9. summaries of the monographic/habilitation work

Habilitation work - scientific publications (not less than 10) in publications that are referenced and indexed in world-famous databases with scientific information

1. OUTCOME AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA AT THE HEMATOLOGY CLINIC OF UMHAT "SVETA MARINA" VARNA. *I. Micheva1, V. Gerov1, S. Dimitrova1, Ya. Petrov1, A. Antonov1 , T. Chervenkov2, I. Reznik.* Hematology. Vol XL VIII, 1-2/2022. (8,6) *Scopus*

With the introduction of new therapeutic options, the outcome of patients with multiple myeloma (MM) has improved significantly in recent years1. The inclusion of drugs such as immunomodulators and proteasome inhibitors in the induction therapy significantly increases the speed and depth of the responses achieved and leads to a prolongation of remissions and overall survival. Autologous stem cell transplantation (ASCT) is the standard of care in adult patients with newly diagnosed multiple myeloma (MM). Clinical trials confirm the usefulness of ASCT even in the era of new drugs.

The aim is to retrospectively analyze for a period of 6 years the response, overall survival (OS) and progression-free survival (PFS) in patients with multiple myeloma (MM) after ASCT performed in the transplant unit at UMBAL "Sveta Marina" Varna.

ASCT was performed in 48 patients with MM at a mean age of 60.1±7.4 years (27 men at the age of 59.0±7.7 years and 21 women at the age of 61.4±7.0 years). According to ISS there were 8 patients in first, 18 - in the second, and 22 in the third clinical stage. Before ASCT, 19 patients achieved complete response (CR), 6 - very good partial response (VGPR), 22 - partial response (PR) and one was in progressive disease (PD).

The conditioning regimen was Melphalan 200 mg/m2; in 16.7% of patients the dose was reduced to 140 mg/m2 due to renal failure. The average number of CD34+ peripheral stem

cells trnansfused was 3.21±1.19x106/ kg. Mean time to neutrophil and platelet engraftment was 11±1 and 12±1.8 days, respectively. After ASCT, CR+VGPR were found in 62.5% of patients. Post-ASCT consolidation therapy was administered in 18.8% of the patients, maintenance therapy with Bortezomib in 43.8% and Lenalidomide in 22.9% of patients. At a median follow-up of 25 months, median OS and PFS were 68.2 and 38 months, respectively. Median OS was significantly prolonged in patients in I clinical stage compared to those in II and III stage (p=0.04) and in patients achieving CR+VGPR before and after ASCT, compared to those with PR (p=0, 02). Median PFS in patients achieving CR and VGPR was significantly higher compared to patients with PR (p=0.0009). The probability of achieving 3.5-year survival after ASCT for those who achieved CR+VGPR was 79.4% versus 21.8% for patients with poorer response (P<0.005).

The results of this retrospective study confirm the efficacy and safety of ASCT in patients with MM. Achieving CR+VGPR is a prognostic factor for prolonged OS and PFS.

2. BLOODSTREAM INFECTIONS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES: ETIOLOGY AND ANTIMICROBIAL RESISTANCE PROFILE , T. Stoeva, D. Niyazi, I. Micheva, Hematology. Vol XL VIII, 1-2/2022. (20т.) Scopus

Blood infections are among the most common complications in patients with oncohematological diseases (HCD). Although a significant reduction in mortality in neutropenic patients from this type of infection has been reported from 25% in the 1970s to 6% in recent years due to the use of broad-spectrum antibiotics and the proper administration of antimicrobial chemotherapy, one adverse consequence of this is the modern a trend towards a steady increase in the proportion of infections (including those of the blood) associated with multi-resistant bacteria. The aim of the research is to study the etiological spectrum and antibiotic resistance of bacterial pathogens, the causes of blood infections in patients with oncohematological diseases (OCH) from the Hematology Clinic of the St. Marina Medical University Hospital, Varna for a six-year period (2015 - 2020.), comparing the results with those of a previous 5-year study. In the period 2015 - 2020 2828 blood cultures of patients with OCD were examined. 316 non-recurrent, clinically relevant isolates were isolated from 298 patients. For the period of the study, Gram-negative bacteria were proven to be the causative

agents of bacteremias in 54.7% of cases, with the leading species being E. coli (14.5%), Enterobacter spp. (12%) and Klebsiella spp. (10.1%). Gram-positive bacteria were found in 37.6%, with staphylococci (22.4%) and enterococci (9.5%) being the most common isolates. The proportion of ESBLs producers was respectively: 68.8% for Enterobacter spp., 53.3% for Klebsiella pneumoniae and 19.5% for E. coli. Resistance to carabapenems was proven most often in isolates of Acinetobacter baumannii (85.7%), Pseudomonas aeruginosa (11.8%) and Klebsiella spp. (10%). The share of methicillin-resistant S. aureus (MRSA) and CoNS is 12% and 90%, respectively. Vancomycin-resistant enterococci and staphylococci are not proven. The level of fluoquinolone resistance among enterococci is between 88% and 100%. A continuing trend for the dominance of Gram-negative bacteria in the etiological spectrum of blood infections in patients with OCD, a persistent high level of ESBL producers among members of the Enterobacteriaceae family and multi-resistant Enterococcus faecium was found. A high proportion of carbapenem-resistant Acinetobacter baumannii was found. and methicillin-resistant CoNS. A new, negative trend is the emergence of invasive carbapenem-resistant isolates from the Enterobacteriaceae family.

3. **V. Miteva**, **C. Ruseva**, **T. Chervenkov**, **I. Micheva**. Chromosomal abnormalities and their prognostic significance in patients with multiple myeloma. – Medical Review, 2022;58(1):38-43 (15т.) *Web of Science (CABI)*

A retrospective analysis of clinical and laboratory data from the time of diagnosis, including karyotypes, of 110 newly diagnosed MM patients for the period 2016-2020 was performed. The aim of the study was to determine the frequency of cytogenetic aberations and to evaluate their prognostic value at diagnosis. Cytogenetic analysis (CG) was performed on 97 (88%) newly diagnosed patients. It was successful in 83 (86%), and in 68 (82%) of them a normal karyotype was established, and in 15 (18%) – various aberrations. Fluorescence in situ hybridization (FISH) was also performed in 30 patients, and in 17 (57%) of them the combination of CG and FISH was applied, and in the remaining 13 (43%) - FISH alone. In 9 (30%) del(17)(p13) was detected, and in the remaining cases the result was normal. A statistically significant difference was found in the median survival in patients with normal and pathological karyotype, 34 and 8 months, respectively ($p = 0.0493$). No difference was found in median survival between patients with hyperdiploid and non-hyperdiploid karyotypes ($p =$

0.3042). Among the most frequently detected disorders are trisomies, and one patient was found to have a deletion in chromosome 8, del(8)(p21), for which recent data have been reported to be associated with resistance to bortezomib treatment. Numerical and structural chromosomal abnormalities are an important prognostic factor, especially in combination with some other significant laboratory findings. The presence of a non-hyperdiploid karyotype is considered a poor prognostic marker in contrast to the standard risk of hyperdiploidy. Our experience confirms the need to combine FISH with another type of analysis to increase the comprehensiveness of genetic evaluation.

4. Niyazi D, **Micheva I**, Markovska R, Stoeva T. Phenotypic and molecular detection of slime producing *Staphylococcus* spp. obtained from blood samples of patients undergoing hematopoietic stem-cell transplantation. Acta Medica Bulgarica. 2022 (SJR_{2020} 0.120, Q4) **IF 0,204 (15)** *Scopus*

Bacterial bloodstream infections are among the most important infectious complications in patients after hematopoietic stem-cell transplantation (HSCT), most often associated with the preslococci (CoNS), bacteria with weak virulent potential (S. epidermidis, S. hominis, S. haemolyticus, etc.). The colonization of the CVC is an important risk factor for catheter-associated infections and bacteremia.

The aim is to investigate the slime production in isolates of Staphylococcus spp., associ- ated with bacteremia in patients after hematopoietic stem-cell transplantation (HSCT) and to determine the relationship between the slime production and ica genes carriage, as well as the correlation of ica and methicillin resistance. Between 2019 and 2020, twenty-one clinically significant Staphylococcus spp. isolates were obtained from blood cultures of 17 patients after HSCT. The species identification and the susceptibility to cefoxi- tin were determined by BD Phoenix M50. Two phenotypic tests (Congo red agar, CRA; Chris- tensen's method, TT) and PCR for icaA and icaD were used to detect slime production. A PCR method was also used to detect the mecA, mecC genes. In the studied group of 21 isolates (S. epidermidis, n = 12; S. haemolyticus, n = 4; S. hominis, n = 2; S. aureus, n = 3), the phenotypic tests were positive in 13 isolates. Ten isolates (47.6%) were identified as carriers of ica genes (S. epidermidis, n = 9, and S. haemolyticus, n = 1). Five isolates (23.8%) were detected as slime producers by all three

methods. The mecA gene was identified in 18 isolates (85.7%). All ica positive isolates were also mecA carriers. A relatively high proportion of the blood isolates of Staphylococcus spp. were slime producers, associ-ated with ica genes. A combination of both phenotypic and genetic methods should be used to detect alternative routes of slime production. The co-expression of ica and mecA is associ-ated with the occurrence of difficult-to-eradicate isolates.

5. S. Dimitrova, I. Micheva. Interleukin-6 and its relation to the clinical and laboratory characteristics of patients with myelofibrosis. – Medical review, 57, 2021, № 5, 43-47. **(30т)** ***Web of Science (CABI)***

Inflammatory cytokines are key mediators in the pathological relationship between the tumor microenvironment and clonal hematopoiesis in MF. Interleukin-6 (IL-6) plays a key role in a number of pathophysiological mechanisms in the development, clinical presentation, prognosis and progression of MF. In this study, serum IL-6 levels in MF patients and its association with clinico-laboratory characteristics of myelofibrosis (MF) patients were analyzed. MF is a myeloproliferative disease characterized by a heterogeneous clinical picture characterized by bone marrow fibrosis, ineffective extramedullary hematopoiesis and excessive splenomegaly. Inflammatory cytokines are key mediators in the pathological relationship between the tumor microenvironment and clonal hematopoiesis in MF. Interleukin-6 (IL-6) plays a key role in a number of pathophysiological mechanisms in the development, clinical presentation, prognosis and progression of MF. 68 MF patients and 12 healthy controls were analyzed. Serum levels of IL-6 were investigated by the ELISA method. The results were processed statistically using dispersion, comparative and correlation analysis. Mean IL-6 levels in all MF patients were found to be statistically significantly higher compared to healthy controls. A significant difference was found between healthy controls and patients with MF - fibrotic phase and between healthy controls and patients with postpolycythemic MF. IL-6 levels were significantly higher in patients with fibrosis grade 3 compared to other grades. A significant directly proportional relationship between IL-6 and the number of hemotransfusions was demonstrated. The study demonstrates the key role of IL-6 in the development of fibrosis in MF and the possibility of targeting it in order to therapeutically influence and control the disease.

6. Micheva I, Gerov V, Dimitrova S, Efraim M. Efficacy of Inotuzumab Ozogamicin plus Ponatinib Followed by Allogeneic Stem Cell Transplantation in a Patient with Relapsed Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia. Case Rep Hematol. 2021 May 27;2021:1717506. (15т.) *Web of Science; Scopus*

The paper presents a case of a patient with Ph + ALL with T315I mutation successfully treated after early relapse with inotuzumab plus ponatinib, followed by allogeneic HSCT and ponatinib maintenance with a review of the current therapeutic options for ALL.
Acute lymphoblastic leukemia, Ph + is an aggressive disease with a poor prognosis. Despite the inclusion of tyrosine kinase inhibitors (TKIs) in therapeutic strategies, patients with relapse after chemotherapy plus TKIs have a short overall survival (OS) and a lower chance of reaching hematopoietic stem cell transplantation (HSCT), which remains the only curative approach . Therefore, new drugs, such as potent newer-generation TKIs, antibody-drug conjugates, bispecific monoclonal antibodies, and CAR-T, are being developed and studied in patients with Ph + ALL alone or in combinations. However, the combination of inotuzumab plus ponatinib has limited application . In our case, it proved to be a potent combination inducing a complete molecular response in extremely high-risk Ph + ALL with T315I mutation. Post-transplant TKI maintenance therapy may play an important role in reducing the rate of hematologic relapse and improving disease-free survival in such challenging and difficult-to-treat cases.

7. Niyazi, D., Stoeva, T., Atanasova, S., Markovska, R., & Micheva, I. (2021). Invasive pulmonary aspergillosis in patients with haematological malignancies and hematopoietic stem cell transplantation: a single-center study. *Folia Medica*, *63*, 941. DOI: 10.3897/folmed.63.e65248 (IF-0,84) (12т.) (SJR_{2021} 0.203, Q4) *Web of Science; Scopus*

The aim of this study was to evaluate the clinical significance of the Aspergillus Galactomannan antigen (GM) test for the diagnosis of invasive pulmonary aspergillosis (IPA) in patients with hematological malignancies, including patients undergoing hematopoietic stem cell transplantation (HSCT). Patients with hematologic malignancies and those undergoing hematopoietic stem cell transplantation (HSCT) are at high risk of developing severe, life-threatening infections with a mortality rate of up to 55%. Invasive pulmonary aspergillosis

(IPA) is one of the most important infectious complications with an incidence of 12% in individuals with hematologic malignancies and between 2% and 8% after autologous and allogeneic stem cell transplantation. Depending on the underlying disease, IPA-related mortality can be as high as 88%.

