ABSTRACTS
ORAL PRESENTATIONS

01. CLINICAL AND GENETIC ANALYSIS OF THE BCR-ABL NEGATIVE CHRONIC MYELOPROLIFERATIVE DISEASES IN INITIAL DIAGNOSIS: SINGLE CENTER EXPERIENCE

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OBJECTIVE: Chronic myeloproliferative diseases (CMPD) are clonal stem cell disorder characterized by the uncontrolled proliferation of myeloid lineage cells in the bone marrow. Polycythemia vera (PV), essential thrombocytosis (ET), and primary myelofibrosis (PMF) are bcr-abl negative CMPD. After the identification of JAK2V617F mutation, the classification of CMPD has changed and the existence of this mutation has been included in the World Health Organization (WHO) diagnostic criteria. Thereafter, MPL and calreticulin mutations were defined in the 2016 WHO revision classification of CMPD, with the showing of them in the pathogenesis of CMPH.
METHODS: In this study, the demographic characteristics, subtype, risk status and mutation analysis of patients diagnosed with bcr-abl negative CMPD were investigated, between July 2017 and June 2018.
RESULTS: Twenty-eight patients were diagnosed with CMPD. Twelve (42.9%) of them were PV, whereas sixteen (57.1%) were ET. Seventeen (60.7%) of the patients were female, eleven (39.3%) were male and the median age was 53 (38-82) years. Medians of hemoglobin (Hb), Hematocrit (Ht), leukocyte and platelets were 18 (14.2-20.4) gr/dL, 56.5% (44.4%-65.2%), 12400/mm^3 (5,300-18,900) and 390000/mm^3(151,000-609,000) in patients diagnosed with PV and 13.8 (12-16.8) gr/dL, 42.2% (36%-48.5%), 10,500/mm^3 (6,500-21,800) and 759,000/mm^3 (451,000-1,189,000) in ET, respectively. Splenomegaly was noted in 11 (55%) [6 (55%) PV, 5 (45%) ET] patients. JAK2V617F mutation was detected in sum of eighteen patients, nine of them (75%) diagnosed with PV and rest of them (N=9, 56.3%) diagnosed with ET. Calreticulin mutation was detected in four patients diagnosed with ET and JAK2V617F negative (57.1%). MPLW515K/L wasn't detected in any of patients. Neither of mutations was detected in three of the patients, so they were triple negatives. At the time of diagnosis, 15 (53.5%) patients were at high risk and cytoreductive treatment was started. Portal vein thrombosis was present in two patients with PV at the time of diagnosis and who were also JAK2V617F mutation positive. Five patients had a medical history of thromboembolic event (four patients with coronary artery disease and one patient with ischemic cerebrovascular event), all of which were JAKV617F mutation positive.
CONCLUSION: Pathogenesis, classification and risk groups of CMPDs have been well characterized with the identification of some genetic mutations in recent years. The first of these somatic mutations is JAK2617F, found to be approximately 95% positive in PV, approximately 50-60% in ET and PMF, whereas calreticulin and MPL mutations aren`t detected in PV. According to literature, calreticulin mutation is positive approximately 25% and 17%, while the MPL mutation is approximately 6% and 10% in the ET and PMF, respectively. The positivity of calreticulin was found to be associated with a lower risk of thrombosis, it was associated with lower Hb/Ht tand higher platelet levels. The effect of any mutation on leukemic transformation and surveillance is unclear. JAK2V617F, CALR and MPL are the most frequently identified somatic mutations in the pathogenesis of CMPD, which are now important in the diagnosis, risk classification and follow-up of the disease and gain importance in the personalization of patients' treatments.

02. APOPTOSIS, CELL CYCLE PROFILING AND DIFFERENTIATION STUDY OF LOW-DOSE DECITABINE AND/OR BORTEZOMIB IN THE AML CELL LINE KASUMI-1, WITH EMPHASIS ON THE POSSIBLE ABOLISHMENT OF DECITABINE- PROMOTED RESISTANCE PHENOTYPE

