB (17.6%), RAEB-t (16.8%) and CMML (5.9%). In the group analyzed by them, the cases with RA predominate again (*Triantafyllidis I et al, 2012).* In contrast to our results and those of Elizabeth Souto and Irina Triantafyllidis et al., another retrospective study by Lee and co-authors found that RAEB patients predominated in the FAB classification, followed by RA patients. The analysis found that 36% of patients had RA, 8% – RARS, 40% – RAEB, 12% – RAEB-t and 4% – CMML.

In an analysis of the WHO2008 classification, researchers found a pre-

dominance of patients with RCMD followed by RAEB1 and RAEB2. (*Lee J H et al, 2003)*. In our WHO analysis, we found a prevalence of patients with RCMD followed by RAEB2.

A retrospective study by Müller-Berndorff et al found that the survival and prognosis in patients with multilinear dysplasia with / without ring sideroblasts differed significantly from that in unilateral dysplasia with / without sideroblasts *(Müller-Berndorff H et al, 2006)*. Our analysis also showed a difference in survival between the MDS-SLD (*39 months*) and MDS-MLD (*28 months*) groups. We found the longest survival in patients with MDS-5q syndrome (*44.8 months*).

In addition to the classification systems, prognostic scoring systems for risk stratification in patients with MDS have been developed and verified over the years. Bektaş and team conducted an analysis in 101 patients with MDS for the period 2003–2011 in order to compare the prognostic scoring systems. The results of their study found that according to IPSS, low-risk patients were 30.7%, intermediate-1 risk patients -40.6%, intermediate-2 risk patients -19.8% and high-risk patients were 8.9%. They prove the predominance of patients at intermediate risk. In our analysis, we also found a predominance of patients at intermediate risk according to IPSS.

In the Bektaş study, according to IPSS-R patients were divided into 5 groups: very low risk-17.8%, low risk-22.8%, intermediate risk-24.8%, high risk-17.8% and very high risk 16.8% of the analyzed patients. In the group analyzed by them, patients with intermediate risk predominate. The data from our analysis are similar. We found a predominance of patients at intermediate risk according to IPSS-R.

In the risk stratification according to WPSS, Bektaş and colleagues report that 7.9% are in the group of very low risk, 30.7% – low risk, 24.8% – intermediate risk, 25.7% – high risk and 10.9% of patients – very high risk. They found an increase in the percentage of patients in the high-risk group when comparing WPSS with IPSS-R. They proved the significance of WPSS ($p < 0.001$) and IPSS-R ($p = 0.037$) in determining overall survival (*Bektaş Ö et al, 2016)*. The results of our analysis also found a predominance of high-risk patients according to WPSS. We found the shortest survival in high-risk patients (9.3 months) according to IPSS ($p < 0.001$), while according to IPSS-R and WPSS at very high risk (8.9 months and

9.4 months) ($p < 0.001$).

The inclusion of age and gender as prognostic factors and their respective interactions contribute to the improvement and individualization of the prognosis. A retrospective multicenter study by Nösslinger and authors examined and analyzed 897 patients with primary MDS in order to determine the prognostic impact of age and gender on survival and to investigate their modulating effect on IPSS outcomes. They found that the low-risk group was dominated by male patients and had a shorter survival rate than women. Their analysis proves that the high-risk group is dominated by younger (≤ 66 years) patients, mostly female (*Nösslinger T et al, 2010)*. In contrast to their results, we found that according to IPSS, the relative share of women in the low-risk group is higher (35.0%), while those with intermediate-1 risk predominate in men (42.1%).

In addition to the classification and risk stratification systems, it has been found that some clinical and biological indicators provide an easy and fast prognostic score for determining the risk and survival in diagnosing patients. (*Bowles KM et al., 2006)*.

A large number of studies prove that the anemic syndrome is a prognostic factor with a negative impact. Malcovati et al. analysed data from 1,344 patients with MDS and demonstrated a significant reduction in overall survival at hemoglobin levels < 90 g / l in men and < 80 g / l in women. These hemoglobin levels increase the risk of morbidity and mortality, mainly due to an increased risk of cardiac complications. *(Malcovati L et al, 2011)*. In a study by Elizabeth Souto and colleagues, hemoglobin values were analysed in the same way as in the work of Sanz et al. (*Sanz G F et al, 1989*), comparing the survival curves of three groups of patients with hemoglobin < 80 g / l, 80–100 g / l and > 100 g / l. No statistical significance of the anemic syndrome has been demonstrated in these groups of patients (*Elizabeth Xisto Souto et al, 1997)*. In our analysis, we did not find a relationship between hemoglobin levels and survival, but we can say that patients with hemoglobin < 80 g / l have a significantly shorter survival than others (16.3 months and 20.7 months, respectively) ($p < 0.05$). In other studies, in the distribution of patients in two groups with Hb < 60 g / l and Hb > 60 g / l, the multivariate analysis showed a statistically significant difference in mean survival (24.4 months versus 49.6 months). These results confirm

that the hemoglobin level is an important prognostic indicator. *(Coiffier B et al, 1987; Mufti G J et al, 1985;. Kerkhofs H et al, 1987; Sanz G F et al, 1989; Riccardi A et al, 1988; Goasguen J E et al, 1990; Tricot G et al, 1986; Van Der Weide M et al, 1988)*.