Between January 2016 and June 2019, ninety patients were tested for GM. A total of 134 blood samples and 19 bronchoalveolar lavage (BAL) samples were analyzed using the Platelia Aspergillus Ag Enzyme-Immuno Assay (Bio-Rad Laboratories). All patients were divided into five groups based on their GM scores. A positive GM antigen test was found in 16 patients (17.7%). Of these, ten had positive serum samples (group I). Five patients with negative serum samples had positive BAL results (group II). One patient had both serum and BAL samples positive (group III). Fifteen GM positive patients (9 from group I, group II and III) were categorized as probable IPA. Thirty-six patients (40%) negative for GM (group IV) were considered possible IPA. IPA was excluded in 38 patients (42.2%) (group V). Therapy was initiated in all 15 patients considered to be cases of probable IPA. IPA was the immediate cause of death in 3 cases (25%).

To the best of our knowledge, this is the first study in Bulgaria evaluating the clinical significance of the GM test for the diagnosis of IPA in patients with hematological malignancies, including patients undergoing HSCT.

The Aspergillus Galactomannan Enzyme-Immuno Assay is a reliable method that can be used both for diagnosis and for monitoring the response to antifungal therapy in cases of IPA, as well as a screening tool in neutropenic patients with various risk factors. In the absence of contraindications, the simultaneous examination of serum and BAL fluid is recommended. The use of both serum and BAL GM tests, regular follow-up of patients with the Aspergillus GM test, along with imaging findings and clinical data greatly improve the diagnosis of IPA.

8. Gercheva, L., Dimitrova, S., & Micheva, I. (2016). Impact of the impaired iron homeostasis on the pathogenesis of anemia in primary myelofibrosis. Journal of IMAB–Annual proceeding scientific papers, 22(1), 1083-1085. SJR 0,225 (IF-0,787). (20т.) *Web of Science; Scopus*

The aim of this study is to evaluate the relation between anemia, bone marrow fibrosis, prognostic score, survival, and parameters of iron metabolism. Anemia is a well established prognostic factor in primary myelofibrosis (PMF). Recent data suggests that markers of

abnormal iron homeostasis, which are known to be affected by both iron overload and inflammation, may be involved in the pathogenesis of anemia in PMF.

We included 72 patients with PMF. The following parameters were analyzed: degree of bone marrow fibrosis, hemoglobin level, MCV, components of iron homeostasis (total iron binding capacity (TIBC), ferritin, serum iron). The prognostic score was determined according to IPSS and DIPSS. We found significant correlation between the level of hemoglobin and degree of bone marrow fibrosis and prognostic score. The MCV was analyzed in 56 of the patients and was found low in 9,5 % of them. However, there was no significant correlation between degree of fibrosis and the lower MCV. Our study shows negative correlation between hemoglobin level, serum ferritin and TIBC. The change in serum ferritin level is an indirect sign of significance of hepcidin and interleukins levels for severity of anemia in patients with PMF. Our results reveal the possible role of the impaired iron metabolism in the pathogenesis of anemia in PMF.

9. Chaushev, B., Micheva, I., Mechmed, M., Balev, B., Bocheva, Y., Ivanova, D., & Dancheva, j. (2016). 18f-fdg pet/ct in the diagnosis of an extranodal relapse of diffuse large B-cell lymphoma (DLBCL): a clinical case with a literature review. Nuclear Medicine Review, 11-13. (IF 0,55) SJR 0,216 (8.6т.) *Web of Science*

We present a case of a 52-year-old man with generalized diffuse large B-cell lymphoma (DLBCL) with multiple extranodal sites involvement detected by ^{18}F-FDG PET/CT, demonstrating the high specificity of the method. Diffuse large B-cell lymphoma (DLBCL) is the most common form of NHL. It can arise in lymph nodes or outside of the lym- phatic system, in the gastrointestinal tract, testes, thyroid, skin, breast, bone, or brain. The involvement of specific extranodal sites has shown a prognostic value in the rituximab era. The ex- tranodal involvement of the lung/pleura, liver, lower urinary tract or bone marrow was a statistically significant poor prognostic factor. Our case ia a man diagnosed with FL with progression after 4 courses of R-CHOP into DLBCL. After three lines of treatment, he achieved complete response. A year later the patient presented with relapse with multiple extranodal lesions detected by ^{18}F-FDG PET/CT - a huge parenchymal lesion, adjacent to the chest wall in the left apical region with increased uptake of ^{18}F-FDG with SUV $_{max}$ 8.6, another soft

tissue lesion at the upper pole of the right kidney (SUV_{max} 4.1), and bone involvement of Th8 and Th9 with SUV_{max} 3.0. PET hypermetabolic lesions in lung and in right kidney were verified histopathologically .

PET-CT has a significant advantage for the diagnosis of diffusely infiltrating organs. As a hybrid imaging modality it allows accurate localization of disease and may be beneficial for the detection of unexpected extranodal sites of disease or exclusion of disease in the presence of nonspecific or equivocal extranodal CT findings.

10. Ilina Micheva, Trifon Chervenkov, Canka Ruseva, Liana Gercheva. Chronic myelomonocytic leukemia - review and clinical experience of the hematology department UMHAT "St. Marina" - Varna. J of Imab. 2016 Jan-Mar;22(1):1091-1095 SJR 0,225 (IF-0,787). (15т.) *Web of Science; Scopus*

Chronic myelomonocytic leukemia (CMML) is a clonal hematologic malignancy characterized by absolute peripheral monocytosis, ineffective hematopoiesis, and an increased risk of transformation to acute myeloid leukemia.

The aim of this study is to analyze the cases with Chronic myelomonocytic leukemia (CMML) diagnosed in the Hematology clinic, UMHAT “St. Marina”, Varna with assessment of risk, prognosis and survival.

The results from cytology, flow cytometry, histology, and genetics are re-estimated. For the risk stratification the CPSS was used.

Fifteen patients with CMML, 12 men and 3 women, with median age of 69,8 years were included in the study. According to the leukocyte count 12 were myeloproliferative (CMML/MP) and 3 myelodysplastic CMML (CMML/MD). The flow cytometry of peripheral blood and bone marrow was characterized by CD14, CD64, CD16 and CD56 expression. According to the histology of the bone marrow 2 cases were described as MDS, 1 as MDS/MPN, the rest as MPN with fibrosis in two of the cases. The cytogenetic risk was high in 5 patients and low in 10. According to CPSS one patient was with low risk, 3 with intermediate 1, 9 with intermediate 2 and 2 with high risk. Acute myeloid leukemia transformation occurred in 9 patients within median period of 13.1 months. The median

survival after transformation was 2,5 months. The median survival in the whole group was 21.4 months.

The study demonstrate that CMML is an aggressive disease. The prognosis of patients with CMML is poor, with low survival and high risk of transformation. The therapeutic options are limited.

Summaries of scientific works

Publications and reports published in scientific publications, referenced and indexed in world-renowned databases of scientific information

11. Yahya D, V. Miteva, **Micheva I**, C. Ruseva , L Angelova. Cytogenetic analysis of patients with hematological malignancies. Cytology and genetics. Q3, **IF 0.643** (6т.) *Scopus*

With this study, we aim to summarize and assess the activity and performance of the Cytogenetic sector of the Laboratory of Medical Genetics – Varna, regarding the conventional cytogenetic analysis of bone marrow samples from patients with onco(hematological) diagnoses. We have performed the analysis with the G-banding technique on 2,653 samples from patients of age 0-93 years by the current European recommendations and the International System for Human Cytogenomic Nomenclature. The greater part of these samples (90.9%) was with an indication of a hematological malignancy, most commonly Acute myeloid leukemia, Myelodysplastic syndrome, Acute lymphoid leukemia, Chronic myeloid leukemia, and Multiple myeloma. Analysis was successful in 2,215 (83.5%) - from those normal karyotypes were found in 1492 (67.4%) and pathology in 723 (32.6%). Regarding the latter, the most common were complex karyotypes (30.6%), Philadelphian chromosome (21.3%), trisomy 8 (5.9%), and deletion in the long arm of chromosome 5 (4.3%).
Cytogenetic analysis is a method with great impact on the evaluation of many hematological malignancies and for this reason, it remains an essential part of routine assessment of these diseases. The disadvantages of it mainly in the field of oncohematological diseases, recognized by the scientific society and confirmed in our own experience, suggest a need for an additional genetic method to overcome these limitations.

12. Dimitrova S, Efraim M, **Micheva I**. Myasthenia gravis as an unusual non-hematological autoimmune manifestation of a relapsed chronic lymphocytic leukemia – clinical case and review of literature. European Review for Medical and Pharmacological Sciences. **IF-3,14 (20т.). Приета за публикация.** *Scopus*

Autoimmune phenomena are well known to complicate chron- ic lymphocytic leukemia (CLL) and occur in 10% to 25% of the cases. Although less common, non-he- matological autoimmune manifestations have been reported. Myasthenia gravis (MG) is an au- toimmune disease of the neuromuscular junction, characterized by fatigable weakness of the extra- ocular,

bulbar, and limb musculature. The co-exis- tence of MG and CLL is exceedingly rare and there are very few cases reported in literature. Our case is of a 63-year-old female patient with a severe form of MG which is likely related to a relapse of CLL. Treatment with combined targeted and immuno- therapy was initiated with acceptable tolerability. Targeted agents and mono- clonal antibodies exert complex activities on the patient's immune system. It will be of interest to assess their role in managing autoimmune com- plications, accompanying CLL.

13. Yahya D, Hachmeriyan M, **Micheva I**, Chervenkov T. Acute myelogenous leukemia – current recommendations and approaches in molecular-genetic assessment. Rom. J. Intern. Med., 2022, 60, 2, 103-114. https://www.sciendo.com/article/10.2478/rjim-2022-0004 **(IF-1,73) (15)** *Web of Science; Scopus*

Acute myelogenous leukemia (AML) is a multistage hematological malignancy affecting the function, growth, proliferation and cell cycle of myeloid precursors. AML is characterized by genetic heterogeneity, rapid evolution, and variable genetic events, which are essential for diagnostic and prognostic evaluation as well as evaluation of therapeutic response.
This review presents a summary of the latest recommendations for genetic-molecular testing in AML, the preferred genetic methods, their general advantages and limitations. The possibilities of methods such as SNP-microarray, NGS, Sanger sequencing, multiplex ligation-dependent probe (MLPA), PCR are sidcussed. Since none of these methods is actually totipotent, we aim to shed light on the often difficult choice of the appropriate genetic assay.

14. V. Miteva, C. Ruseva, T. Chervenkov, **I. Micheva**. Cytogenetic findings in 126 patients with Multiple Myeloma – a retrospective single-centre study. ARCHIVES OF HELLENIC MEDICINE. Accepted for publication IF 0,2 Scopus

This is a retrospectiv study of newly diagnosed patients with multiple myeloma in the Laboratory of Medical genetics, UMHAT "St.Marina",Varna,Bulgaria for a period of 8 years (January 1st 2013 through December 31st 2020). All of the patients were assessed by conventional cytogenetics and fluorescence in situ hybridisation (FISH). The following variables were studied at diagnosis in each patient age, sex, bone marrow plasma cell infiltration, type of MM, ISS stage and karyotypes.
A total of 126 adult patients meeting the International Myeloma Working group diagnostic criteria of multiple myeloma were identified. Conventional karyotyping was performed on 113

(90%) of 126 patients, 17 (15%) of whom had also FISH analysis. The other 13 (10%) of 126 were analysed with FISH only. Karyotyping was successfully performed on 96 (85%) out of 113, 17 (15%) had no metaphase growth for chromosome analysis. With normal karyotype are 77 (68%) out of 113 patients. Abnormal karyotypes were found in 18 (16%) - 11(61%) of them had complex karyotypes and 7 had karyotypes with single anomaly (39%). 13 patients were analysed only with FISH, 9 (69%) of them had normal results, 4 (31%) had deletion on chromosome 17 - del(17)(p13).

On the whole 30 patients had FISH analysis, 7 (23%) of them with del(17)(p13).

In all 11 patients with complex karyotypes bone marrow plasma cell infiltration is >30%. In 6 out of 7 patients with a single anomaly in the karyotype bone marrow plasma cell percentage is >30%.The median overall duration of survival of the patients after diagnosis was 32 months. The median duration of survival between patients with normal and abnormal results from conventional cytogenetic analysis shows statistical significance (p=.03).

Our study demonstrates that despite the fact that the conventional cytogenetic analysis and FISH are performed on non-enriched plasma cells they still have informative value. Moreover, they are essential for risk stratification in MM patients at time of diagnosis. They can be used to detect progression of the disease or when there is no response of the treatment.

15. Геров В., Герова Д., **Мичева И.,** Галунска Б. Дисрегулация на костното ремоделиране – основен патогенетичен механизъм на костната болест при множествения миелом. Хематология. Том XL VIII, 1-2/2022.