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OBJECTIVE: We investigated the possible reversal of decitabine's (a DNA methyltransferase inhibitor) resistance phenotype in AML, in terms of cell viability and differentiation by combining decitabine (D) with an oxidative stress inducing agent, such as the proteasome inhibitor bortezomib (BZ).
METHODS: Kasumi-1 AML (M2) cells were treated with low-dose D (10nM, 50nM, 100nM, 200nM and 400nM), with or without BZ (10nM). Apoptosis and cell cycle were evaluated after 24h of treatment through FACS (Annexin V/PI and DAPI staining, respectively). Expression levels of CD193, CD11b, CD13, CD14, CD15, CD16 and CD117 surface differentiation markers were evaluated after 6 days of treatment through FACS. One-way Anova and LSD/ Bonferroni methods were applied for the statistical analysis of the results.
RESULTS: Our data indicate significant alterations in cell death and cell cycle stages in Kasumi-1 cells following D and BZ combination treatment compared to the control (untreated cells) and single treatments. Apoptosis was statistically significantly increased compared to control (15.15%) after only 100nM and 400nM of single D treatment (27.2% and 28.25%, p=0.037 and p=0.026, respectively) and after D/BZ treatment for all D concentrations tested. Compared to single treatments, the D/BZ combination significantly increased apoptosis by 101.92%, 72%, 100.9%, 113.12% and 96.63% (p=0.009, p=0.019, p<0.000), respectively. Cell cycle profiling also highlighted a much greater sensitivity of Kasumi-1 cells in D/BZ combinations compared to single treatments, with a significant increase in the G1 population by 11.84% (p<0.000), 12.55% (p<0.000), 6.62% (p=0.021), 8.38 (p=0.006) for all D/BZ combinations (from lower to higher) tested, except 400nM D/BZ. A significant decrease in S phase was observed after all combination treatments tested, compared to single ones, by 36.26%, 39.25%, 26%, 30.99% and 30.8% (lower to higher D concentration) (p<0.000), while finally, G2/M population was increased by 56.35% (p<0.000), 85.34% (p<0.000), 34.29% (p=0.02), 81.92% (p<0.000) and 138.45% (p<0.000), respectively. Finally, in terms of differentiation, FACS analysis mostly revealed a decreased expression of the myeloid progenitor surface antigen CD117 [21.25%, 3.9%, 6.15%, 8.45%, 5%, p<0.000 and 21.75%, 25.55%, 3.15%, 25.05%, 19.7% p<0.000 from lower to higher D and D/BZ concentration, respectively vs 80.1% of control] and an increased expression of the myeloid cell lineage antigen CD15 [79%, 82.3%, 74.9%, 76.9%, 79.1%, p<0.000 and 65.2%, 72.5%, 75.3%, 62%, 71.9%, p<0.000 from lower to higher D and D/BZ concentration, respectively vs 36.5% of control], after both D and D/BZ treatments compared to untreated cells.
CONCLUSION: Our data indicate that the addition of BZ -a proteasome inhibitor which, among others, is capable of inducing oxidative stress- to low-dose D significantly enhances apoptosis and decreases live cell population of Kasumi-1, with the combinations of 100nM and 200nM of D with BZ appearing as the most successful ones. Moreover, cell cycle profiling revealed that D/BZ treatment synergistically leads to G1 and G2 arrest, hence prohibiting cells to either synthesize DNA (S phase) or proceed to mitosis. On the contrary, D seems to promote monocytic and granulocytic differentiation of Kasumi-1 cells more effectively alone rather than in combination with BZ.