The results of our analysis revealed a difference in hemoglobin levels according to IPSS-R ($p < 0.001$) and WPSS ($p = 0.003$). The anemic syndrome is the most pronounced in the group with intermediate risk. In contrast to our results, a study by Jelena Kao et al found that hemoglobin levels were a statistically significant predictor of overall survival in the intermediate-1 and intermediate-2 risk subgroups of IPSS. ($p < 0{,}0001$) (*Kao J M et al, 2008)*.Unlike them, we found no difference in hemoglobin levels according to IPSS. Based on relatively little evidence from the studies, it is established that there is a strong clinically significant relationship between the anemic syndrome degree and cardiovascular morbidity and mortality in MDS.

A study by Shi and colleagues in 2020 examined the prognostic significance of MCV in patients with MDS. They analysed 321 newly diagnosed patients with primary MDS for the period 2009–2017 who have not been transfused. They found that the mean overall survival of patients with MCV ≤ 100 fl was shorter (27 months versus 72 months, $p < 0.001$). This proves that MCV ≤ 100 fl is an independent variable *(Shi Z X et al, 2020)*. Our analysis did not confirm a relationship between survival and MCV. We can say that patients with MCV < 100 fl have a shorter survival than the others (*16.4 months ± 17.6 months* and *20.4 months ± 25.5 months*, respectively). Similar results are presented in literature, where a study by Hong Wang and colleagues investigated the importance of MCV in patients with MDS with an abnormal karyotype. They confirm that MCV < 100 fl ($p = 0.026$) is an independent risk factor that affects patient survival (*Wang H et al, 2010*).

In the analysis of the indicators of PB we found a predominance of patients with leukopenia. We proved that there is a significant difference between the groups in terms of overall survival and leukocyte count. Survival was shortest in patients with leukocytes < 4×10^9 / l and > 10×10^9 / l (*15.7 months*) in contrast to the group with normal leukocyte count (*24.3 months*). The results of the analyses of Elizabeth Souto et al. prove that

the leukocyte count can be considered as a significant prognostic marker for survival (p = 0.0214) (*Elizabeth Xisto Souto et al, 1997).* It has been found that not only leukocyte count is related to survival. Jacobs and colleagues analysed 503 patients with MDS (excluding del (5q)) to determine the prognostic value of ALC. It demonstrates that patients with ALC ≥ 1.2 x 10^9 / l have a better overall survival than patients with ALC < 1.2 x 10^9 / l. The mean overall survival ranged from 26.6 to 18.5 months. Therefore, ALC at diagnosis is an independent predictor of overall survival *(Jacobs N L et al, 2010).* In our analysis, no significant difference in ALC values was found, as well as relations toward survival. The role of ANC as a prognostic marker was confirmed with its inclusion in IPSS-R. The results of our study found a weak positive relationship between ANC and overall survival. Survival increases with increasing neutrophil count.

In deepening the analyses regarding the changes in the PB, we found significant differences in the platelet count. In the study by Sanz and colleagues, there was a significant difference in survival according to platelet count – 50 x 10^9 / 1, 50–100 x 10^9 / 1 and 100 x10^9 / 1 (*Sanz G F et al, 1989)*. After dividing the patients into three groups following the example of Sanz and colleagues, we found in our study that patients with platelet levels < 50 x 10^9 / l had the shortest survival (*12.7 months ± 19.8 months*), followed by patients with platelets – 50–100 x 10^9 / l (*14.9 months ± 19.1 months*). The longest survival was in patients with a platelet count > 100 x 10^9 / l (*22.8 months ± 23.5 months*) (p = 0.019). The data are comparable with the results of studies by Sanz and Varela. Varela and colleagues found that patients with platelets < 20 x 10^9 / 1 had a poor prognosis (*Varela B L et al, 1985).* In 2011, Ali Al Ameri and colleagues conducted a study on the prognostic significance of platelets in 2517 patients with MDS and proved the importance of the biomarker for survival. *(Al Ameri A et al, 2011)*. In contrast to our results and those in the literature, in a study by Elizabeth Souto et al., platelet count did not prove to be an important prognostic factor. (*Elizabeth Xisto Souto et al, 1997).* According to Kristian Bowles and colleagues, platelet count and MPV are two indicators of the blood that have an independent prognostic value. (*Bowles KM et al., 2006).*

Early detection of progression in low-risk patients is an important decision point in intensive care. A study of Wimazal and team conducted in 221

patients with primary MDS identified LDH as the most appropriate and accessible follow-up. They found that elevated LDH levels at diagnosis were associated with an increased likelihood of developing AML and decreased survival ($p < 0.05$). In addition, elevated LDH is accompanied or followed by other signs of disease progression, such as thrombocytopenia or PC myeloblasts. Multivariate analysis found that high LDH levels were an independent prognostic indicator (*Wimazal F et al, 2008)*. In another study, Zhang and colleagues analysed the level of LDH in 163 patients between 2001 and 2009 in order to determine its prognostic significance. They retrospectively investigated the relationship of changes in LDH levels with the prognosis, survival and progression, as well as with the indicators of PB, the number of myeloblasts in BM and the karyotype. They prove that the median survival time of patients with elevated LDH levels (> 240 U / L) is 25.6 months, which is significantly shorter than that of patients with normal LDH levels (56.8 months) ($p < 0.05$) (*Zhang Y Q, et al, 2011)*. The results of our analysis found a weak negative dependence, which shows that with increasing LDH levels, overall survival decreases.