The purpose of this review is to present some of the main pathogenetic mechanisms for the development of myeloma-induced bone disease MBD. Bone remodeling is an important process that occurs throughout human life in order to maintain bone homeostasis. In multiple myeloma (MM), the second most common hematologic malignancy, clonal plasma cells accumulate in the bone marrow, which profoundly affect the bone remodeling processes. Up to 80% of newly diagnosed patients have osteolytic bone lesions, leading to a significantly increased risk of pathological fractures and impaired quality of life. An imbalance in the processes of bone remodeling with a predominance of bone resorption over bone formation underlies the pathogenesis of MBD. Multiple intra- and intercellular signaling pathways such as RANKL/RANK/OPG, Notch and Wnt/β-Catenin signaling are involved, as well as a variety of chemokines, signaling and effector molecules such as DKK-1, sclerostin, periostin, activin A and transcription factors.

16. **С. Атанасова, И. Мичева. Епигенетични механизми при миелодиспластичен синдром. Медицински преглед. 58, 2022, № 6, 26-32. Sv. Atanasova, I. Micheva**. Epigenetic mechanisms in myelodysplastic syndrome – Medical review (Med. pregled), 58, 2022, № 6, 26-32.

Epigenetic mechanisms are closely related to oncogenesis and disturbances in these processes have been found in many malignant diseases. There are three main mechanisms for epigenetic regulation - DNA methylation, histone modifications and microRNA synthesis, and all three mechanisms are present in the pathogenesis of myelodysplastic syndrome (MDS). Mutations in genes responsible for DNA methylation, histone modifications, the role of micro RNAs in the pathogenesis of MDS were examined in detail. Azanucleotides were the first epigenetic drugs approved for the treatment of MDS. As a result of the many studies and research on epigenetic mechanisms, we are seeing progress in the discovery of new prognostic markers, as well as in the development of new therapeutic targets.

17. Efraim M, Micheva I. The role of comorbidities and clinical frailty scale in prognostic stratification of patients with myelodysplastic syndrome. Hematology (Bulgaria). 2021;57(1–2):17–23. (30т.) *Scopus*

Despite the expanding knowledge in the field of MDS and the improvement of prognostic scales, the choice of therapeutic approach remains a serious challenge. Current scoring systems, however, are of limited utility in therapy selection. The currently existing systems for risk stratification of oncological diseases, as well as of MDS, do not include the so-called patient prognostic factors such as general health status, presence of comorbidities and their degree of manifestation. Comorbidities may precede MDS or occur during treatment without being an adverse event related to it. Clinical studies indicate that the number and degree of manifestation of comorbidities influence the outcome of malignant disease. It is important to note that older MDS patients are less likely to receive active treatment as well as high-quality care. Three independent factors that are not included in risk stratification systems can be potentially decisive in determining the therapeutic approach - index of "vulnerability", comorbidity and quality of life. The Charlson comorbidity index, comorbidity index specific for patients before hematopoietic stem cell transplantation (HCT) (HCT-comorbidity index (HCT-CI)), MDS-specific comorbidity index (MDS-CI), the assessment of comorbidity for adults 27

(Comorbidity Evaluation-27 (ACE-27)). Comorbidities and degree of “vulnerability” play a significant role in determining survival in patients with MDS. Patients with severe forms of comorbidities have a 50% reduced survival, regardless of age and IPSS risk group. The use of comorbidity and "vulnerability" scales would facilitate the choice of a therapeutic approach in patients with MDS.

18. Dimitrova S, **Micheva I**. Pegylated asparginase in the treatment of acute lymphoblastic leukaemia in adults. Hematology (Bulgaria). 2021;57(1–2):63–7. (30т.) Scopus

The paper presents a historical overview of the use of L-asparginase in the treatment of patients with acute lymphoblastic leukemia (ALL). The treatment of acute lymphoblastic leukemia in adults is a serious challenge. While in the pediatric population a long-term remission is achieved in 90% of the patients, the results in adults are less encouraging. Despite the high incidence of response from the induction therapy, only 30-40% of adult patients with ALL remain in long-term remission. Although L-asparaginase is a standard in the treatment of children, its implementation in adult protocols is a matter of discussion due to the higher frequency of adverse effects. The introduction of pegylated asparaginase as a standard in the protocols for the treatment of patients with ALL is associated with a significant advance in optimizing therapy, keeping an acceptable balance between benefit and risk in all age groups.

19. **В. Митева, М. Хачмериян, Т. Червенков, И. Мичева. Цитогенетични аберации при пациенти с множествен миелом.** Медицински преглед, 57, 2021, № 5, 28-35. V. Miteva**,** M. Hachmeriyan**,** T. Chervenkov**,** I. Micheva**.** Cytogenetic aberrations in patients with multiple myeloma. – Medical review, 57, 2021, № 5, 28-35. **(15т.) Web of Science**

MM is characterized by complex and diverse chromosomal aberrations, similar to those found in solid tumors. Hyperdiploidy, with multiple trisomies of odd-numbered chromosomes and translocations involving the immunoglobulin heavy chain gene (IgH) locus, are two primary genetic events implicated in the pathogenesis of MM. The most common secondary cytogenetic abnormalities are monosomies, deletions and MYC translocations. The frequency of secondary aberrations tends to increase during the transition between MGUS to SMM or MM. Translocations t(4;14) and del(17)(p13) are the most common ones and are associated with poor prognosis, deletion del(13)(q14) is considered as a major prognostic factor. On the other

hand, patients with the translocation t(11;14) are considered to have a good prognosis. One of the most common structural aberrations involves chromosome 1 and its presence in the karyotype increases the risk for progression.

20. Cappellini MD, Viprakasit V, Taher AT, Georgiev P, Kuo KHM, Coates T, Voskaridou E, Liew HK, Pazgal-Kobrowski I, Forni GL, Perrotta S, Khelif A, Lal A, Kattamis A, Vlachaki E, Origa R, Aydinok Y, Bejaoui M, Ho PJ, Chew LP, Bee PC, Lim SM, Lu MY, Tantiworawit A, Ganeva P, Gercheva L, Shah F, Neufeld EJ, Thompson A, Laadem A, Shetty JK, Zou J, Zhang J, Miteva D, Zinger T, Linde PG, Sherman ML, Hermine O, Porter J, Piga A; BELIEVE Investigators. A Phase 3 Trial of Luspatercept in Patients with Transfusion-Dependent β-Thalassemia. N Engl J Med. 2020 Mar 26;382(13):1219-1231. doi: 10.1056/NEJMoa1910182. (IF 91,245)

The results of a randomized, double-blind, phase 3 trial comparing best maintenance plus luspatercept versus placebo in adult patients with transfusion-dependent β-thalassemia are presented. Luspatercept, a recombinant fusion protein that binds to selected ligands of the transforming growth factor β superfamily, can improve erythroid maturation and reduce the transfusion burden (total number of red blood cells transfused) in such patients. The primary end point was the percentage of patients who had a reduction in the transfusion burden of at least 33% from baseline during weeks 13 through 24 plus a reduction of at least 2 red-cell units over this 12-week interval. Other efficacy end points included reductions in the transfusion burden during any 12-week interval and results of iron studies. A total of 224 patients were assigned to the luspatercept group and 112 to the placebo group. Luspatercept or placebo was administered for a median of approximately 64 weeks in both groups. The percentage of patients who had a reduction in the transfusion burden of at least 33% from baseline during weeks 13 through 24 plus a reduction of at least 2 red-cell units over this 12-week interval was significantly greater in the luspatercept group than in the placebo group (21.4% vs. 4.5%, $P<0.001$). During any 12-week interval, the percentage of patients who had a reduction in transfusion burden of at least 33% was greater in the luspatercept group than in the placebo group (70.5% vs. 29.5%), as was the percentage of those who had a reduction of at least 50% (40.2% vs. 6.3%). The least-squares mean difference between the groups in serum ferritin levels at week 48 was -348 μg per liter (95% confidence interval, -517 to -179) in favor of luspatercept. Adverse

events of transient bone pain, arthralgia, dizziness, hypertension, and hyperuricemia were more common with luspatercept than placebo.

21. Димитрова Ст, **Мичева И.** Първична миелофиброза – участие на регулатори на железния метаболизъм – хепсидин и инфламаторни цитокини в патогенезата на анемичния синдром. Медицински преглед. 2020;56(4):5-11. (30т.) Web of Science

The publication examines the likely mechanisms involved in the development of the anemia in PMF. One of the suspected factors is the dysregulation of iron homeostasis. Iron metabolism impairment plays a key role in the pathogenesis of several disorders like solid tumors, chronic inflammatory diseases and ageing. Peptide hormone hepcidin is a main regulator of iron metabolism. Through the hepcidin-ferroportin line, it controls the levels of extracellular iron in the organism. It was found that newly diagnosed patients with PMF have higher hepcidin levels in comparison to healthy controls, which correlates with severity of anemia, red blood cell transfusion requirements and ferritin levels. High hepcidin levels are predictors of inferior prognosis, independently of the risk profile. Nevertheless, hepcidin regulation is complex and multifactorial. It is influenced by many physiological and pathological processes like inflammatory stimuli, hypoxia, dis-hema¬to¬poiesis, oxidative stress. Because of the complicated and multistage hepcidin regulation, its role in anemia development is ambiguous. Cytokine dysregulation in parallel with iron metabolism impairment participates in anemia development in PMF. Elevated serum levels of IL-8 and IL-6 are found, as the overexpression of IL-8 is observed in different hematopoietic cell lines and is an independent prognostic factor for survival.

22. Niyazi D, Micheva I, Stoeva T. Bacterial and fungal complications in patients undergoing hematopoietic stem cell transplantation. Hematology (Bulgaria). 2020;56(1):26–31. (20т.) (SJR_{2020} 0.101, Q4) *Scopus*

This review presents in detail the main risk factors for the development of infectious complications in patients who have undergone autologous and allogeneic HSC transplantation, as well as the most important bacterial and fungal infections characterizing its various stages. The risk factors for the development of infectious complications were examined, such as factors from the patient's side, from the donor's side and those related to the transplantation

itself. Among the most significant factors are older age, previous HSCT, as well as the development of invasive infections caused by Candida spp. and Aspergillus spp. before transplantation, the older age of the donor, use of myeloablative conditioning regimen, degree of HLA incompatibility, source of stem cells, use of immunosuppressive agents such as anti-thymocyte globulin (ATG) and alemtuzumab. Among the most important risk factors for infectious complications after transplantation are the duration and severity of neutropenia.The most common causes of bacterial and mycotic infections, their place in the different phases of SCT and their importance for the outcome of transplantation are presented. In the pre-engraftment period, bacteria are the leading pathogens in this phase, while fungi and Herpes simplex virus (HSV) are less common. The main sources of bacterial and mycotic infections for the patient are the normal microbial flora of the gastrointestinal tract, represented by Gram-negative bacteria, mainly belonging to the family Enterobacteriaceae and fungi of the genus Candida, as well as central venous catheters connected to representatives of normal skin flora – mostly Gram-positive bacteria (coagulase-negative staphylococci). In the early post-engraftment period, infectious complications are associated with various pathogens, but with a dominance of intestinal bacteria leading to life-threatening conditions, adenoviruses, BK and respiratory viruses, Pneumocystis jirovecii, Candida spp. and Aspergillus spp. In this period, symptomatic CMV infection usually presents as either life-threatening pneumonia or enterocolitis in pretransplant seropositive patients with GVHD. In the late post-engraftment period, infectious complications are usually observed mostly among recipients of allogeneic HSCT and specific risk factors, which necessitates their long-term follow-up. These complications relate mostly to the pathogens characteristic of the early post-engraftment period, Varicella Zoster Virus and encapsulated bacteria.

Knowledge of the risk factors and the most common infectious pathogens during each period after HSCT supports decision-making about the need for prophylaxis, allows differential diagnosis of clinical symptoms and the initiation of adequate and timely therapy of relevant infectious complications .

23. Goranova-Marinova V, Ignatova K, Ganeva P, Spasov E, **Micheva I,** Radinov A, Petrova R, Tzvetkova G, Hadzhiev E, Goranov St, Gercheva L. Therapeutic results in patients with Hodgkin’s lymphoma treated with brentuximab vedotin-bulgarian experience. Hematology (Bulgaria). 2017;53(1–2):60–5. (5.45т.) Scopus

The aim of this study is to analyze the overall response rate (ORR), type of response, event free survival (EFS) and the prognostic factors affecting them in patients with Hodgkin's lymphoma treated with Brentuximab Vedotin (BV). Fourty-four (44) Hodgkin lymphoma patients treated with BV in 5 hematology clinics in Bulgaria were studied. The male / female ratio is 1.1 / 1, the mean age is 39.98 +/ – 12.48. The most common histological variant is nodular sclerosis 31 (70.5%). In the II clinical stage there were 13 patients (29.5%), III – 16 (36.4%), IV – 15 (34.1%). B-symptoms had 36 patients (81.8%), extranodal involvement – 17 (15.9%) and large mediastinal tumor mass – 14 (31.8%). All patients before treatment with BV received 4 (2-12) treatment lines, 50.0% – 3 lines, 27.3% – 4 lines, and 9.1% > / = 5 lines. Stem cell transplantation was performed in 33 patients (75.0%), two of which were two autologous STCs, and in one auto/ allo SCT. ORR is 60.0% (N=24) – complete response was achieved in 10 (25.0%) and partial in 14 (35.0%) patients. Stable disease was registered in 7 (17.5%) and progression in 9 (22,5%). Patients achieving at least a partial therapeutic response prior to commencement of BV treatment significantly improved their response at the end of treatment: 15 (62.5%) versus 9 (37.5%) with previous progression and stable disease ($P < 0.05$, R = + 0.496). PFS for the entire group is 10 months. The median survival (MS) was not reached by the end of the study. Age, sex, histological variant, clinical stage, number, type and outcome of previous treatment lines did not affect the duration of PFS. The only significant prognostic factor that determines a remarkably longer remission is the qualitative therapeutic response: in the CR + PR group, the median PFS is not reached by the time of the analysis, while in patients with stable disease and progression EFS is 7 months ($P <0.001$). BV is an effective drug with manageable toxicity. The results of the treatment of our patients con- firm the accumulated international experience. An additional therapeutic response and prolonged progression- free survival give a chance to these previously doomed patients with Hodgkin's lymphoma.