03. DECITABINE TREATMENT IN PATIENTS WITH AML AND MDS; SINGLE CENTRE EXPERIENCE

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OBJECTIVE: Decitabine inhibits the function of DNA methyltransferases by incorporation into the DNA and prevent the methylation of cytosine during cell division, resulting in genome-wide demethylation. This drug is widely accepted as the treatment option for myelodisplastic syndrome (MDS) and elderly patients with acut myeloid leukemia (AML).The goal of this study was to identify the safety and efficacy of decitabine in MDS and AML patients in our center.
METHODS: This single-center, retrospective study examines outcomes of decitabine treatment in AML and MDS patients. Among 38 patients, 25 patients were MDS and 13 patients were AML. Decitabine administered 20 mg/m2 by intravenous infusion daily for 5 consecutive days every 4 weeks. Treatment response was assessed by monthly complete blood counts and bone marrow examination after four cycles. In addition survival, overall response rate (ORR),hematologic improvement (HI [only for MDS]) and drug toxicity were analyzed. Response assessed by 2006 International Working Group criteria for MDS. Patients who underwent allogeneic stem cell transplantation (ASCT) were not included in the assessment of survival.
RESULTS: Results for MDS and AML were assessed separately. 25 MDS patients were enrolled. Median age was 67 years (range 23-82). All patients had International Prognostic Scoring System score of ≥ 0.5. Median overall survival (OS) and progression free survival (PFS) were 21 months and 14 months respectively. At 1, 2 and 5 years OS were 62.9%,47.1% and 17.7% respectively. At 1, 2 and 5 years PFS were 54.9%, 45.8% and 30.5% respectively. The ORR was 48% (11 marrow complete remissions [mCRs] plus 1 partial response [PR]) and the overall improvement rate (OIR) was 64%, which included 48% HI. OIR was 62.5% for intermediate-1 patients and 75% for intermediate-2 and high-risk patients. 88% of patients experienced stable disease (SD) or better. HIs were detected by the end of median two cycles. The OS and PFS outcomes of patients whom achieved response were observed statistically significant better than the others ($p<0.013$ and $p<0.005$, respectively). 13 AML patients were enrolled. Median age was 75 years (range 18-85) and 6 of 13 AML patients experienced CR (n=4) or PR (n=2) (ORR: 46.2%). Median OS and PFS were not reached due to 76% of patients (n: 10) are still alive and 38% of patients (n: 5) have been still receiving treatment. At 1 year OS and PFS were 61.4% and 77.8%, respectively. Adverse events were evaluated for all 38 patients. Grade 3 or higher neutropenia, thrombocytopenia and febrile neutropenia, occurred at rates of 36.8%, 13.2%, 33%, respectively. Seven of 25 MDS patients and one of 13 AML patients underwent ASCT.
CONCLUSION: The response to decitabine treatment was found to be associated with survival benefit in MDS patients. At the same time our study shows that approximately half of AML patients achieved response. There is no serious adverse event observed except grade 3-4 hematological toxicity. As a result,decitabine is effective and safe with acceptable toxicity in AML patients who can not tolerate standard chemotherapy regimens and in intermediate or high risk MDS patients.

04. IMMUNOSUPPRESSION SHOULD NO MORE BE THE FIRST-LINE TREATMENT FOR PATIENTS WITH HYPOPLASTIC MARROW FAILURE SYNDROMES OVER THE AGE OF 50 YEARS AND SHOULD BE SUBSTITUTED BY ELTROMBOPAG

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OBJECTIVE: Immunosuppression is considered the initial treatment of choice for patients with aplastic anemia and other immune-mediated hypoplastic marrow failure syndromes. Recently, eltrombopag, a Thrombopoietin-mimetic receptor agonist has been approved as second-line treatment, for patients with aplastic anemia, following failure of immunosuppression. We retrospectively analyzed results of the various treatment strategies applied to patients with hypoplastic marrow failure syndromes in Western Greece.
METHODS: Between 1.1.1988 and 30.6.2018, 116 patients with hypoplastic marrow failure syndromes were diagnosed, and among them 32 received ATG/ALG-based immunosuppression as initial treatment (Group A), 21 received non ATG/ALG-based immunosuppression (Group B) and 6 received eltrombopag (Group C). These 59 patients were 26 male and 33 female with a median age of 50 years. Non-ATG/ALG immunosuppression was Cyclosporin-A combined with corticosteroids for a minimum of 9 months, unless unacceptable toxicity emerged. Cyclosporine-A was also administered to patients treated with ATG/ALG. Criteria for choosing of ATG/ALG-based or non ATG/ALG-based immunosuppression were age, fitness, comorbidities and patient's willing. Eltrombopag was given as initial treatment in elderly or unfit patients the last 3 years.
RESULTS: There was a significant impact of age on treatment-related complications and mortality in both patient groups received immunosuppression. We compared treatment outcome in the 3 Groups, between patients younger or equal to, and older than 45 year-old, at three time points: 6 and 12 months post-treatment start, and at the last follow up. At 6 months post-treatment CR had been achieved by 13/20 younger and 3/12 older patients from Group A, by 2/4 younger and 4/17 older patients from Group B, and by 2/6 older patients from Group C. Early treatment-related death was observed in 1/20 younger and 5/12 older patients from Group A, in 6/17 older patients from Group B and in no patient from Group C. Taken together at 6 months post-treatment CR had been achieved by 12/24 younger (62.5%) and by 7/29 older (24.1%) patients received immunosuppressive treatment and by 2/6 older patients (33.3%), treated with eltrombopag. Treatment-related mortality was 4.2% (1/24) among younger patients and 37.9% (11/29) among older patients treated with immunosuppression and by no patient treated with eltrombopag. At 12 months post-treatment CR rates for younger and older patients, respectively, were 70% (14/20) and 25% (3/12) in Group A, 50% (2/4) and 23.5% (4/17) in Group B, and 33.3% (2/6) in older patients of Group C. After a median follow-up of 39.4 months (range 1-363 months) 18 patients (56.3%, 15 younger or 75% and 3 older or 25%) are alive from Group A, 5 patients (29.4%, 4 younger or 100% and 1 older or 5.9%) are alive from Group B, and 4 older patients (66.7%) are alive from Group C.
CONCLUSION: Immunosuppressive treatment with ATG/ALG and/or Cyclosporin-A is too toxic for patients with hypoplastic marrow syndromes over 45-50 year old, and for this age group should no more be considered first-line treatment of choice but it should be applied after failure of eltrombopag, which is quite safe treatment for all patient groups.