In an analysis of the number of dysplasias in BM, we found a weak negative correlation with overall survival. We demonstrated a decrease in survival with an increase in the number of dysplasias in BM. Similar results were reported in the Irina Triantafyllidis study, where the number of dysplasias showed a direct relationship with the number of cytopenias and was associated with a worse prognosis and quality of life. They specify that in patients with unilinear cytopenia or without cytopenia, the overall survival is up to 36 months. Patients with bi – or pancytopenia have a total survival of 32 and 26 months, respectively *(Triantafyllidis I et al, 2012)*. The results of our analysis show that in unilinear dysplasia the survival rate reaches *33.3 months*, in bilinear dysplasia up to *12.0 months* and in multilinear dysplasia up to *17 months.*

In the analysis of the relationship between the percentage of myeloblasts in BM and survival, we found a moderate negative relationship. As the percentage of myeloblasts in BM increases, survival decreases. Similar are the results of a study by Elizabeth Souto et al. in which they proved that the percentage of myeloblasts in BM is the most significant prognostic factor. Their results prove that in < 5% myeloblasts in BM the median

survival reaches *84.7 months*. In the presence of 5–10% blasts, the median survival was reduced to *35.3 months*, while in > 10% myeloblasts it was only *7.2 months*. *(Souto E X et al, 1997)*. They show that the percentage of myeloblasts combined with cytogenetic findings are the most important prognostic factors for determining survival in patients with MDS. Their importance as an important prognostic marker is confirmed by many other authors *(Coiffier B et al, 1987; Mufti G J et al, 1985; Tricot G et al, 1985; Kerkhofs H et al, 1987; Goasguen J E et al, 1990)*.

BM fibrosis is found in 12–50% of cases and some authors suggest that its presence may have a negative impact *(Lambertenghi-Deliliers G, 1991)*. Our analysis showed that BM fibrosis was found in 16.2% of patients. We found no difference in survival according to the degree of fibrosis in BM.

The incidence of cytogenetic abberations in primary MDS reaches 50% *(Olney H J & Le Beau M M, 2001)*. In our analysis, we found a normal karyotype (46, XX / XY) in 59% of the patients, while in 35% we proved the presence of cytogenetic abnormalities and in 6% no metaphases for analysis. There was a statistical difference in the analysis according to cytogenetic abnormalities and survival. We found the shortest survival in patients with del (9q) (*2 months*) and the longest in del (5q) (*39.6 months*). A study by Heim et al. conducted with 1100 de novo and 200 secondary cases of MDS reported the most common aberrations – del (5q) -27%, trisomy 8 (+8) – 19%, monosomy 7 (-7) – 15% , while in secondary MDS for monosomy 7 (-7) -41%, del (5q) -28% and monosomy 5 chromosome (-5) – 11% *(Heim S, 1992)*. Another large study was conducted by Schanz and colleagues in 2,109 patients. They found a normal karyotype in 55.1% of the analyzed patients. Among the most common cytogenetic abberations are complex karyotype-7%, del (5q) – 6.5% and trisomy 8 (+8) in 4.7% *(Schanz J et al, 2012)*. Similar results were obtained in our analysis – 59% of patients had a normal karyotype, followed by a complex karyotype (11%), del (5q) -7% and trisomy 8 in 3% of the analyzed patients.

So far, the clinical and prognostic significance of JAK2 ^{V617F} mutations in MDS remains unclear. *(Ohyashiki K et al, 1991)*. Mutations in the JAK2 gene are common in patients with chronic myeloproliferative diseases. The JAK2 ^{V617F} mutation is less common in patients with AML and MDS. The data in the literature are quite heterogeneous. According to Ingram and

colleagues, the incidence of JAK2 ^{V617F} mutations reaches 6.7% in patients with MDS *(Ingram W et al, 2006)*. Malcovati and co-authors report a 40% frequency in the MDS / MPD group *(Malcovati L et al, 2009),* while according to Lee et al., it rises to 53% in RARS *(Lee J W et al, 2006)*. In our analysis, the JAK2^{V617F} mutation was detected in 6% (n = 1) of the patients studied. According to Schmitt-Graeff et al., the JAK2^{V617F} mutation in MDS correlates with a low risk of progression to AML and prolonged overall survival. *(Schmitt-Graeff A H et al, 2008)*.

Horiike and colleagues were the first to report the presence of the FLT3-ITD mutation in 3% of MDS patients studied in 1997. *(Horiike S et al, 1997*). Similar data were obtained from Shih et al., when they analysed 150 patients (RAEB-T not included) and found the presence of the FLT3-ITD mutation in 2.5% of the subjects. *(Shih L Y et al, 2004[1]*). In 2007, Bacher et al. investigated the presence and role of the FLT3 mutation in MDS. All three studies showed that FLT3 mutations were more common in cases progressing to AML and were associated with poorer prognosis and reduced overall survival. Our analysis confirmed the presence of the FLT3 mutation in 7% of patients, noting that these were cases of RAEB, with short survival and rapid progression to AML.

In contrast to our results, Wimazal and colleagues identified eosinophilic and basophilic count as factors for optimizing the prognosis in MDS. *(Wimazal F et al,2010)*. We found no difference in survival according to eosinophils, basophils, monocytes and lymphocytes.