24. EP505 Daniel Primo, Jaime Pérez Oteyza, Juan Miguel Bergua, José Ángel Hernández-Rivas, Elena Ruiz, Susana Vives, Aurelio López, Carlos Javier Cerveró, Milan Jagurinoski, Roman Hájek, Atanas Radinoff, **Ilina Micheva**, Rebeca González Rolfe, María Calbacho, John Muth, Julian Gorrochategui, Antonio Valeri, Jan Davidson, Joaquín Martínez-López, Joan Ballesteros. Bispecific T-cell engager antibodies may contribute to reactivate pre-existing tumor specific T-cells. Hema Sphere, 2020;4;S1. EHA Library. Ballesteros J. 06/12/20; 294424; **IF 1,56** https://library.ehaweb.org/eha/2020/eha25th/294424/joan.ballesteros.bispecific.t-cell.engager.antibodies.may.contribute.to.html

Bispecific T-cell engaging antibodies (BsAbs) redirect T cells to kill tumor cells by proximity independent of intrinsic antigen-specific TCR recognition. It was found that after incubation of acute myeloid leukemia (AML) samples with CD3xCD123 BsAb, isolation of BsAb-activated T cells by fluorescence-activated cell sorting (FACS), removal of BsAb by washing steps, and addition of new autologous blasts, these BsAb-activated T cells in the absence of BsAb were able to kill the blasts.

The aim of the study was to identify patients with TCR-dependent blast-T cell interaction after in vitro incubation with CD3xCD123 BsAb on bone marrow (BM) from AML patients, for potential selection of patients with a more durable response after BsAb therapy.

16 bone marrow samples were tested with CD3xCD123 BsAb (Creative Biolabs) at 120h at different doses.

T-cell activation and effective tumor cell lysis following BsAb exposure was demonstrated in 62% of samples (10/16). In this group of responding patients, the TCR-HLA interaction was evaluated in the presence of blocking antibodies and showed partial blocking of the TCR-dependent interaction in 40% of samples (4/10) patients. We speculate that direct contact between T cells and blast cells by BsAb may in some cases directly reactivate pre-existing tumor-specific antigen T cells from the BM to recognize and kill AML blasts themselves. This secondary T-cell response requires cross-presentation by classical APCs or possibly blasts directly, independent of BsAb, which can be blocked by anti-HLA or TCR antibodies. Reactivating the immune system would provide these patients with the benefits of long-term responses.

25. S Dimitrova, **I Micheva**, D Gerova, L Gercheva. The role of hepcidin and dysregulated iron homeostasis in the pathogenesis of anemia in myelofibrosis: pf667. Hemasphere 3, 286. **IF 0,86** Stela Dimitrova, **IlinaMicheva,** Daniela Gerova, Liana Gercheva**. THE ROLE OF HEPCIDIN AND DYSREGULATED IRON HOMEOSTASIS IN THE PATHOGENESIS OF ANEMIA IN MYELOFIBROSIS** EHA Library. Dimitrova S. 06/14/19; 266466; PF667 **(IF-0,86) (15т.)**

The aims is to analyze hepcidin, IL-6, IL-8 levels and parameters of iron metabolism in a random cross-section of patients with different forms and stages of myelofibrosis. We analyzed 36 patients with MF – 75 % with primary MF(PMF) (prefibrotic PMF – 38,9% and overt PMF – 36,1%) and 25% with secondary MF (post-PV -11,1% and post-ET – 13,9%); median age of the group was 68,5 years (range 39-88); male: female ratio- 1,57:1. According to DIPSS: 5,6% were Low; 52,8% were Intermediate 1; 33,3% were Intermediate 2 and 8,3% were High risk.

Regarding treatment 30,6% of the patients received hydroxyurea, 8,3% - Interferon, 19,4% - Ruxolitinib and 41,7% - best supportive care. Patients who were transfused with ≥3 RBC units/month were 19,5% and those receiving < 3 RBC units/month - 80,5%. All patients were divided in 3 groups according to the time from diagnosis: newly diagnosed (30,6%), 1 to 5 years (47,2%) and > 5 years (22,2%) from diagnosis. Serum ferritin, Fe, TIBC and parameters of CBC were measured as a part of routine clinical assessment. Serum hepcidin and serum concentrations of IL 6 and IL8 were measured in duplicate by ELISA (My BioSource, San Diego, USA) in all patients and 14 healthy controls. The levels of hepcidin in MF patients are not found statistically different from levels in healthy controls. No difference is found in hepcidin level within the three risk groups according to DIPSS (Intermediate 1, Intermediate 2 and High), neither between the subtypes of disease (proliferative or fibrotic stage of primary myelofibrosis and secondary myelofibrosis). However, we find significant difference between hepcidin levels within the three groups according to disease duration. Patients with newly diagnosed MF have higher levels of hepcidin compared to those with prolonged evolution ($F=7,09$, $R= -0,476$, $R^2= 0,226$, $p=0,003$). Patients receiving > 3 RBC transfusions/month present with lower hepcidin compared to transfusion independent patients ($10,10 \pm 6,67$; $31,15 \pm 44,37$; $p < 0,05$). We also find significantly lower hepcidin level in patients receiving cytoreductive or target treatment compared to patients without any therapy ($17,74 \pm 21,99$; $43,05 \pm 58,46$; $p<0,05$). Unexpectedly, higher levels of ferritin and serum iron are associated with low hepcidin. In multivariate analysis a significant straight correlation between hepcidin level and IL6 ($R=0,427$, $p=0,009$) and between IL6 and IL8 levels in the whole cohort ($R=0,500$, $p=0,002$) is found.

Publications and reports published in non-refereed peer-reviewed journals or published in edited collective volumes

26. Niyazi D, **Micheva I,** Savova D, Georgieva M, Ismail E, Stoeva T. Species diversity and antimicrobial resistance of Staphylococcus spp. isolates associated with catheter-related bloodstream infections in patients after hematopoietic stem cell transplantation. Scripta Scientifica Medica. 2022 Apr 13;54(2). [Online First]. (5)

The aim of this study was to investigate the species diversity of clinically significant Staphylococcus spp. isolates, obtained from blood cultures of patients with central venous

catheter (CVC) following hemato- poietic stem cell transplantation (HSCT) during the period January 2019–December 2020, as well as to test their susceptibility to a set of antimicrobials. In the collected group of 21 isolates, 85.7% were coagulase-negative staphylococci (CoNS): S. epi- dermidis, n=12; S. haemolyticus, n=4; S. hominis, n=2, and 3 isolates were identified as S. aureus. Methicil- lin resistance was 85.7% and was detected only in the CoNS group. In decreasing order, the resistance rates in CoNS group were as follows: 100% for penicillin and cefoxitin >83.3% erythromycin >72.2% ciprofloxacin >61.1% gentamicin >44.4% for clindamycin and trimethoprim/sulfamenthoxazole. No resistance to vanco- mycin, teicoplanin, and linezolid was found. S. aureus isolates demonstrated preserved susceptibility to all antimicrobials with exception to penicillin. In the present study, CoNS were identified as the most common cause of catheter-related bloodstream infections (BSIs) in patients following HSCT, with S. epidermidis being the predominant species. All CoNS isolates were methicillin-resistant, but also exhibited reduced susceptibility to other antimicrobials and thus causing difficult-to-treat infectious complications in patients after HSCT.

27. Геров В, Герова Д, **Мичева И**, Галунска – Калчева Б. Костни биомаркери при множествен миелом. MEDINFO. 2022;4 (7,5)

Biomarkers are an integral part of the diagnosis, follow-up and prognosis of MM. In light of the increased knowledge of the pathogenesis of MM and of the relationship of myeloma cells with cells of the bone marrow microenvironment, the characterization of new bone biomarkers is needed, with the help of which it would be possible both to stratify risk groups more precisely and to develop new targeted therapies. The importance of basic biomarkers related to hypoxia and neoangiogenesis in multiple myeloma as well as biomarkers related to osteoclast and osteoblastic function - RANKL, DKK-1 and sclerostin, periostin, vitamin D - was discussed.

28. Атанасова С, **Мичева И.** Нарушения в метилирането при миелодиспластичен синдром – терапевтични възможности. MEDINFO. 2022;4 (15)

The course of MDS and the risk of transformation are closely related to molecular genetic and epigenetic disorders. Among the most frequently mutated genes in MDS are those encoding enzymes responsible for epigenetic regulation, which is indicative of a role in the pathogenesis of MDS. DNA methylation is the best-studied epigenetic mechanism in MDS, whose disturbances can result in suppression of genes involved in cell cycle regulation, apoptosis, and

DNA repair. Disturbances in the genes responsible for DNA methylation in MDS, such as TET2, DNMT3A and IDH1/2, as well as therapeutic options for influencing DNA methylation disorders, were discussed.

29. D Yahya, V Miteva, I Micheva, T Ruseva, L Angelova. Cytogenetic analysis of patients with hematological malignancies. Preprint from Research Square, 07 Apr 2022
    DOI: 10.21203/rs.3.rs-1466211/v1

We aim to summarize and assess the activity and performance of the Cytogenetic sector of the Laboratory of Medical Genetics – Varna, in regards to conventional cytogenetic analysis of bone marrow samples from patients with various haematological diagnoses. Another purpose is to evaluate the tendencies noticed over a period of eleven years in order to draw conclusions and share our experience. We performed retrospective analysis on all bone-marrow-derived samples in our centre during the period 2010-2020. We evaluated 2,653 results in total from patients of age 0-93 years. Samples were stained with the G-banding technique in accordance with the current European recommendations and the International System for Human Cytogenomic Nomenclature. Haematological malignancy was the most frequent indication (90.9%) with predominance of acute myeloid leukaemia, myelodysplastic syndrome, acute lymphoid leukaemia, chronic myeloid leukaemia, and multiple myeloma. Analysis was successful in 2,215 (83.5%) - from those normal karyotype was found in 1492 (67.4%), and pathology - in 723 (32.6%). Regarding the latter, most common were complex karyotype (29.9%), Philadelphian chromosome (21%), trisomy 8 (6.1%) and deletion of the long arm of chromosome 5 (4.4%). The method holds a great inuence over the evaluation of haematological malignancies and thus it remains an essential part of standard work-up of these diseases.

30. D. Yahya, M. Stoyanova, M. Hachmeriyan, L. Angelova, **I. Micheva**, T. Chervenkov. Molecular genetic markers in acute myelogenous leukemia—the good, the bad, and the intermediate. Варненски медицински форум, т. 11, 2022 **(5)**

The application of molecular genetic markers in the work-up of various diseases carries a great significance for current clinical and diagnostic practice. This routine only became possible after the finalization of the Human Genome Project with mapping of genes, essential for human pathology. Incorporating high-resolution genetic techniques and a widely available

international database has also had a major contribution to the evolution of this process. Regarding acute myelogenous leukemia, in particular, the knowledge of the most common and weighty diagnostic, prognostic, and predictive molecular genetic markers is already included in the well-established recommendations. With this review, we aim to summarize and present information regarding the most common molecular variants currently as well as their significance for a personalized approach.

31. Yuzeir S, Stefanova N, **Micheva I.** VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION IN ADULT NEWLY DIAGNOSED NON-HODGKINS'S LYMPHOMA PATIENTS. KNOWLEDGE - International Journal. 2022;50(4):533–537. (IF-1,82).

The aim of the study is to investigate the level of VEGF expression in newly diagnosed patients with aggressive and indolent NHL and to look for the relationship with clinico-pathological characteristics and prognostic parameters. Through an immunohistochemical reaction, the expression level of VEGF, as a marker of bone marrow angiogenesis, was determined in 60 patients with histologically verified NHL before chemotherapy treatment and in 20 healthy controls. Results: Of the total of 60 NHL patients examined, 32 were men and 28 were women. The distribution according to the type of lymphoma is as follows: 24 are with Diffuse B-large cell NHL, 8 with Mantle cell NHL, 2 patients with Peripheral T-cell NHL, 9 with Marginal zone NHL, 10 with Follicular NHL, 1 patient with Plasmablastic and 6 with Small cell NHL. A statistically significant correlation was found between the group of patients with NHL and the group of healthy controls ($p<0.001$). In the group of patients with indolent NHL, two times higher levels of VEGF were recorded compared to the group of aggressive NHL ($p= 0.016$). A significant significant dependence was found in the group of patients with high LDH values ($p= 0.015$), compared to the control group. No association was found between VEGF and hemoglobin, leukocytes, platelets, beta2 microglobulin and B symptoms. Multivariate analysis proved a significant difference between the two studied groups in terms of IPI ($p=0.005$), finding a moderate correlation between the type of lymphoma and the risk profile of the patients ($r=0.399$; $p=0.002$). Patients with intermediate- and high-risk NHL and elevated VEGF levels have an unfavorable prognosis and short overall survival. VEGF plays a vital role in the development and progression of NHL. Based on the obtained results, our hypothesis is that VEGF overexpression represents a promising potential prognostic factor in NHL.