05. PAROXYSMAL NOCTURNAL HEMOGLOBINURIA: ONE CENTER EXPERIENCE

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OBJECTIVE: Paroxysmal Nocturnal Hemoglobinuria (PNH) is a disease characterized by chronic persistent hemolysis, multi-organ damage and eventually multiple organ failure. It is a rare disease. Due to variable and complicated clinic, there are delays in the diagnosis. Morbidity and mortality are high and can be prevented in case of early recognition. For this reason, we aimed to keep a projection for the future by determining which risk groups the disease is more frequent.
METHODS: The most risky groups in terms of PNH were identified in the literature. A total of 116 patients (70 female and 46 male) who were determined to be included in these risk gorups in Manisa province were examined for PNH clone. For this purpose, PNH scanning was performed by the FLAER method which is accepted as the gold standard today.
RESULTS: PNH clones were detected in 12 of 116 patients included in the study. Clones were found more frequently in patients with elevated LDH with bone marrow aplasia. PNH clone was found in patients with intravascular hemolysis. PNH clones were not detected in patients with portal, splenic and mesenteric vein thrombosis. Eculizumab was initiated in four patients who detected a PNH clone. Seven patients were followed up with clones by 6 months interval.
CONCLUSION: PNH is a progressive, morbidly and mortal disease with variable onset and course. Patients with bone marrow aplasia/hipoplasia and patients with unexplained cytopenia, MDS-refractor anemia, thrombosis with unusual sites associated with LDH elevation or intravascular hemolysis should be screened for PNH. In our study, in patients with thrombosis, the PNH clone may have been found to be less than the deficiency of our criteria or patient count. There is a need for large-scale and multicenter studies involving more patients to determine the frequency of the disease and to identify risk groups.

06. SYSTEMIC MASTOCYTOSIS: MANAGEMENT AND OUTCOME; DATA ANALYSIS FROM THE GREEK REGISTRY

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OBJECTIVE: Systemic mastocytosis (SM) is a rare hematologic malignancy with diversity of clinical manifestations that results in both delayed diagnosis and treatment. The aim of this analysis was to present the characteristics and management of SM cases diagnosed in Greece during the last 30 years.

METHODS: This study was organized by the Myeloproliferative Neoplasm Working Party of the Hellenic Society of Hematology. The medical files of SM patients, who were diagnosed in Greece between 1987 and 2018, were retrospectively evaluated.

RESULTS: Overall, 60 SM patients (median age 52.0 years) were studied. Median time of symptoms onset to diagnosis was two years. Twenty-one patients were categorized as indolent SM (ISM), 20 as SM with an associated hematological neoplasm (SM-AHN), 18 as aggressive SM (ASM)