Rami Komrokji and colleagues analysed 767 patients to determine the role of albumin. Hypoalbuminemia has been identified as an independent prognostic factor in patients with MDS *(Komrokji R S et al, 2012)*. Their results are confirmed in a study by Praveen Malayath from 2019, which proves that low serum albumin levels in patients with MDS determine an unfavorable course of the disease and increased mortality. *(Praveen Malayath, 2019)*. In contrast, we found no difference in patient survival according to albumin values, but we can say that it is an unfavorable prognostic factor.

Simona Gatto and colleagues determine second place in importance of beta2 microglobulin in MDS after cytogenetic disorders in terms of overall survival *(Gatto S et al, 2003)*. Similar results were provided by Frank

Neumann and colleagues confirming the data that B2MG levels > 2ng / ml significantly reduced overall survival and increased the risk of AML progression in the high-risk group. *(Neumann F et al, 2009)*. Our results did not reveal a relationship between beta2 microglobulin levels and patient survival, but found a significant role in the transformation of MDS into AML.

We found no evidence in survival dependent on number of blood transfusions, ferritin and serum levels of vitamin B12.

Analyses by Sanz et al. show that the percentage of myeloblasts in BM, age, platelet count, leukocytes and hemoglobin levels are important prognostic indicators. *(Sanz G F et al, 1989).* In a multi-regression step analysis, we identified the main factors influencing overall survival in patients with MDS and found that myeloblasts in BM, age, platelet count, and serum iron levels had the greatest impact. Coiffier and colleagues reported similar results. They prove the following indicators with prognostic significance – age, percentage of myeloblasts in BM and PB and platelet count. At the same time, they specify the indicators with prognostic significance for progression in AML – hemoglobin level, the percentage of myeloblasts in BM and the presence of blasts in PB. (*Coiffier B et al, 1987.)* Of interest to our analysis is the fact that serum iron levels correlate slightly positively with overall survival, which shows that with increasing serum iron levels, the survival of patients with MDS also increases. Another study by Jose Falantes and colleagues conducted on 332 low-risk patients identified adverse prognostic factors that are associated with survival and the risk of progression in AML. They prove the importance of the severity of cytopenias, age, the percentage of myeloblasts in BM and transfusion dependence. Cytogenetic abnormalities are identified as a major factor (*Falantes J F et al, 2013)*.

A study by Shi and colleagues in 2004 dynamically followed 151 patients with MDS and with transformation to AML. They analyse factors such as clinical manifestation of disease, PB and BM, cytogenetic abberations, immunophenotypic characteristics, treatment response and patient prognosis. Transformation into AML was reported in 13.9% of patients. In contrast, in our analysis, transformation was found in 22.4% of patients. Shi and co-workers report a mean time to transformation of 5 months and

a short survival after 6 months. In contrast, we found a mean time to transformation of 16.3 ± 19.8 months. Survival data after transformation into AML are similar. Patients had a 6-fold shorter life expectancy than before the transformation. They report 5 main factors related to the risk of transformation: age (< 40 years), pancytopenia, > 15% myeloblasts in BM, at least two cytogenetic abnormalities and treatment with combination chemotherapy (*Shi J et al, 2004)*. Interestingly, they found no differences in the time of leukemic transformation when comparing RA, RAEB and RAEB-t.

Our analysis specified the lowest transformation rate in RA patients and the highest in RAEB. A similar result was provided by Vallespi and colleagues who studied 101 patients with MDS. The aim of their study is to analyse the risk of transformation between the subtypes of the FAB classification. They prove that patients with RA have the best prognosis and report the most unfavorable prognosis in patients with RAEB-t. They confirm that the FAB classification is easy to apply and defines well the MDS subgroups (*Vallespi T et al, 1985)*. Bennett and colleagues prove the highest transformation rate in RAEB-t (60–100%) in contrast to RA (10–20%) *(Bennett JM et al, 1982)*. Greenberg and colleagues analysed 7,012 patients by FAB and 5,504 patients according to WHO classifications. When determining the risk group according to IPSS, they reported the best survival in low-risk patients (5.7 years) and the shortest survival in high-risk patients (4 months) *(Greenberg P et al, 1997)*. The shortest time to progression in AML is in high-risk patients (2 months). The good trend is maintained for low-risk patients (9.4 years). Jabbour et al. retrospectively summarized data from 2 clinical trials conducted in 162 MDS patients treated with decitabine. Detection of hemoglobin > 100 g / l, platelets > 50×10^9 / l and absence of abnormalities on chromosome 5 or 7 determine longer survival. Patients whose disease progresses to AML have RAEB and a high-risk IPSS group. The data show the highest percentage of transformed patients with RAEB according to FAB. IPSS analysis found the highest rate of progression in patients with intermediate-2 risk (*Jabbour E et al, 2013)*. A similar result was found in our study. According to IPSS, the most common is transformation in patients with intermediate-risk.

Quintás-Cardama and colleagues develop a prognostic model for deter-

mining prognostic markers for survival and risk of AML transformation in patients with secondary MDS. They analysed 281 patients and identified 7 factors that independently predicted short survival: age ≥ 65 years, ECOG, unfavorable cytogenetics (−7) and / or complex karyotype), WHO subtype (RARS or RAEB-1/2) , hemoglobin (< 11 g / dL), platelets (< 50×10^9 / dL) and transfusion dependence (*Quintás-Cardama A et al, 2014)*.