32. **Мичева И.** Obinutuzumab в лечението на фоликуларен неходжкинов лимфом. МЕДИК плюс. 2022;1 (30 т.)

An up-to-date review of the most significant results of randomized clinical trials with Obinutuzumab in follicular NHL, defining the drug's place in the treatment of this type of NHL, is presented. Future therapeutic directions are also outlined.

33. Dimitrova S, **Micheva I.** Therapeutic approach in the treatment of newly diagnosed elderly patients with secondary acute myeloid leukemia—a clinical case and review of the literature. Journal of the Union of Scientists - Varna Medicine and Ecology Series. 2021 Mar 1;26(2):20–6. (15)

Therapy-related acute myeloid leukemia (t-AML) is defined as a distinct subtype of AML whose pathogenesis is associated with the effects of prior cytotoxic chemotherapy or radiotherapy. The results of therapeutic intervention in patients with t-AML are limited, and optimal treatment in these patients is an even greater challenge. We present a case of a 68-year-old woman who was hospitalized for isolated anemia. The patient was diagnosed with breast carcinoma in 1996 and ovarian carcinoma in 2014, which are in remission. From the diagnostic procedures performed, a previous therapy-related myelodysplastic syndrome (t-MDS) with a complex karyotype was proven. The patient was stratified as high risk according to the R - IPSS. Two months later, evolution in t-AML was demonstrated. Treatment with a hypomethylating agent was started. In the course of the treatment, the patient died with the picture of sudden cardiac death. The choice of therapy in adult patients with t-AML is difficult and multifactorial. Combinations of already approved agents and new molecules will in the future improve and diversify therapeutic choices in adult patients with high-risk AML.

34. Yuseir S, Shtereva B, **Micheva I,** Gercheva L. PROGNOSTIC SIGNIFICANCE OF PLATELET- NEUTROPHIL COMPLEXES IN NON-HODGKIN'S LYMPHOMA PATIENTS. American Scientific Journal. 2021;1(54):14–7. (7,5) Index Copernicus ISSN 2707-9864

The aim of the study was to investigate PNC levels in patients with indolent and aggressive non-Hodgkin's lymphomas (NHL) and their relationship with clinico-laboratory parameters. A total of 88 newly diagnosed patients with histologically verified NHL and 20 healthy controls

were studied. Of the patients with NHL, 34 were with Diffuse B-large cell NHL, 9 with Mantle cell NHL, 4 patients with Peripheral T-cell NHL, 15 with Marginal zone NHL, 10 with Follicular NHL, 1 patient with Plasmablastic, 1 with Lymphoplasmacytoid and 14 with Small cell NHL. PNC levels were determined before initiation of immuno-chemotherapy by flow cytometric analysis of venous blood. Mean PNC levels were statistically significantly higher in patients with NHL compared to the group of healthy controls ($p<0.01$). A significant difference in PNC levels was found in aggressive compared to indolent NHL ($p=0.05$). A significant linear relationship between circulating PNC and platelet values and absolute neutrophil count was demonstrated. The analysis showed an inversely proportional relationship between PNC and hemoglobin values ($r = 0.463$; $p < 0.001$). No correlation was found with the leukocyte count, LDH, beta 2 microglobulin, the presence of B symptoms and the absolute lymphocyte count. High levels of PNC may serve as an independent negative prognostic marker in the development of NHL.

35. Yuzeir S, Varbanov H, Yordanova Ts, **Micheva I.** Langerhans Cell Histiocytosis with Multisystem Involvement in a Young Woman: A Case Report and Literature Review. Journal of Clinical Case Reports. 2021;11(7):1452. (IF 0,532). (7,5т.) Google Schola

Langerhans Cell Histiocytosis (LCH), also known as Histiocytosis X (HX), is a group of hyperplastic cellular diseases of unknown causes. LCH could affect bones, lungs, central nervous system, liver, thymus, skin, and also lymph nodes. The diagnosis of LCH is difficult to enforce and rarely found in adults, with just about 5 cases per million per year. The present study reports the case of a young woman with LCH with multisystem involvement, including that of the bone, orbit, pulmonary system and central nervous system. The patient received chemotherapy for 6 months and exhibited rapid improvement in the involved systems. The last PET/CT showed metabolic activity in the right iliac bone. One year after completion of the therapy, the patient returned to the hospital showing deteriorating health. The clinical case is interesting not only because of the registered clinical, morphological, and imaging data of histiocytosis but also because of the unclear prognostic and diagnostic importance of this phenomenon.

36. **Мичева И.** Polatuzumab vedotin в лечението на релапсирал или рефрактерен дифузен В-едроклетъчен лимфом. PRO MEDIC. 2021:3 (30 т.)

The review presents the therapeutic options for diffuse large B cell lymphoma (DLBCL) which accounts for about 25% of the newly diagnosed cases of non-Hodgkin's lymphomas. Despite significantly improved survival after the introduction of immunochemotherapy, about half of the patients relapse or have refractory disease. The outcome of patients, ineligible for transplantation is very poor due to limited ability to control the disease. Only a small proportion of them respond to salvage therapy after relapse. Improving outcome in these patients requires the incorporation of novel drugs into the therapeutic approaches. Polatuzumab vedotin is the first in class antibody-drug conjugate (ADC) targeting CD79b, which represents a promising new option for the treatment of relapsed or refractory DLBCL patients, ineligible for autologous stem cell transplantation.

37. Efraim M, **Micheva I.** Sociodemographic Characteristics Of Survival In Patients With Myelodysplastic Syndrome. Varna Medical Forum. 2021 Sep 1;10(2):507–10.. (15т.)

The purpose of the present study is to analyze the impact of some sociodemographic characteristics on the survival of patients with MDS. We analyzed 219 patients with MDS, who were admitted in the Clin- ic of Hematology, “Sv. Marina” University Hospital, Varna for a period of 10 years (2010–2020). Survival was assessed by age, sex, FAB, and WHO2016 subtype and the risk group was defined by IPSS, IPSS-R, and WPSS. There is a significantly higher survival rate in wom- en and an inversely proportional relationship between survival and age. Patients with RAEB and RAEB-t have the lowest survival as well as patients with high and very high risk. MDS presents with significant dif- ferences in survival between subtypes, age, and sex. The outcome of the disease varies according to the risk group determined by the established scales for risk stratification, and the most accurate in the prognosis is IPSS-R.

38. Ефраим М, **Мичева И**. Нови биомаркери в диагнозата и прогностичната оценка на пациенти с миелодиспластичен синдром. MEDINFO. 2021; 04. (15т.)

Cytogenetic and molecular markers - fundamental in determining the diagnosis and prognosis in patients with MDS - were reviewed. Analyzes showed that mutations of CBL, IDH2, ASXL1, DNMT3A and TP53 genes were independent prognostic biomarkers associated with shorter survival. Their incorporation into the MDS classifications and prognostic scoring

systems is pending. The search for additional biological markers, as potential targets for influencing MDS, will also allow personalization of the treatment of this complex and heterogeneous disease.

39. Efraim M, **Micheva I**. The role of MDS-CI in the prognostic assessment of patients with myelodysplastic syndrome. Journal of the Union of Scientists - Varna Medicine and Ecology Series. 2021 Nov 21;26(1):25–30. 15)

The MDS comorbidity index (MDS-CI) is designed to predict the impact of comorbidities on the outcome of the disease. The aim of our analysis is to assess the prognostic value of MDS-CI within the WHO prognostic scoring system (WPSS) subgroups. We applied MDS-CI in 219 patients with MDS, diagnosed and treated in the Clinic of Hematology of St. Marina University Hospital, Varna, Bulgaria between May 2010 and May 2020. WPSS was used for prognostic stratification. The mean age was 70.7±10.2 years (35–93 years). In patients with very low/low risk according to WPSS, we found significant difference in terms of survival between MDS-CI=0 and MDS-CI>2 (69.2±43.0 vs. 38.3±42.1 months, $p<0.001$). Similar difference was found within the intermediate/very high risk groups ($p<0.001$). MDS-CI adds prognostic value to the es- tablished WPSS. Combining both systems allows refining the prognostic assessment and survival of MDS patients.

40. S Dimitrova, L Gercheva, D Gerova, **I Micheva.** Controversial impact of hepcidin metabolism in the pathogenesis of anemia in myelofibrosis. American Journal of Internal Medicine 2020, 8 (4), 153-158. (15т.) CrossRef

The aim of the study was to analyze parameters of iron metabolism and inflammation in patients withdifferent forms and stages of myelofibrosis. Thirty-six patients with primary MF, post-polycythemia vera and post-essentialthrombocythemia MF and fourteen healthy controls were included in the study. The hepcidin level in the patient group was found statistically lower compared to healthy controls(27,64±41,56 ng/ml; 111,13±49,56 ng/ml; F=2,81, $p<0,001$). Patients with newly diagnosed MF had significantly higher levels ofhepcidin compared to those with prolonged evolution: between 1 and 5 years ($p=0,005$) and >5 years ($p=0,038$). Transfusiondependent patients presented with lower hepcidin compared to transfusions independent (10,10±6,67 ng/ml; 31,15±44,37 ng/ml;$p=0,026$). In patients receiving cytoreductive or target treatment hepcidin level was significantly lower compared to patients

onbest supportive care (17,74±21,99 ng/ml; 43,05±58,46 ng/ml; p=0,037). No difference was found in hepcidin level within the riskgroups according to DIPSS, neither between the subtypes of disease (primary MF and secondary MF). Higher hepcidin positivelycorrelated with leukocytosis and age. A significant highlypositive correlation was found between hepcidin and IL-6 and weaker between hepcidin and IL-8. A significant straight correlation was demonstrated between IL-6 and IL-8 and a negative betweenserum iron and IL-6 and IL-8. The hepcidin regulation is complex andmultifactorial. Its role in pathogenesis of anemia in myelofibrosis is controversary. Probably it has a higher impact in early stagesof the disease and depends on treatment and transfusions.

41. Dimitrova S, **Micheva I.** The role of hepcidin in the pathogenesis, severity of clinical manifestation, and prognosis of patients with myelofibrosis. Journal of the Union of Scientists - Varna Medicine and Ecology Series. 2020 Dec 11;25(1):18–23. (15т.)

    The aim of the study was to analyze the serum levels of hepcidin in patients with PMF and its impact on the clinical course, prognosis, and outcome of the disease. A total of 68 patients with PMF and 12 healthy controls were analyzed. Serum hepcidin levels were measured by ELISA. Then mean hepcidin levels in patients with PMF were statistically significantly higher compared to healthy controls. (99.05 ng/mL; 20.57 ng/mL; F=7.95; p=0.006). High levels of hepcidin correlated with high risk according to DIPSS (p=0.046), carrier of JakV617F mutation (p=0.022), fibrotic phase according to WHO 2016 (p=0.062), and the number of blood transfusions per month (p=0.005). Higher hepcidin levels were not relevant to overall survival. Hepcidin is a biological marker the monitoring of which in the course of MF would help for a more accurate clinical and prognostic assessment of the disease.

42. Ванкова, Д., Капинчева, И., **Мичева**, И., Петрова, И., Петрова, М., Матев, Б., Божинова, Д., Радкова, Ж., 2020. Интегративни научни подходи към комплементарна и алтернативна медицина (КАМ) - изследване на концепция, контекст и качество на живот, Асклепий 2020, том XVI, стр. 102-107. (7.5т.)

Integrative scientific approaches are increasingly relevant in modern research environments. They require transdisciplinarity (combining knowledge and methods from different scientific fields), synthesis (combining diverse concepts and information into new knowledge and understanding) and collaboration (enabling researchers to work together). The presented

project on the topic "Scientific approaches to complementary and alternative medicine (CAM) - research on concept, context and quality of life" applies all the possibilities of integrative science, betting on teamwork and topicality of the subject.

43. **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. (6т.)

The aim of the study was to assess the efficacy of azacitidine treatment in patients with MDS and AML.Twenty-seven patients with MDS and AML treated in the Clinic of Hematology at Sv. Marina University Hospital, Varna were included in the study and followed for 18 months. Azacitidine was administered subcutaneously at a dose of 75 mg/m2 for 7 days. Disease assessment was performed on the 3rd month, 6th month, and at progression.
Their median age was 71.5 years. Nine had refractory ane- mia with excess of blasts II (RAEB II), 5 had chronic myelomonocytic leukemia II (CMML II), 1 was with unclassifiable MDS (MDS-U), and 12 with AML. The median number of administered cycles was 6 (1-19). Eleven patients completed 6 cycles of azacitidine. Partial response was achieved in 9 patients (33%) (7 MDS and 2 AML), stable disease in 8 (29%) (5 MDS and 3 AML). Progressive disease was observed in 10 patients (37%). The response correlated with the type of the disease (p=0.03), cytogenetic risk (p=0.01), and survival (p=0.000). At 18 months, 60% of MDS patients were alive compared to 41.7% in the AML group. The medi- an time to death in the AML patient group was 2.5 months. The mean overall survival was 10.4 months (12.6 months for MDS patients and 5.4 months for AML patients).The therapy with azacitidine is an option for elderly patients with high-risk MDS. In pa- tients with AML a rapid progression is observed during the first two cycles with mortality rate of 58.3%.