and one with mast cell leukemia (MCL). Regarding SM-AHN, myeloid malignancies were the most common malignancy, including MDS or MDS/MPN (n=9), CMML (n=2), AML (n=1), CML (n=1) and ET (n=1); lymphoid malignancies were also reported and included NHL (n=4), HL (n=1) and B-ALL (n=1). SM and the AHN were diagnosed simultaneously in 13 cases, while the AHN diagnosis preceded SM diagnosis in 4 cases (median time: 22 months); the opposite occurred in three cases (median time: 7.0 months). Most of ISM patients (16/21) did not receive any therapy; one was treated with imatinib, one with hydroxyurea and three received corticosteroids to control the mediators' related symptoms. One of the later had diarrhea without infiltration of the gastrointestinal tract and received consecutively interferon-alpha (IFNα), imatinib and corticosteroids without resolution of the syndrome. Eight patients with SM-AHN received treatment for both the SM and the co-existing hematological neoplasm, six only for the AHN, three only for SM, while four have not required treatment yet. The most common treatment for SM was IFNα (n=6), followed by imatinib (n=4), cladribine (2-CdA) (n=3) and dasatinib (n=1). Patients with ASM received either IFNα (n=8) or 2-CdA (n=5) as first line treatment. Second line treatment included imatinib (n=2), 2-CdA due to IFNα intolerance (n=1) and corticosteroids (n=2). Two patients with vertebrae fractures required surgical intervention. All patients with skeletal involvement received additionally bisphosphonates. The patient with MCL received consecutively 2-CdA, chemotherapy (FLAG-Ida) and dasatinib achieving partial response and proceeded to allogeneic stem cell transplantation. He died 6 months later due to graft versus host disease. With a median follow-up of 31 months the median overall survival in the entire cohort is not reached. The median follow-up for patients with ISM and ASM is 33.5 and 18.5 months respectively, and all of them are alive with adequate disease control. Seven deaths were reported only in the group of patients with SM-AHN. Six patients died due to acute leukemia and one due to infection, indicating that the aggressiveness of the underlying hematological malignancy is the strongest factor that affects survival in this group.

CONCLUSION: SM is a rare disease with variable manifestations and outcome. Novel targeted therapies seem to improve outcome, but accurate diagnosis, according to WHO classification, remains important for appropriate management.

07. HOW DO WE MANAGE PATIENTS WITH POSITRON EMISSION TOMOGRAPHY POSITIVITY AFTER FRONTLINE TREATMENT OF HODGKIN LYMPHOMA?

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OBJECTIVE: Hodgkin Lymphoma is relatively rare and has become a highly curable disease in the past decades. The aim of this retrospective study was to analyze the clinical impact of watch-and-wait approach after routine end-of-treatment Positron Emission Tomography without obvious progression or refractoriness.
METHODS: Adult Hodgkin Lymphoma patients followed at Hematology Departments of Hacettepe University and Ankara Numune Training and Research Hospital were reviewed retrospectively for analysis. Demographic data, disease stages, pathological classifications, first-line treatments, end-of-treatment Positron Emission Tomographies were recorded. Univariate analyses were performed via Chi-square and t-tests. Multivariate analyses were done by Cox regression analysis.
RESULTS: A total189 Hodgkin lymphoma patients were in close follow-up. 26 patients had positive end-of-treatment Positron Emission Tomography results. Median age at diagnosis was 31 (19-73), whom 18 (69%) were male. Twenty-one (84%) had advanced stage disease. All patients received ABVD regimen except one patient, who received brentuximab front line due to comorbidities. Nineteen patients had 18-flouro-deoxy-glucose positivity suitable for biopsy but biopsy was performed in 7 cases. Two of them were consistent with lymphoma other 5 had benign results. Watch-and-wait approach was applied for 5 patients with benign pathologies and all of them were alive without progression with a median follow-up of 18 (1-40) months. Nineteen patients had no biopsies, 5 of them were considered as primary refractory and received second line treatments, 1 was lost-to follow-up, 13 was followed with watch-and-wait approach. Two of 13 cases had progressed and needed second line treatment with a median time to progression of 3.5 months. Median overall survival was 41 (3-64) months for watch-and-wait cases and 46 (40-56) months for second line treatment cases (cases without biopsy) and 3 year overall survival was not different between treatment and watch-and-wait cases (p=0.234).
CONCLUSION: The results of our present study revealed that false positivity is not a negligible problem in positron emission tomographies in Hodgkin lymphomas and if no other sign of active disease was found watch-and-wait approach may protect the patients from potentially harmful unnecessary biopsies and hazardous over-treatment.