Does the inclusion of patient-specific factors other than age improve risk stratification? Very often age and concomitant diseases influence the choice of therapy. Established risk stratification scales are based primarily on disease-related factors. Complementing them with patient-related factors such as comorbidity and 'frailty' may improve prognosis.

Comorbidities have been found to increase with age. The majority of patients with MDS have ≥ 1 comorbidities that precede or follow their diagnosis. Comorbidities are a significant factor as they may affect treatment plans, tolerability and treatment outcomes. (*Extermann M, 2000)*. Although the majority of patients with MDS have comorbidities that affect outcomes, including survival, none of the commonly used prognostic scales include them as a prognostic factor. (*Breccia M et al, 2011; Sperr W R et al, 2010; Wang R et al, 2009)*.

It is necessary to discuss the development of personalized risk stratification systems, which include the most important factors related to the disease and the patient. The best model for including comorbidities as a prognostic factor in current scales has not been identified yet.

The "frailty" scale combines the two prognostic groups of factors and thus significantly improves risk stratification. According to Gregory Abel, an increased level of "frailty" in patients is most often associated with increased mortality. (*Abel G A & Klepin H D, 2018)*. CFS can be useful in identifying "frail" patients and determining the risk of adverse outcomes. (*Turner G et al, 2014)*. Rockwood and colleagues develop a 7-point scale for clinical "frailty" and apply it to 2,305 elderly patients. The Rockwood, Clinical Frailty Scale (CFS) is designed to provide clinicians with an easily applicable model that stratifies elderly patients according to the level of "frailty". (*Rockwood K et al, 2005)*. Kazuki Sakatoku et al. conducted a retrospective analysis in 118 patients with MDS. They examined the prognostic significance of the "frailty" scale and comorbidity. The degree

of “frailty” was assessed using CFS. Comorbidity was determined using CCI and MDS-CI. They demonstrated that combining the “frailty” and comorbidity scales with IPSS-R may help to predict more accurately overall survival, especially in low-risk MDS patients. They proved the importance of CFS as an independent prognostic indicator in analysis against IPSS-R (*Sakatoku K et al, 2019)*. We also found a weak positive relationship between IPSS-R and CFS, which shows that increasing the rate of CFS reduces survival in all risk groups according to IPSS-R. In the analysed group of patients, we found a decrease in survival with increasing CFS score. In our study, survival in patients at low risk and CFS > 5 (*22 months*) was shown to be comparable to that in patients at high risk and CFS = 0 (*20 months*).

Retrospective studies have been performed to analyse the role of comorbidities. The assessment of comorbidity was performed using the known scales for determining the comorbid indices – CCI (*Charlson M E et al, 1987*), HCT-CI (*Sorror M L et al, 2005),* ACE-27(*Naqvi K et al, 2011)* и MDS-CI (*Della Porta M G et al, 2011)*.

Concomitant diseases are rarely systematically analysed in patients with MDS. Wang and team conducted a large population-based study in 1708 patients to assess the role of comorbidity in the survival of newly diagnosed patients with MDS. They found a median survival of 18 months, with 51% of patients having comorbidities and a significantly higher risk of death. They found that the risk of death increased with increasing an CCI score. The study confirms comorbidity as an important and independent prognostic factor for survival in patients with MDS (*Wang R et al, 2009)*. The data from our analysis confirm the role of comorbidities as a prognostic factor. In an analysis of the classification and risk stratification systems, we found that with increasing CCI, survival decreases.

Zipperer et al. studied 171 patients with MDS according to comorbidities using CCI and HCT-CI and evaluated their association with IPSS. They confirmed that HCT-CI has a prognostic value in IPSS intermediate and high-risk groups. HCT-CI is reported to be superior to CCI, including IPSS. According to a study by Zipperer and colleagues, low-risk patients (48%) predominate (HCT-CI = 0)(*Zipperer E et al, 2009)*. The results of our analysis established a predominance of patients with intermediate risk (40.7%) (HCT-CI = 1–2). They report that both systems are to be prognos-

tic, but HCT-CI more clearly distinguishes between low, intermediate and high risk patients (HCT-CI = 0 (low risk), HCT-CI = 1–2 (intermediate risk) and HCT -CI ≥ 3 (high risk). The median survival of the different risk groups according to HCT-CI was 68, 34 and 25 months, respectively ($p < 0.001$). Our results also found a reduction in survival with an increasing risk on the HCT-CI scale. The mean survival according to the HCT-CI score was 19.5, 18.3, and 2.0 months, respectively.

In a study by Zipperer and colleagues, according to CCl – 65% of patients had no comorbidities and 22% had at least one comorbidity. Our analysis is also dominated by patients without comorbidity (36.1%), and with at least one comorbidity are 31.1% of patients. Our results differ from those of Zipperer et al. They found that patients with CCI = 0 had a median survival of 42 months, while those with CCI = 1 – 15 months. Interestingly, patients with CCI ≥ 2 had a survival of 19 months compared with CCI = 0 ($p = 0.006$). Our analysis found a progressive decrease in survival with an increasing CCI score.

A study by Rozema and team focused on assessing overall survival for comorbidities. An observational study was conducted involving 291 patients diagnosed between 2005 and 2017 in Friesland. They confirm significantly better survival in patients with CCI < 4, age < 65 years, female and low-risk MDS. The study proves that as the number of comorbidities increases, survival decreases (*Rozema J et al, 2021*).