Reports published in scientific publications, referenced and indexed in world-renowned databases of scientific information

44. Ivanova M, Tsvetkova G, Tsvetelin L, **Micheva I**, Hadjiev E, Shivarov V. HLA class II immune editing in JAK2V617 and CALR mutation driven oncogenesis. In HLA 2022 May 1 (Vol. 99, No. 5, pp. 449-449). 111 RIVER ST, HOBOKEN 07030-5774, NJ USA: WILEY. **Impact factor (2021):8,762, Scopus, Web of Science**

We aimed at the elucidation of the putative protective role of HLA-II alleles for the development of JAK2V617F and CALRmut driven MPNs. We performed NGS typing with the Holotype HLA kit (Omixon) of 139 JAK2V617F positive, 46 CALR exon 9 mutation positive MPN patients and 1083 healthy controls. We did not find any differences between HED per locus and per group. Two alleles: HLA-DPB1*03:01, DQB1*04:02 and two haplo- types: DRB1*11:01~DQB1*03:01~DQA1*05:05~DPB1*02:01,DRB1*11:03~DQB1*03:01~DQA 1*05:05~DPB1*04:02 were significantly depleted in MPN patients compared to controls. Additionally, we observed HLA-II alleles and haplo- types with statistically increased frequencies in CALRmut and JAK2V617F positive patients. Gene expression analy- sis showed lower HLA-DQA1, HLA-DMA and CD74 expression in JAK2V617F and CALRmut MPN CD34+ cells as compared to normal CD34+ cells. Furthermore, JAK2V617F positive CD34+ showed down-regulation of HLA-DMB, HLA-DRB1 and HLA-DRA genes. In conclusion this study provides first immunogenetic evidence that HLA class II may restrict JAK2V617F and CALRmut driven oncogenesis. Specific HLA-II alleles might be a predictive marker for response to immunotherapies upregulating HLA expression.

45. **I. Micheva**, V. Gerov, S. Dimitrova, Y. Petrov, I. Resnick·P549 A single center experience of haploidentical stem cell transplantation with unmanipulated graft-case series of eleven patients. Bone Marrow Transplantation (2022) 57:100–416; https://doi.org/10.1038/s41409-022-01798-0

Haploidentical stem cell transplantation (haplo-SCT) is considered a clinical therapeutic option for patients who are indicated for allogeneic stem cell transplantation. The number of patients transplanted with a haplo-relative increases each year worldwide due to its feasibility and accessibility. The recent advances in the field of haplo-HSCT allow a large number of patients with high risk hematological diseases to benefit from this treatment despite not having a matched donor. We present 11 patients (female/male: 1/1.2; mean age 46 years (28-62), 6 with acute myeloid leukemia, 4 with acute lymphoblastic leukemia, and 1 with idiopathic aplastic anemia) who underwent SCT from a haploidentical donor for a period of 3 years. In 10 of the patients it was a first transplantation, and in one - second. 54.5% of patients were transplanted in first complete remission (CR1), 27.3% - in second complete remission (CR2), and one - in progressive disease. The stem cell source in all patients was peripheral stem cells. In 81.8% we performed myeloablative conditioning regimen, in 18.2% - conditioning with reduced intensity. As part of prophylaxis of graft-versus-host disease (GvHD) we administered post-

transplant cyclophosphamide to 10 patients and antithymocyte globulin to 1 patient. The mean number of transfused CD 34 (+) cells was 6.53 x 106/kg (3.76-9.73). All patients achieved engraftment – of the neutrophils on D + 19 (13-25) and of the platelets on D + 22 (14-35). 36.4% of the patients had acute GvHD grade II-IV, 18% - grade IV. At the time of the analysis, 72.2% of patients were alive. 45.5% achieved remission of the disease, 27.3% developed relapse. Graft failure was observed in one patient. Causes of death included acute myocardial infarction, BKV encephalitis and relapse. Haploidentical SCT is an acceptable option in absence of a compatible donor and necessity of well-timed treatment.

46. D. Niyazi, **I. Micheva**, T. Stoeva. P321Fecal colonization in patients following hematopoietic stem cell transplantation by multidrug-resistant bacteria: a single center study on microbial spectrum and resistance profile. Bone Marrow Transplantation (2022) 57:100–416; https://doi.org/10.1038/s41409-022-01798-0

Nowadays antimicrobial resistance is one of the most important medical and epidemiological problems. Patients after hematopoietic stem-cell transplantation (HSCT) are considered a high-risk group for infectious complications, caused by multidrug-resistant bacteria (MDR). The enteric microbial flora is considered a major source for these complications. The routine fecal screening for colonization by MDR bacteria helps the adequate choice of empirical antibacterial therapy, especially in the era of the COVID-19 pandemic and the increased consumption of antimicrobial agents. The aim of this study is to investigate the spectrum of the MDR gut colonizers and to detect the genes associated with resistance to beta-lactam and glycopeptide agents. During a two-year period (November 2019 – November 2021), 74 patients were studied. A total of 44 non-duplicate MDR bacterial isolates were obtained from fecal samples of 28 patients after HSCT. All studied samples were inoculated on media containing cefotaxime (1 mg/L), CHROMagar^{TM} CPE (BD BBL^{TM}, USA) and blood agar (BD BBL^{TM}, USA) and were incubated at 37˚C for 24 hours. Species identification and antimicrobial susceptibility were determined by the Phoenix Automated System (BD, USA). PCR was used to identify the genes, encoding resistance to 3rd generation cephalosporins, carbapenems (*bla*$_{SHV}$, *bla*$_{CTX-M}$, *bla*$_{TEM}$, *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{OXA-48}$) and glycopeptides (*vanA, vanB, vanC, vanD*). DNA was obtained by SaMag Bacterial DNA Extraction Kit (Sacace, Italy). Epidemiological typing by ERIC and RAPD PCR was performed to determine the genetic relationship between the isolates. The PCR products were resolved by gel electrophoresis.

A total of 44 MDR fecal isolates were collected: *Enterococcus faecium*, n = 14; *E. coli*, n = 10; *Pseudomonas* spp., n = 8; *Enterobacter cloacae* complex, n = 7; *Klebsiella pneumoniae*, n = 4 and *Serratia marcescens*, n = 1. All *E. faecium* were vancomycin and teicoplanin - resistant and *vanA* positive. In the group of Gram negative bacteria, resistant to 3rdgeneration cephalosporins and/or carbapenems, the following genes were identified: bla_{SHV}(n = 4, *K. pneumoniae*), bla_{CTX-M} (*E. coli*, n = 9; *K. pneumoniae*, n = 3; *E. cloacae* complex, n = 7; *S. marcescens*, n = 1), bla_{TEM} (*E. coli*, n = 5; *K. pneumoniae*, n = 2; *E. cloacae* complex, n = 6; *S. marcescens*, n = 1), bla_{VIM} (*Pseudomonas* spp., n = 6; *E. cloacae* complex, n = 1). In 13 isolates (46.7%) more than one resistance gene were found: bla_{CTX-M} + bla_{TEM} (n = 13), bla_{CTX-M} + bla_{TEM} + bla_{SHV} (n = 2) and bla_{CTX-M} + bla_{TEM} + bla_{VIM} (n = 1). The epidemiological typing of *E. faecium*, *E. coli* and *Enterobacter cloacae* complex revealed both unique profiles and clusters of closely related strains, demonstrating identical profiles. All *K. pneumoniae* isolates exhibited unique ERIC profiles. A high rate of fecal colonization by MDR bacteria was detected (38.3%). Resistance to 3rd generation cephalosporins was associated with bla_{SHV}, bla_{CTX-M}, bla_{TEM}genes and carbapenem resistance - with bla_{VIM}. Glycopeptide resistance was mediated by *vanA* gene. The identification of epidemiologically related isolates is an indication for possible intra-hospital dissemination of these MDR pathogens and a risk factor for nosocomial infections associated with these problematic bacteria.

47. D. Yahya, T. Ruseva, M. Hachmeriyan, L. Angelova, T. Chetvenkov, I. Micheva. Cytogenetic profile of newly diagnosed patients with acute myeloid leukemia - a single centre retrospective study. 54th European Society of Human Genetics (ESHG) Conference. **European Journal of Human Genetics (2022)**, vol 3. Sup. 1 https://doi.org/10.1038/s41431-021-01026-1 (***2-year Impact Factor*: 4.246***) **(IF-4,58)**

We conducted a retrospective study on newly diagnosed adult patients with AML who underwent conventional cytogenetic analysis(CCA) in the Laboratory of Medical genetics, Varna between 01.2019-12.2020. A total of 74 patients were tested using bone marrow samples and G-banding technique. CCA was performed accordingly with the International System for Human Cytogenomic Nomenclature 2016. Karyotyping was successful in 63 (85.1%) of the

evaluated patients with 11 (14.9%) samples lacking metaphase plates. CCA showed normal karyotype(NK) in 34 (54%), and abnormal karyotype in 29 (46%) cases. According to the European LeukemiaNet risk stratification 2017, 6 (9.5%) of the patients were with favorable, 44 (69.8%) with intermediate, and 13 (20.6%) with adverse risk. The two-year study showed overall survival of 64%, 24% and 10%, respectively, which correlated with the risk groups.
Conclusions: CCA is a basic method in AML diagnosis, incorporated in classification and risk stratification. Due to technical problems the method is not always informative. Also, given the known molecular genetic markers, significant for diagnosis, prognosis and monitoring, it is highly recommended to combine karyotyping with molecular genetic analysis.

48. Valentina Miteva, Tsanka Ruseva, Dinnar Yahya, Marya Levkova, Milena Stoyanova, Mari Hachmeryan, Ilina Micheva, Lyudmila Angelova. (2022, April). Incidental findings in cytogenetics-the old new. In *EUROPEAN JOURNAL OF HUMAN GENETICS* (Vol. 30, No. SUPPL 1, pp. 560-561). CAMPUS, 4 CRINAN ST, LONDON, N1 9XW, ENGLAND: SPRINGERNATURE. **(IF-4,58)**

Materials and Methods: For a period of 10 years among 1554 bone marrow karyotypes performed on both children and adults with haematological disorders, subsequent follow up was suggested in 9 patients. Cytogenetic analysis of peripheral blood lymphocytes was successfully conducted in 7 of them. Genetic counselling was performed.
Results: Suspected chromosomal aberration based on bone marrow result has been confirmed in 6 patients (0.4%) - two robertsonian translocations (14;21) and (13;14), two monosomies X, one paracentric inversion and a ring 18 chromosome. Three of the findings lead to diagnosis of unsuspected chromosomal disorder and the other three required genetic counselling in first grade relatives at risk with possible further impact on their reproduction.
Conclusion: Incidental findings have always been a feature of medicine. A proper follow up of every abnormal result should be considered. Depending on the patients' will and the neat collaboration between genetic counsellors and other hospital clinicians a number of families can receive proper genetic care.

49. Minarik J, Micheva I, Usenko G, Mikala G, Simeonova K, Thuresson M, Jerling M, Xie H, Pour L. PORT (OP-109): Phase 2, Randomized, Pharmacokinetic (PK), Cross-

over Study of Peripheral Vs Central Intravenous Administration of Melflufen in Patients with Relapsed/Refractory Multiple Myeloma (RRMM). Blood. 2021 Nov 23;138:4772.

Here we present the results from the PORT study (NCT04412707), which aimed to compare the PK of melphalan after central and peripheral melflufen administration and to assess the local tolerability of peripheral melflufen administration. The overall efficacy and safety of melflufen in patients with RRMM were also evaluated.

Melflufen was only administered via a central venous catheter (CVC); however, the use of a peripheral venous catheter (PVC) may be preferred by patients if safety and tolerability are acceptable.

Systemic exposure to melphalan was found to be similar after melflufen PVC and CVC administration. The differences between PVC- and CVC-related pharmacokinetic parameters for melflufen and desethyl-melflufen are considered to be of no clinical consequence because their duration of plasma exposure is short. There were no local reactions after the application of melflufen PVC.

50. Minarik J, Micheva I, Usenko G, Mikala G, Masszi T, Simeonova K, Andersson B, Jerling M, Xie H, Pour L. P-205: PORT (OP-109): Phase 2, randomised, Pharmacokinetic (PK), cross-over study of peripheral vs central intravenous administration of Melflufen in patients with Relapsed/Refractory Multiple Myeloma (RRMM). Clinical Lymphoma Myeloma and Leukemia. 2021 Oct 1;21:S151-2.