08. BRENTUXIMAB VEDOTIN IN RELAPSED/REFRACTORY HODGKIN`S LYMPHOMA; TWO-CENTER IZMIR EXPERIENCE

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OBJECTIVE: The treatment of relapsed or refractory Hodgkin Lymphoma (R/R HL) is still a major therapeutic challenge. Approximately 30%-40% of patients with advanced disease are refractory to front line therapy or will relapse after first-line treatment. The standard management of these patients is salvage chemotherapy followed by high-dose chemotherapy and autologous stem cell transplant (ASCT). The best prognostic factor is the status of disease before ASCT; in particular, the normalization of positron emission tomography (PET) scan. The use of Brentuximab Vedotin (BV), an anti-CD30 antibody-drug conjugate, represents a promising approach for these patients.
METHODS: In this retrospective case note review we enrolled patients at 2 clinical sites from Izmir who were diagnosed with R/R HL and received BV between 1 January 2014 and 1 June 2018. Patients were included without any limitations regarding cycle number of BV, performance status and any other features. Patient`s name, age, date of diagnosis, stage, previous chemotherapies, date of relapse, cycles and dose of BV, adverse side effects, responses, final status of the patient were all gathered from the files. BV was given at a dose of 1.8 mg/kg intravenously over 30 min every 3 weeks. Response was assessed by PET/CT.
RESULTS: A total of 17 patients were treated with BV during the examined period. Before therapy initiation 70% of our patients had advanced-stage disease and all of them had B symptoms. Patients` median age at diagnosis was 33 years. Nodular sclerosis was the most frequent (65%) histologic subtype, approximately half of the patients (54%) had primary refractory disease. The main characteristics of the patients were specified at the Table 1. Six patients have experienced grade 2 to 3 cytopenias and G-CSF injections were sufficient. The most common treatment-related adverse events were cytopenias, fatigue and myalgia. Response assessment after ≥4 cycles of BV was performed with PET/CT and the analysis showed an ORR of 58.8% (n=10/17) with CR in 1 patient (5.9%) and PR in 9 patients (52.9%). The median number of BV cycles was 8 (range, 2-31), and the median time to best response was the fourth cycle. At a median follow-up of 23 months, median overall survival was 23.6 (95% CI, 2-52) months. At the time of data analysis 12 patients (70%) were alive. In general, the treatment was well tolerated with no dose reduction required.
CONCLUSION: Best responses to BV are observed after 2-5 cycles, early during the treatment course and the decision for further consolidation with a transplant should be taken early during treatment when best responses are achieved. BV is an effective treatment for R/R HL patients after failure of ASCT, not only within clinical trials but also in everyday clinical practice.

09. PROGNOSTIC FACTORS IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA (PMLBCL) UNDER RITUXIMAB-CHOP (R-CHOP) CHEMOTHERAPY WITH OR WITHOUT RADIOTHERAPY (RT)

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OBJECTIVE: The identification of prognostic factors for the outcome of patients with PMLBCL treated with R-CHOP±RT.

METHODS: The study included 262 patients with PMLBCL, who were treated with R-CHOP±RT (6-8 cycles) in a multicenter setting in Greece and Cyprus. The following potential prognostic factors were evaluated: Age (median 32; range 16-82; >60 years only 4%), gender (female 66%), B-symptoms (29%), stage III/IV (11%), infradiaphragmatic disease (6%), extranodal involvement (E or IV, 38%), pleuritis (32%), pericarditis (28%), any serositis (44%), bulky disease (≥10 cm; 60%), performance status (PS) ≥2 (15%), LDH levels (elevated 83%), anemia (39%), leukocytosis ≥10x109/L (25%), ESR ≥50 mm/h (40%), albumin <4 g/dL (45%), age-adjusted IPI (aaIPI; ≥2 in 20%). The Kaplan-Meier method, log-rank comparisons and Cox's regression models were used for statistical analysis. The endpoints were Freedom From Progression (FFP) and Lymphoma Specific Survival (LSS).

RESULTS: The median follow-up of currently alive patients was 59 months (2-198). Among 61 failures, 58 occurred within 2 years (all within 4 years) from diagnosis. The 5-year FFP was 75%. With 30 deaths recorded (including 2 unrelated deaths), the 5-year overall survival (OS) was 87%. The aaIPI identified a small minority of patients (aaIPI=3; 3% of total) with a 5-year FFP of 38% vs. 74%, 77% and 87% for those with aaIPI 2,1 and 0 (p=0.02), being therefore of questionable value. Indeed, when aaIPI was split as 0-1 vs 2-3, the absolute difference in 5-year FFP was statically significant but not clinical meaningful (79% vs 66%, p=0.04). A modified aaIPI (aaIPI-E) was also evaluated, scoring 1 point not only for stages III/IV but for IE/IIE as well. The modified aaIPI-E (0-1 vs 2-3) was a more powerful prognostic factor for FFP compared to the conventional aaIPI (5-year FFP 87% vs 61%). On univariate analysis, any extranodal involvement (E/IV), elevated LDH, any serositis, involvement of ≥2 extranodal sites, bulky disease, infradiaphragmatic disease, leukocytosis and albumin <4 g/dL were significantly associated with inferior FFP. In multivariate analysis of FFP, any extranodal involvement (E/IV) and any serositis were independent PFs (hazard ratios 2.2 and 1.9; p=0.01 and p=0.04 respectively). None, 1 or 2 of these factors were present in 41%, 36% and 24% of the patients with 5-year FFP of 90%, 69% and 60% (p=0.0001). LSS at 5 years was 98%, 84% and 75% respectively (p=0.0005).