The study by Sperr and colleagues examined the effect of comorbidities on the survival and development of AML. 419 patients with de novo MDS for the period 1985–2007 were retrospectively analysed. Patients with MDS were stratified by CCI and HCT-CI. Sperr et al found that HCT-CI was an important prognostic factor for overall survival (OS, $p < 0.05$) as well as event-free survival (EFS, $p < 0.05$), while CCI was prognostic for overall survival ($p < 0.05$), but not for EFS. Comorbidity was found to be an independent prognostic factor in patients with low or intermediate-risk MDS ($p < 0.05$) for OS and EFS. *(Sperr W R et al, 2010)*. In an analysis of CCI and risk scales, we found longer survival in patients with low CCI scores. Increasing the CCI score leads to reduced survival.

The most common used comorbidity index in patients with MDS is MDS-CI. It was developed in 2010 by the Italian research group and vali-

dated using data from the Düsseldorf MDS register. It uses factors related to the patient. Numerous studies have been conducted to demonstrate that comorbidities are an important prognostic factor in decision-making in patients with MDS.

Zipperer et al. investigated the role of MDS-CI and its prognostic significance in combination with IPSS-R. The retrospective study included 1161 patients who received therapy other than allo-SCT. According to the MDS-CI risk groups, the mean survival was 39, 24 and 15 months, respectively, for the low-, medium – and high-risk groups ($p < 0.001$). Our results also found that according to MDS-CI, the mean survival of patients was 21.8, 17.7 and 8.5 months, respectively, for low-, medium – and high-risk groups. The trend of decreasing survival with increasing risk of MDS-CI is confirmed. Zipperer and colleagues reported the most common comorbidities – heart disease (37%), followed by solid tumors (10%) and lung (9%), kidney (7%) and liver (4%).In contrast, in our group of analysed patients, we found that the most common comorbidities are again heart disease, but are followed by kidney, lung, liver disease and at the end the most common solid tumors. Analyses by Zipperer and colleagues found that the mean survival according to IPSS-R was 105, 70, 36, 14 and 8 months for the very low, low, intermediate, high and very high risk groups. In the cohort of patients we analysed, the mean survival according to IPSS-R was 62, 41, 26, 12 and 9 months for the very low, low, intermediate, high and very high risk groups. The low-risk group of IPSS-R is divided by MDS-CI into three risk groups with a survival of 92, 63 and 36 months. In detailed analysis, we also divided patients from the low-risk IPSS-R group into three MDS-CI risk groups with a median survival of 36.2, 28, and 7 months. The present study demonstrates that MDS-CI is an independent prognostic marker of IPSS-R (*Zipperer E et al, 2014)*. Other research groups have also shown that MDS-CI is an independent prognostic factor added to IPSS-R. (*van Spronsen M F et al, 2014).*

Valleari et al. conducted a study in 318 patients to assess the impact of age, comorbidities, and risk group (IPSS and IPSS-R) in clinical practice. They prove the presence of comorbidity in 55.7% of patients. It turns out that age is a negative factor in terms of survival. Demonstrate MDS-CI with prognostic significance in low-risk groups of IPSS and IPSS-R.

MDS-CI is an independent prognostic factor for overall survival. HCT-CI has not been shown to be a factor in survival (*Balleari E et al, 2015).*

Italian groups find that MDS-CI can more accurately determine the life expectancy of patients with MDS stratified according to WPSS. In studies by Breccia and Della Porta et al., MDS-CI has been shown to be an independent prognostic indicator by WPSS. (*Breccia M et al, 2011[1]; Della Porta M G et al, 2011)* Breccia and colleagues compared all three scales for assessing comorbidity. Breccia et al. found that better risk stratification can be done by WPSS and MDS-CI. The study included 450 patients with the aim of assessing the prognostic significance of comorbidities in patients with very low / low risk and intermediate and high / very high risk according to WPSS. They found that in the very low / low risk group there was a significant difference in survival (48.5 months for MDS-CI = 0 and for MDS-CI > 2–20.4 months ($p = 0.002$)). In the intermediate risk of WPSS, similar significant differences in overall survival were found (32.3 months for MDS-CI = 0 to 18.3 months for MDS-CI > 2 ($p = 0.001$)). No significant differences were found in patients with high / high risk WPSS. In our analysis, we found similar results, and in contrast, we found a decrease in survival with increasing MDS-CI in the very high risk group. In the very low / low risk group, there was a significant difference in survival (69.2 months in MDS-CI = 0 to 20 months in MDS-CI > 2). For intermediate risk by WPSS, we found similar significant differences in overall survival (32.4 months for MDS-CI = 0 to 8.3 months for MDS-CI > 2). At very high WPSS risk, we found similar significant differences in overall survival (12.0 months for MDS-CI = 0 to 1.0 month for MDS-CI > 2).

Breccia and colleagues reported a higher percentage of patients at intermediate risk for WPSS (41.5% according to Della Porta versus 18% in the Pavia study). In our analysis, the patients with intermediate risk according to WPSS are 28.6%. In contrast, in our study, the percentage of patients with high / very high risk of WPSS was higher (50.4%). They demonstrate that MDS-CI is able to distinguish MDS patients with very low / low and intermediate risk of WPSS in terms of overall survival and risk of non-leukemic death. Their results confirm the data from the analysis of Della Porta and colleagues. Breccia et al.demonstrate the prognostic significance of comorbidities.*(Breccia M et al, 2011[1]*).