Melphalan flufenamide (melflufen) is a peptide–drug conjugate with unique PK properties that rapidly penetrates cells, where it is metabolised to melphalan either directly or through an intermediate metabolite, desethyl-melflufen. Melflufen has only been administered via central venous catheter (CVC); however, peripheral venous catheter (PVC) administration may be preferred by patients if safety and tolerability are acceptable. The ongoing PORT study aims to assess the PK, safety, tolerability and efficacy of melflufen CVC vs PVC administration in patients with RRMM (NCT04412707). Patients (following ≥2 lines of prior therapy) were randomised (1:1) to melflufen 40 mg (with oral dexamethasone 40 mg [20 mg for patients aged ≥75 years] weekly on Days 1, 8, 15 and 22) either via PVC in cycle 1 then CVC in cycle 2 (Arm A) or via CVC in cycle 1 and then PVC in cycle 2 (Arm B). From cycle 2 (Arm A) or cycle 3 (Arm B) onwards, patients received melflufen via CVC. PK sampling was performed

frequently during and after the 30-minute melflufen infusion. PK parameters after CVC and PVC administration were compared using bioequivalence methods. At data cutoff (2 June 2021), 27 patients had received melflufen (median age 67 years; 48.1% male), of whom 19 patients received at least two doses and were evaluable for PK analysis. Melphalan Cmax, AUC0–t, and AUC0–inf were all bioequivalent for CVC and PVC administration, as demonstrated by a 90% confidence interval (CI) for the ratio of means within 80–125%. For melflufen, the ratio of means was 107–117% for the PK parameters, with all upper 90% CIs above 125%. For desethyl-melflufen, AUC0–t and AUC0–inf were bioequivalent and the 90% CI for Cmax was marginally above the upper limit (127%). Melflufen disappeared rapidly from plasma after the end of infusion, with an average half-life of 5–7 minutes. Melphalan Cmax was observed on average 7–9 minutes after the end of melflufen infusion for both routes of administration, which reflects the delay in distribution of melphalan from tissues to plasma. No PVC-related local reactions were reported. The overall melflufen safety profile was in line with previous studies.

51. S. Dimitrova, **I. Micheva**. PB 1123Interleukin-6 and its relation to clinical and laboratory characteristics of patients with myelofibrosis. HemaSphere, 2021;5:S2 doi: 10.1097/HS9.000000000000056

The aim of the study is to analyze serum IL-6 levels in patients with MF and its relation to clinical and laboratory characteristics of patients. We analyze 68 patients with MF (overt MF -27 patients, prefibrotic MF – 25 patients, post-polycythemia vera (post-PV) MF -9 patients and post-essential thrombocytemia (ET) MF- 7 patients) and 12 healthy controls. Degree of fibrosis in the bone marrow (grade 0-3), carrier of Jak2 V617F mutation, spleen size by ultrasound examination and peripheral blood count parameters (hemoglobin, leukocytes, platelets, MCV) are analyzed in all patients. We measure serum IL-6 levels by ELISA.
Levels of IL-6 are significantly higher in patients with MF compared to healthy controls (26.96 ± 46.73 pg/ml; -0.03 ± 1.68pg/ml; t = 1.99; p = 0.05). A significant difference is found between healthy controls, patients with overt MF (p = 0.036) and patients with post-PV MF (p = 0.028). IL-6 levels are significantly higher in patients with grade 3 fibrosis in comparison to the other grades (60.4 ± 77.3 pg /ml; p = 0.025). There is a strong significant correlation between IL-6 and the number of blood transfusions per month. No correlation is found with hemoglobin, leukocyte count, MCV, spleen size, Jak2 mutation, DIPSS, symptomatic disease and treatment.

IL-6 is a key mediator in the pathogenesis and clinical evolution of MF. Its involvement in the establishment of a cytokine risk model of MF, makes it a potential target for therapeutic response, control and monitoring of the disease.

52. R. Lukanov, S. Dimitrova, V. Miteva, **I. Micheva**. PB1686 Conventional cytogenetic analysis in patients with multiple myeloma-single center study. HemaSphere, 2021;5:S2 doi: 10.1097/HS9.00000000000005

The aim of the study is to define the role of conventional cytogenetics in the disease phenotype, time to next treatment (TNT) and overall survival (OS). We analyzed 103 patients with newly diagnosed MM between February 2012 – December 2020. The cytogenetic risk was determined according to the Mayo Algorithmic Approach for Stratification of Myeloma and Risk-Adapted Therapy (mSMART) classification v.3.0. Patients with high risk are 8,7%, standard risk-82,5%. In 8,7% metaphases for cytogenetics are not obtained. Patients are distributed in 8 groups based on karyotype: normal karyotype-74,8%, hypodiploids-6,8%, hyperdiploids-2,9%, diploid-1,9%, high risk deletions-2,9%. One patient is with pseudodiploid karyotype and one is with complex karyotype. By crosstab descriptive analysis approximately half of the patients with standard cytogenetic risk are stratified in ISS 3-54,1% (n=46). In the high risk group 81,8% are categorized in ISS 3. Regarding the karyotype classification, in ISS 1, 20 of the patients are with normal karyotype and only 2 have pathological karyotype: one is hypodiploid and one is with del(21)(q22). In the ISS 2, from 19 patients 17 are with normal karyotype and 1 is hyperdiploid. In ISS 3 the distribution is heterogeneous with normal karyotype in 40; hypodiploids in 6; hylerdiploid in 2, pseiudodiploid in 1, diploid in 2, high risk deletions in 2 and complex karyotype in one patient. The mean OS of the whole group is 39,7±4,2 months. We found significant difference in the mean OS based on the cytogenetic risk - 40,8±4,4 months in standard risk and 19,6±6,9 in high risk group ($p<0,05$). We found a significant difference in the time to third treatment (TTT) within the cytogenetic risk groups ($p<0,001$). The mean TTT in patients with standard risk is 2,1±5,2 months compared to 0 months in high risk group. We found a tendency showing that patients with standard risk have longer time to second treatment (TST) compared to those with high risk (12,9±18,8 months; 4,5±9 months; $p=0,6$). There is no difference between number of therapies, cytogenetic profile and myeloma type according to the karyotype. The cytogenetic profile in MM is of utmost importance for risk stratification. Deviations from the normal karyotype detected by G-banding are found in approximately 20% of patients. Despite the importance of FISH-based risk

stratification of MM, the role of conventional cytogenetics should not be underestimated, giving the opportunity of detecting a vast majority of cytogenetic abnormalities.

53. M Efraim, **I Micheva**. AS06-Prognosis/AS06a-Prognostic factors of outcome and risk assessment: PROGNOSTIC SIGNIFICANCE OF COMORBIDITIES AND FRAILTY IN RISK STRATIFICATION OF PATIENTS WITH MYELODYSPLASTIC SYNDROME. . Leukemia Research. 2021 Sep 1;108:106681-4. **IF 3,156**

MDS is a heterogeneous group of hematopoietic stem cell clonal diseases characterized by ineffective hematopoiesis, cytopenia, and an increased risk of transformation into AML. Disease-related factors are included in the established prognostic scoring systems-IPSS, IPSS-R and WPSS. The addition of patient-related factors such as frailty and comorbidities can improve prognostication. To assess and incorporate frailty (Clinical frailty scale (CFS)) and comorbidities (Charlson comorbidity index (CCI), Hematopoietic stem cell-comorbidity index (HCT-CI), MDS-comorbidity index (MDS-CI)) within the IPSS, IPSS-R and WPSS for risk stratification and survival.

We applied CFS, CCI, HCT-CI and MDS-CI in 219 patients with MDS, diagnosed and treated in the hematology clinic of UMHAT "St.Marina" Varna, Bulgaria between May 2010-May 2020. IPSS, IPSS-R and WPSS were used for risk stratification.

We analysed the distribution of patients according to the CFS. 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" were 38.4% (n = 84) of patients. We found significant difference in mean survival according to the comorbidity indexes and CFS independently of IPSS-R, IPSS and WPSS (p<0,001). A statistically significant difference in overall survival was also found in the risk assessment against IPSS and CCI (p <0.001), HCT-Cl (p <0.001), MDS-Cl p <0.001) In the analysis with WPSS and CFS, we found a decrease in survival with an increasing CFS score. The mean survival of patients with low WPSS risk and CFS> 5 (very “frail") was comparable to that of very high WPSS risk and CFS = 2 ("unfrail") (13 ± … and 11.7 ± 9.7 months, respectively) CFS and comorbidities provide prognostic value to the established prognostic systems. Incorporation of comorbidity index into existing prognostic scoring systems may improve prognostication in MDS.

54. **I. Micheva**, S. Dimitrova, V. Gerov, T. Nikolova, I Reznik. Outcome of patients with non-Hodgkin lymphomas and Hodgkin disease after salvage autologous stem cell transplantation. Hema Sphere, 2020;4;S1. EHA library. Micheva I. 06/12/20; 298243; pb2329. **IF 1,56**

The aim of this study was to evaluate the prevalence and outcome of patients with refractory non-Hodgkin lymphomas (NHL) and Hodgkin disease (HD) after salvage ASCT. Among 44 patients with NHL and HD undergoing ASCT in the Transplant unit at the University Hospital, Varna between 2015 and 2019, 23 (52%) patients, refractory after 1-7 lines of treatment were included in the study. 13 patients were with HD, 7 with diffuse large B cell lymphoma (DLBCL), 3 with peripheral T cell lymphoma (PTCL). All patients were stage III and IV at diagnosis. At the time of ASCT, 11 patients were with PR and 12 with PD. Patients were mobilized after DHAP (n=14), ICE (n=5), Hyper CVAD (n=1), G-CSF (n=2), and IGEV(n=1). 65,2% (n=15) of the patients achieved the target number of > 2x10 6/kg CD34+ cells after 1 apheresis, 34,8% (n=8) - after two apheresis with mean harvest of 9,1x106 /kg (range 2,02-41,5 x106 /kg). The conditioning regimens were BEAM (n=12), TECAM (n=9), BeEAM (n=2). 39% (n=9) of the patients achieved CR (6 with HD and 3 with DLBCL) and 61% (n=14) - PR 3 months after ASCT. Independently of the response, 10 (43%) patients received consolidation treatment with brentuximab (9 with HD and 1 with PTCL) within 3-12 months after ASCT. Two patients with PR achieved CR during the consolidation. 39% (n=9) of patients progressed within 6 months after ASCT (2 HD, 2PTCL, 5DLBCL). Three patients progressed during consolidation; two of them with HD and PTCL proceed to allogeneic stem cell transplantation, one patient with HD received PD-1 inhibitor. The five-years survival rate is 78%. Reason for death was PD in five patients and infectious complication in one.High dose chemotherapy and ASCT is still a method of choice for refractory lymphoma patients. Consolidation strategies after ASCT can substantially improve the outcome in salvage settings.

55. Veselina S Goranova Marinova, Kalina Ignatova, Penka Ganeva, Emil Spasov, **Ilina Micheva,** Atanas Radinov, Raya Petrova, Gergana Tzvetkova, Eugueni Hadzhiev, Stefan E Goranov And Liana Gercheva. Therapeutic results in patients with Hodgkin's lymphoma treated with brentuximab vedotin —bulgarian experience. Blood 130 (Suppl 1), 5193-5193 **IF 22,11**

The aim of this study is toanalyze the overall response rate (ORR), type of response, event free survival (EFS) and the prognostic factors in patients with Hodgkin's lymphoma treated with Brentuximab Vedotin (BV). Fourty-four (44) Hodgkin lymphoma patients treated with BV in 5 hematology clinics in Bulgaria were studied. The male / female ratio is 1.1 / 1, the mean age is 39.98 +/- 12.48. The most common histological variant is nodular sclerosis 31 (70.5%). In the II clinical stage there were 13 patients (29.5%), III - 16 (36.4%), IV -15 (34.1%). B-symptoms had 36 patients (81.8%), extranodal involvement - 17 (15.9%) and large mediastinal tumor mass - 14 (31.8%). All patients before treatment with BV received 4 (2-12) treatment lines, 50.0% -3 lines, 27.3% - 4 lines, and 9.1% > / = 5 lines. Stem cell transplantation was performed in 33 patients (75.0%), two of which were two autologous STCs, and in one auto/ allo SCT. ORR is 60.0% (N=24) - complete response was achieved in 10 (25.0%) and partial in 14 (35.0%) patients. Stable disease was registered in 7 (17.5%) and progression in 9 (20,5%). Patients achieving at least a partial therapeutic response prior to commencement of BV treatment significantly improved their response at the end of treatment: 15 (62.5%) versus 9 (37.5%) with previous progression and stable disease ($P < 0.05$, $R = + 0.496$). PFS for the entire group is 10 months. The median survival (MS) was not reached by the end of the study. Age, sex, histological variant, clinical stage, number, type and outcome of previous treatment lines did not affect the duration of PFS. The only significant prognostic factor that determines a remarkably longer remission is the qualitative therapeutic response: in the CR + PR group, the median PFS is not reached by the time of the analysis, while in patients with stable disease and progression EFS is 7 months ($P <0.001$). BV is an effective drug with manageable toxicity. The results of the treatment of our patients confirm the accumulated international experience.