CONCLUSION: In the largest patient series reported so far in the literature, long-term disease control and OS with R-CHOP±RT in PMLBCL were 75% and 87% respectively. The conventional aaIPI was moderately predictive of the outcome. The combination of any extranodal involvement (E/IV) and any serositis (pleuritis and/or pericarditis) defined a subgroup, comprising 1/4 of the patients, with ~40% risk of failure and 25% risk of disease- related death, who can be suitable for treatment intensification or incorporation of novel agents in the 1st line. Furthermore, a very low risk subgroup (absence of both factors), comprising >40% of patients with only 10% failure rate and minimal disease-specific mortality (2%), may not benefit from any treatment intensification, such as R-da-EPOCH.

10. INVESTIGATION OF POSSIBLE RELATIONSHIP BETWEEN TUMOR METABOLIC ACTIVITY (PET / CT-SUVMAX) AND KI-67 PROLIFERATION INDEX IN OUTPATIENT DIFFUSE LARGE B CELL LYMPHOMA CASES

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OBJECTIVE: DLBCL is the most common subtype of NHL. Life is short, aggressive if not treated. However, cure is provided by systemic chemotherapy. Treatment with combination chemotherapies has been successful for many years. These treatments have important prognostic factors that determine disease-free and overall survival rates. Prognostic indexes based on these factors are used both in the selection of treatment and in the development of new treatment modalities. In recent years, the addition of rituximab to these combination treatments has shown a positive effect on both disease-free survival and overall survival. The use of prognostic indexes has been questioned after the use of rituximab. The possible association between IPI score parameters, SUVmax value measured in PET / CT imaging, total metabolic tumor volume measurement (TMTV), and Ki-67 proliferation indexes examined in biopsy pathology of the relevant tissue in newly diagnosed and untreated DLBCL cases we aimed to measure the power of the parameters to predict disease survival.

METHODS: Our patient files were scanned retrospectively. The clinicopathologic features and prognostic factors of the patients were recorded. Overall survival status was calculated. 65 patients were included in the study.

RESULTS: Patients were grouped according to the cut-off values obtained from statistical analysis according to the 18-month overall survival and it was considered that the TMTV value could be used as an appropriate predictor of overall survival. There was no correlation between SUVmax and Ki-67, and it was seen that these values were not prognostic.

CONCLUSION: Prospective, multicenter, randomized patients with more patients to generalize and support these outcomes work is needed. As a result, it is thought to be more accurate to analyze all of the clinical, imaging and biologic factors without assessing a single prognostic criterion such as IPI to predict survival.

11. BEAC (CARMUSTINE, ETOPOSIDE, CYTARABINE, AND CYCLOPHOSPHAMIDE) IN AUTOLOGOUS HEMATOPOIETIC CELL TRANSPLANTATION FOR LYMPHOMA: A SAFE AND EFFECTIVE ALTERNATIVE CONDITIONING REGIMEN