The results of the analyses of Della Porta and colleagues also found that comorbidities are very common in patients with MDS and have a significant impact on the outcome of the disease. The study population included a cohort of 840 patients diagnosed with MDS in Pavia, Italy, and a validation cohort of 504 patients from Düsseldorf, Germany. Initially, analysis was performed using the two available indices – CCI and HCT-CI. They found that neither of these two indices adequately determined the risk in newly diagnosed patients with MDS. They demonstrate that MDS-CI further stratifies the forecast in WPSS risk groups. A multivariate analysis by Della Porta et al found that five groups of diseases (cardiac, moderate to severe liver, severe lung, kidney, solid tumors) were independently associated with the risk of non-leukemic death. They divide patients into 3 risk groups (65% low, 29% intermediate and 6% high) and determine overall survival and non-leukemic death, regardless of age, gender, WHO classification, cytogenetics and transfusion dependence. In our analysis, according to MDS-CI, patients are also divided into three groups – low risk (43.8%), intermediate (45.2%) and high risk (11%). In the analyzes of Della Porta and colleagues, low-risk patients predominated, while in our study, patients with intermediate risk predominated. They found that all three MDS-CI risk groups differed in overall survival (*Della Porta M G et al, 2011)*. We also found that all three MDS-CI risk groups had different overall survival. The importance of comorbidities for the prognosis determined by MDS-CI is observed mainly in patients with very low, low and intermediate risk of WPSS and IPSS-R. The study demonstrates that MDS-CI improves the prognostic stratification of patients classified according to WPSS and provides a basis for integrating WPSS and MDS-CI in clinical decision making. MDS-CI significantly affects overall survival and the likelihood of non-leukemic death in patients with very low, low and intermediate risk of WPSS, while not retaining significance in high-risk patients. In our analysis, it was found that all patients at very low risk belong into to the group of MDS-Cl = 0, with an increase in the number of patients in groups 1 to 3 on MDS-Cl with an increase in risk according to WPSS.

No less important is the degree of comorbidity. One of the comorbidity scales that measures their severity is ACE-27. A retrospective study by

Daver et al. in 600 patients at the MD Anderson Cancer Center demonstrated the effect of ACE-27 on survival in patients with IPSS-R – intermediate, high, and very high risk. It was found to have no significant effect on median survival in the low and very low IPSS-R groups. Assessment of comorbidity may improve the prognostic ability of IPSS-R (*Daver N et al, 2014)*. In our analysis, it was found that increasing the risk group for IPSS-R reduces survival in all ACE-27 groups. At ACE-27 = 0 and low risk it is 37.1 ± 35.9 months compared to ACE-27 = 0 and very high risk it is only 7.2 ± 8.4 months.

MD Anderson Cancer Center team developed a new prognostic chart including age, IPSS, and the ACE-27 scale, which divided patients into three groups with different overall survivals (43.0, 23.0, and 9.0 months, respectively). (*Naqvi K et al, 2011)*. Based on the analysis of ACE-27 and IPSS, we found that the degree of comorbidities affects survival. With the appearance and deepening of the severity of comorbidity, survival is reduced in all risk groups. The data from the high-risk group are the most demonstrative (8.3 ± 8.9 months for ACE-27 = 0 compared to 1.0 month for ACE-27 = 3).

The Austrian MDS Working Group has published a scoring system consisting of both patient-related and disease-related factors to assess comorbidity according to HCT-CI, ferritin, IPSS and age. (*Sperr W R et al, 2013)*.

The identification, analysis and inclusion of additional prognostic factors to the classification and risk stratification systems individualizes the determination of the group of patient risk, survival and risk of transformation into AML.

VI. CONCLUSION

MDS is a heterogeneous group of diseases with significant differences in survival. The outcome of the disease varies according to the risk group determined by the established scales for risk stratification. Based on a large number of studies, as well as our analysis, we have shown that age is one of the main risks and negatively predictive factor in terms of survival. The role of classification and risk stratification systems in determining survival and risk of transformation in AML has been demonstrated. However, no index is able to determine the more unfavorable clinical course of the disease.

We defined the role of clinical and biological indicators. We found that age, the percentage of myeloblasts in BM, hemoglobin levels, MCV, platelet and leukocyte count, serum iron, LDH, cytogenetic abberations and the number and degree of dysplasia are of the greatest importance for the course of the disease.

Comorbidities are very common in patients with MDS and have a significant effect on disease outcome. Comorbidities and the degree of "frailty" play a role in determining the survival of patients with MDS. They are an important and independent prognostic factor. The addition of comorbid indices as an additional factor to the established risk scales significantly improves the risk stratification. We have shown that patients with severe comorbidities have a 50% reduced survival, regardless of age and risk group. Combining CFS and comorbidity indices with IPSS-R can help more accurately predict overall survival. CFS is an independent prognostic indicator in IPSS-R analysis. MDS-CI has also been shown to be an independent prognostic factor for overall survival adding to IPSS-R and WPSS. Better risk stratification can be made when determining it according to WPSS and assessing comorbidities according to MDS-CI. Thus, MDS-CI improves the prognostic stratification of patients classified according to WPSS and provides a basis for integrating WPSS and MDS-CI in clinical decision making.