56. Penka Ganeva, Yavor Petrov, Georgi Arnaudov, Veselina Goranova-Marinova, Janet Grudeva-Popova, **Ilina Micheva**, Liana Gercheva, Gergana Tsvetkova, Ewgeni Hadjiev, Atanas Radinov, Branimir Spassov, Raya Petrova. Brentuximab vedotin in relapsed/refractory Hodgkin's lymphoma after an ASCT, Bulgarian experience. Bone Marrow Transplantation 53, 603-603 **IF 4,725**

The introduction of BV onto clinical practice has improved the prognosis and survival of patients with HL, relapsed after ASCT. The primary endpoint was the overall response rate (ORR), type of response, toxicity profile, event free survival (EFS) and overall survival (OS) in patients with HL relapsed after ASCT, treated with BV. Thirty three (33) patients with HL treated with BV relapsed after ASCT in 5 hematology clinics in Bulgaria were studied. The

male / female ratio is 14/19, the mean age is 33.1 (17-63 y). The most common histological type is nodular sclerosis in 26 (78.7%). B - symptoms had 28 patients (84.8%). ASCT was performed in all patients, 2 of which were two auto and in one auto/ allo SCT. The median number of prior anti cancer therapy was 3 (range 2- 5) including ASCT. 21 patients (63.6 %) had received prior radiotherapy. BV was introduced for 21 (64.6%) patients with relapse/progression after ASCT and for 12 (35.3%) as consolidation therapy after ASCT. The median number of cycles was 10 (range 3-16). ORR (CR+PR) was confirmed in 18 patients (54.5 %): CR in 13(39.3%), PR in 5 (15.1 %), SD in 2 (6 %), progression in 13 (39.3 %). The status at the start of BV therapy may be have relation with PFS (P=0.28 1). Patients who were treated with ≤10 BV cycles had a lower OS than those received ≥10 courses (80.8% vs. 87.5% p = 0.060). Patients who were treated with ≤10 cycles of BV had a lower PFS than those with ≥10 cycles. (42.2% vs. 59.3% p = 0.023). Patients who responded with progression after treatment with BV had less OS than those who responded with CR, PR, SD (40.1% vs. 100% p = 0.010). The number of courses conducted with BV is an independent factor that affects OS and PFS. In general the treatment was well tolerated and the toxicity profile was similar to that previously reported. Neurological toxicity was seen in 6, one patients stop the therapy because of allergic reaction; aspergilosis of lung (n=1), hepatitis B reactivation in one. The report indicates that BV as a single agent is effective, safe and good tolerated in standard clinical practice.

57. **I Micheva**, S Dimitrova, V Gerov, T Chervenkov, L Gercheva, I Reznik. Effectiveness of chemo-G-CSF protocols for mobilization of peripheral stem cells in patients with non-hodgkin lymphomas and hodgkin disease-single center experience. Bone Marrow Tansplantation 54, 616-617. **IF 4,725**

We retrospectively analyzed patients with R/R NHL and HD undergoing stem cell collection after chemo-mobilization in the transplant unit at clinic of hematology, UMHAT “Sveta Marina”, Varna.

Forty patients, 29 with NHL (18 DLBCL, 6 PTCL, 4 MCL, 2 FL, 1 PBL) and 11 with HD, median age of 44,5 years, 27 male and 13 female, were mobilized between 2019 and 2021. All patients were stage III and IV at diagnosis. 29 of the patients were mobilized after one line of treatment, six after two lines of treatment and five after 3 and more lines of treatment. At the

time of mobilization 29 (72,5%) were in complete response, 5 (12,5%) were with partial response and 6 (15%) with progressive disease. Overall 36 (90%) patients achieved at least 2 x10^6 CD34(+) cells/kg after 1-3 apheresis with mean harvest of 8,44x10^6 /kg (range 2,0-41,5). Fore patients (3 CY and 1DHAP) failed mobilization. Three patients were successfully mobilized after first ASCT. The mean harvest for patients receiving DHAP was 10,02 x10^6 CD34(+) cells/kg, 3,04 x10^6 CD34(+) cells/kg for CY, 8.56 x10^6 CD34(+) cells/kg for IGEV, 6,76 x10^6 CD34(+) cells/kg for ICE, 10,52 x10^6 CD34(+) cells/kg for CHOEP. The patient mobilized with VTD-PACE achieved 2,2 x10^6 CD34(+) cells/kg after 2 apheresis. 19 of the patients achieved the target number of > 2x10^6/kg CD34+ cells after 1 apheresis, 15 after two, and 2 after three apheresis. The median time to apheresis was 13 days (8-18) without significant difference between the regimens. The mean WBC count at the time of apheresis was 42,8x10^9/L after DHAP, 34,5 x10^9/L after CY, 21,2 x10^9/L after IGEV, 23,1 x10^9/L after ICE, 45,8 x10^9/L after CHOEP. There was correlation between WBC and CD34 harvested cells (p=0.005). Grate 3-4 thrombocytopenia was found in 8 patient (5 DHAP, 1 ICE, 1IGEV, 1 VTD-PACE). Grate 3-4 anemia was registered in 3 patients (2 DHAP and 1 VTD-PACE). No correlation was found between the CD 34+ harvest and the age, number of previous lines chemotherapy, the response before mobilization, the type of the lymphoma and the clinical stage.

Our results demonstrate that the chemo-G-CSF protocols have comparable effectiveness with acceptable toxicity and are superior to CY-G-CSF for mobilizing stem cells in lymphoma patients.

58. Vs Goranova-Marinova, K Ignatova, P Ganeva, E Spasov, G Arnaudov, **I Micheva**, L Gercheva, A Radinov, R Petrova, G Tzvetkova, E Hadzhiev, L Bogdanov, N Tzvetkov, B Spasov, Z Grudeva-Popova, D Tumbeva. Prognostic factors influencing outcome after therapy with brentuximab vedotin in patients with relapsed or refractory Hodgkin's lymphoma. Annals of oncology, vol. 30, supplement 5, 2019. DOI: 10.1093/annonc/mdz251.008 **IF 32,976**

The aim of this study is to analyze the prognostic factors, influencing the outcome after therapy with BV in relapsed or refractory patients with Hodgkin's lymphoma. We studied sixty-four (64) Hodgkin's lymphoma patients treated with BV in six Hematology clinics in Bulgaria. All patients before treatment with BV received median of 4 (2-12) treatment lines, 30 (46.9%)

received =/>3 lines. Autologous stem cell transplantation (autoSCT) was performed in 45 (70.3%) patients, two of which were second autologous SCTs, and in one auto/ allo SCT.
ORR was 60.9% (N = 39). Complete response was achieved in 39.1% (25) and partial response in 21.9% (14) patients. Stable disease was registered in 10.9% (7) and progression in 28.1% (18). PFS for the entire group was 14 months. The median survival (MS) was not reached by the end of the analysis. At least partial therapeutic response was significantly higher in patients with chemosensitive disease before autoSCT (P = 0.006, R = +0.340), these who have undergone autoSCT (P = 0.021, R= -0.335) and when BV was started as consolidation therapy by month 3 after autoSCT (P = 0.02, R = +0.287). The only significant prognostic factor that determines a remarkably longer EFS is the type of therapeutic response itself: in the CR + PR group, the median PFS is not reached by the time of the analysis, while in patients with stable disease and progression EFS is 7 months (P < 0.001).
BV is most effective in patients with chemosensitive disease, and when used as consolidation therapy early, by 3-rd month after autoSCT.

59. **I Micheva**, S Useir, L Gercheva. Efficacy and safety of rituximab biosimilar truxima in patients with chronic lymphocytic leukemia and non-hodgkin's lymphomas: pb1912. Hemasphere 3, 871. 2019. **IF 0,86**

The aim of the study was to assess the efficacy and safety of TruximaTM treatment in combination with chemotherapy in patients NHL and CLL followed for 12 months. A total of fifty one patients, 28 men and 23 women, were treated with ruximaTMin the Clinic of Hematology at “Sveta Marina” University Hospital, Varna. TruximaTMwas applied at a dose of 375 mg/m2every 3 weeks in combination with chemotherapy. Response was estimated at treatment completion. Median age was 68,5 years. 22 patients were with CLL, one Richter,s syndrome, 16 with Diffuse large B cell lymphoma (DLBCL), 4 with Mantel cell lymphoma (MCL), 4 with Ssmall cell lymphoma (SCL), 4 with Marginal cell lymphoma (MZL). TruximaTMwas combined with different chemotherapeutic regimens: CHOP in 11 patients, Bendamustin in 23, FC in 6, MINE in 3, ACVBP in 1, COP in 3, ICE in 1, and Leukeran in 1. In 34 patients TruximaTMwas applied as a first line treatment, in 12 patients as a second line, in 5 cases as a third line. 30 patients have completed their treatment at the time of analysis. Complete response was achieved in 9 (30%) patients, partial response in 17 (56,7%) patients, stable disease in 4 (13,3%). Progressive disease was observed in 1 patient with DLBCL. Grade 3 or 4 hematological toxicity was registered in 5 patients (9,8%). Infusion related reactions

were observed in 5,8% of the patientsat grade 1 and 2. Our experience with the biosimilar over the last year has revealed that TruximaTM is an effective treatment without unexpected side effects. Moreover, cost may be a factor for doctor's choice.

60. **I Micheva,** S Dimitrova, S Rangelova, B Chaushev, L Gercheva. The role of 18f-fdg PET/CT in the initial diagnosis and staging of patients with multiple myeloma: pb2181. Hemasphere 3, 979. 2019. **IF 0,86**

The aim of the study was to evaluate the extent of bone disease and extramedullary involvement at the time of initial diagnosis using 18F-FDG PET/CT. Materials and methods: 42 patients with newly diagnosed MM were included in the study. The stage of the disease was determined according to ISS. 18F-FDG PET/CT was used for the detection of the bone lesions and the stage was reassessed according to Durie–Salmon PLUS SS. According to ISS 17 patients were stage 1, 11-stage 2 and 14-stage 3. According to Durie–Salmon PLUS SS 6 patients were in stage I A, 12 in stage II A, 9 patient in stage IB, 13 patients in stage IIIA and 2 in stage IIIB. 8 of the patients assessed in stage 1 according to ISS were reevaluated in a higher stage according to Durie–Salmon PLUS SS-5 in stage IIA and 3 in stage III A. 4 from the 11 patients in stage II ISS were evaluated as IA and IB according to Durie–Salmon PLUS SS, whether 4 were evaluated as IIIA. 2 from 14 of the patients assessed in stage 3 according to ISS were evaluated in IA and 4 in IIA according to Durie–Salmon PLUS SS. No correlation was found between stage according to Durie–Salmon PLUS SS and the MM Ig type, bone marrow plasma cell infiltration, LDH, and hemoglobin level. No correlation was found between the number of bone lesions and their activity (Deauville scoring system). The two staging systems had equal impact on the survival. 18F-FDG PET/CT has shown to have high sensitivity and specificity in patients with MM. Correct staging using 18F-FDG PET/CT can serve as a basis for clinical assessment and clinical decision-making.

61. B Chaushev, **I Micheva**, P Bochev, J Dancheva, C Yordanova, A Klisarova, Diagnostic accuracy of 18f-fdgpet/ct in detection of bone lesions in patients with multiple myeloma. European Journal Of Nuclear Medicine And Molecular Imaging 43, S319-S319 **IF 7,277**

Multiple myeloma (MM) is the most common cause of primary malignancy in bones. Diagnostic imaging plays a pivotal role in staging and prognostic assessment as well as in

planning and mon- itoring treatment. The aim of our study was to estimate the diagnos- tic accuracy of 18F-FDG PET/CT in 18 patents with MM in the evaluation of the extent of bone disease at the time of initial diag- nosis. Materials and methods: 18 patients (9 males and 9 females; age range 42- 68 years) with newly diagnosed IgG MM were in- cluded in the study. PET/CT was used as staging procedure for the detection of the bone lesions and the stage was determined according Durie - Salmon PLUS Staging Systems. Correlative imaging data was available in most of the cases and included skeletal radiographic survey in 11, CT in 7 and MRI in 2. Results: In 13 patients 18F- FDG PET/CT detected higher number of bone lesions in comparison to the other imaging methods. In 5 patients there was no difference in the number of bone lesions, including the two cases where MRI was performed. According to Durie - Salmon PLUS Staging Systems 18F-FDG PET/CT detected 13 patients in stage I, 4 in stage II and 1 patient in stage III. Conclusion: 18F-FDG PET/CT has shown to have high sensitivity specificity and key prognostic value in patients with MM. It is a superior imaging modality for diagnosis of bone lesions in myeloma compared to conventional radiography.

62. **I Micheva**, S Dimitrova, R Rachev, H Varbanov, M Mehmed, V Gerov, A Antonov, L Gercheva. Bortezomib maintenance treatment in patients with multiple myeloma-a single center experience, Haematologica. 2016/6/:796-796. **IF 7,702**

The aim of the study is to define the role of Bortezomib maintenance therapy (MT) in patients with MM after achieving complete response (CR) or very good partial response (VGPR). Bortezomib has been used as post-autologous stem-cell transplantation (ASCT) consolidation therapy. Other trials have investigated Bortezomib maintenance for ASCT patients, and for those who are ASCT ineligible. Thirty-seven patients with newly diagnosed symptomatic MM with CR or VGPR after Bortezomib based induction regiments were included in the analysis. 23 patients received MT with Bortezomib 1.3 mg/m2 once every 2 weeks. 14 patients remained on follow up until progression. After a median follow up of 31.8 months (range, 10-65 months) none of the patients on Bortezomibe MT progressed. In contrast, in the patients group on follow up, 12 from 14 patients progressed within median time of 24,17 months (range, 7-60 months). No correlation was found between the progression and myeloma type, ISS stage, type of response and ASCT. Our results reveal undoubtedly the impotrance of Bortesomib maintenance in patients achieved CR or VGPR.