Determining risk using the risk stratification scales and comorbidity scales using comorbidity indices significantly improves prognostic assessment in patients with MDS. Combining them allows for more precise risk stratification.

VII. IMPLICATIONS OF RESULTS

Referring to the results of the analysis of the prognostic factors available for study, we came to the following conclusions:

1. Age is a major negative prognostic factor. In the study, the mean age of newly diagnosed patients was 70.
2. According to the FAB classification, RA patients predominate, followed by RAEB. The highest risk of transformation and the shortest survival in RAEB and RAEB-t patients.
3. Compared to the WHO 2008 and WHO 2016 classifications, patients with RCMD / MDS-MLD predominate, followed by RAEB-1 and RAEB-2. The highest frequency of transformations and the shortest survival is in RAEB-2 patients.
4. IPSS and IPSS-R are dominated by patients with intermediate risk, while WPSS is at high risk. Survival is shortest in patients in the high and very high risk groups. According to IPSS, the risk of transformation is highest in patients with intermediate-2 risk, WPSS at high risk, and IPSS-R – very high risk.
5. No significant difference in overall survival was found between the different groups of comorbidity and "vulnerability" scales.
6. Negative moderate relationship between ECOG, CFS and CCI and survival to classification and risk stratification systems has been demonstrated.
7. An inverse relationship between HCT-CI and MDS-CI risk groups and survival to classification and risk stratification systems has been demonstrated
8. There was a weak positive relationship between IPSS-R and CFS, as well as a difference in risk analysis according to WPSS and MDS-CI.
9. Approximately 1/5 of patients with MDS transform into AML, with survival being 6-fold shorter after transformation.
10. The prognostic factors influencing survival are age, leukocyte and platelet count, ANC, number of dysplasias, myeloblast percentage in BM, LDH, serum iron levels and cytogenetic abnormalities.
11. The prognostic value for transformation in AML of the following indicators was proved – leukocyte count, ANC value, higher lymphocyte percentage, lower creatinine values and lower B2MG value.

VIII. CONTRIBUTIONS

Contributions of original character

1. For the first time in Bulgaria an analysis was performed in a large group of patients with MDS with demographic, clinical-laboratory and cytogenetic indicators.
2. For the first time in Bulgaria, an analysis was performed in patients with MDS against the comorbidity scales and the clinical "frailty" scale.
3. For the first time in Bulgaria an analysis was performed in a group of patients with MDS of JAK2 ^{V617F} and FLT3 mutation status.
4. For the first time in the world, a parallel analysis of all scales for determining the comorbidity indexes in patients with MDS and their correlation with the classification and risk stratification systems was conducted.

Contributions of a confirmatory nature

1. The importance of the systems for classification and risk stratification as prognostic factors influencing the risk of transformation and survival in the Bulgarian population has been confirmed.
2. The importance of age, leukocyte and platelet count, number of dysplasias, percentage of myeloblasts in BM, cytogenetic abberations and LDH for survival in the Bulgarian population has been confirmed.
3. The importance of comorbidities as prognostic predictors for Bulgarian patients with MDS has been established.
4. The need to assess comorbidities associated with the risk of disease progression and risk-adapted therapy has been identified.

IX. SCIENTIFIC PUBLICATIONS ON THE TOPIC

1. Ilina Micheva, Vladimir Gerov, Stela Dimitrova, Merlin Efraim, Liana Gercheva. Outcome after azacitidine treatment in patients with high-risk myelodysplastic syndrome and acute myeloid leukemia in the clinic of hematology at St. Marina university hospital, Varna. Scripta scientifica medica, 2018;50(1):31–35
2. M.Efraim and I. Micheva SOCIO-DEMOGRAPHIC CHARACTERISTICS OF SURVIVAL IN PATIENTS WITH MYELODYSPLASTIC SYNDROME; Clinic of Hematology at St. Marina University Hospital, Varna Medical University "Prof. Dr. Paraskev Stoyanov ", Varna IX Scientific Session for teachers and students – published by the Medical University – Varna – Medical forum, 2021,app.1
3. M. Efraim, Assoc.Prof. I.Micheva; New biomarkers in the diagnosis and prognostic evaluation of patients with myelodysplastic syndrome; Clinic of Hematology, University Hospital "St. Marina", Varna – Medinfo IV.2021; 100–104

X. ACKNOWLEDGEMENTS

I express my deepest gratitude to:

- Assoc. Prof. Dr. Ilina Micheva, Ph.D. – research supervisor – for the trust shown in me, for the support, patience, guidance and advice, help and dedication in the development, analysis and preparation of the dissertation. Here is all gratitude and admiration for her merits as a leader and a person.
- To my family and friends – for the endless love, patience, support and faith.

Heartfelt thanks to all who in one way or another supported me and believed in me!

XI. APPENDIX

Clinical card of the participant
• **PROTOCOL №** • **PATIENT:** • **DATE**
Age
Sex
MDS type
ECOG
Comorbidity
FAB
WHO2008
WHO2016
IPSS
IPSS-R
WPSS
CFS
CCI
HCT-CI
MDS-CI
ACE-27
Number of dysplasia
Cytogenetic BM
Creatinine
Total bilirubin
ALAT

ASAT
GGT
LDH
Albumin
Beta 2-microglobulin
Erythropoietin
Feritin
Iron
TIBC
Vitamin B12
BM biopsy
FLT3 status
JAK2 status
Treatment
Survival