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OBJECTIVE: BEAM (Carmustine, Etoposide, Cytarabine, and Melphalan) is considered the gold standard conditioning regimen in refractory or relapsed Hodgkin (HL) and Non-Hodgkin (NHL) lymphoma patients undergoing autologous hematopoietic cell transplantation (AHCT). However, given the limited availability of Melphalan and Carmustine over the last decade, alternative conditioning regimens have been administered in our center, including intravenous Busulfan (BuEM) instead of Carmustine and Cyclophosphamide (BEAC) instead of Melphalan. BuEM has been extensively studied showing high tolerability and efficacy compared to the gold standard BEAM regimen, however there is limited previous data concerning BEAC. Therefore, we aimed to evaluate the safety, toxicity and efficacy profile of BEAC in lymphoma patients undergoing AHCT.
METHODS: We retrospectively studied consecutive lymphoma patients that received BEAC as a conditioning regimen (Carmustine 300 mg/m2, Etoposide 800 mg/m2, Cytarabine 800 mg/m2, and Cyclophosphamide 140 mg/kg) in our center between 2016 and 2017. The control group constituted of consecutive lymphoma patients that had been transplanted using BuEM (Busilvex 9.6 mg/kg, Etoposide 800mg/m2 and Melphalan 140mg/m2) between 2011 and 2013.
RESULTS: In total, 100 lymphoma patients were studied from which 33 patients received BEAC and 67 BuEM. There was no significant difference in baseline characteristics of the two study groups regarding age, lymphoma type, relapsed/refractory disease, chemosensitivity to salvage treatment and infused CD34+ cell dose. Days of neutrophil (p=0.657) and platelet (p=0.572) engraftment or transfusion needs (p=0.114 for red blood cell units and p=0.135 for platelets transfused) were also similar between BEAC and BuEM patients. In terms of safety, BEAC patients had significantly lower infection rates (51.5% versus 91%, p<0.001) and WHO grade 3-4 mucositis (p<0.001), gastrointestinal (p=0.025) and liver toxicity (p=0.013). Regarding outcomes, there was no difference in disease status at day +100 or at last follow-up. Moreover, the percentage of patients receiving adjuvant therapy post-transplant was similar in both study groups (p=0.492). Among them, BuEM patients received only radiation, whereas 6 BEAC patients received also adjuvant therapy with brentuximab according to current indications. With a median follow-up of 7.2 (0.8-22.9) months for BEAC and 44.9 (2.3-77.1) for BuEM, relapse mortality did not differ between regimens (p=0.626). 1-year overall survival (OS) was 92.4% and 87.6% respectively (p=0.186). In the multivariate regression analysis, diagnosis of HL (p=0.001) and chemosensitive disease (p=0.005) were independent predictors of OS.
CONCLUSION: In lymphoma patients who underwent AHCT, BEAC resulted in lower toxicity and similar outcomes compared to BuEM conditioning regimen. The observed outcomes were also comparable to our previously published experience with the gold standard BEAM regimen. Therefore, BEAC could serve as an alternative conditioning regimen in AHCT for lymphoma patients.

12. THE TRANSCRIPTION FACTOR TAP63 EXERTS PRO-SURVIVAL EFFECTS IN CHRONIC LYMPHOCYTIC LEUKEMIA ACTING THROUGH THE BCL2 PATHWAY

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OBJECTIVE: TAp63, the prevalent isoform of TP63 in Chronic Lymphocytic Leukemia (CLL), is implicated in disease pathogenesis as it promotes leukemic cell survival and homing to the bone marrow. In activated normal B cells, the TAp63 binds the BCL2 gene, participating in an anti-apoptotic pathway (axis NF-κB/TAp63/BCL2). Here, we investigated the expression of TAp63 in a large cohort of CLL cases and its potential fluctuation during disease progression. Additionally, we interrogated at the molecular level the interplay betweenTAp63 and BCL2.

METHODS: Using RT-qPCR we quantified TAp63 mRNA expression in 166 untreated CLL patients, (U-CLL:n=89,M-CLL:n=77). Next, we analyzed TAp63 mRNA expression in longitudinal samples of 25 U-CLL cases treated with either FCR or rituximab-chlorambucil. In each case, samples were collected at three 'landmarks'; diagnosis, first progression and first relapse. We investigated links between TAp63 and BCL2 by measuring BCL2 mRNA levels in 56 U-CLL cases from the present cohort. Considering that MEC1 cells express high TAp63 mRNA levels, we generated a stable MEC1 cell line to inducibly downregulate TAp63, using CRISPR/dCas9-KRAB upon treatment with doxycycline. We used 2 different guide RNAs (sgRNA1,sgRNA2) targeting 2 distinct regions of the endogenous TAp63 promoter. After 5 days of induction, the expression levels of both TAp63 and BCL2 were quantified by one step RT-qPCR in Tet-on-dCas9-KRAB-sgRNA-TAp63 MEC1 cells. Finally, we assessed ex vivo the effect of the BCL2 inhibitor Venetoclax in primary CLL cells of both TAp63high (n=8) and TAp63low (n=6) cases. Cell viability was measured by flow cytometry at 24 and 48 hours after treatment.

RESULTS: Significantly higher TAp63 mRNA levels were observed in U-CLL vs M-CLL (FD=13.83, p<0.0001). However, outliers were identified in both subgroups, prompting us to re-classify all cases into TAp63high and TAp63low subgroups using ROC-curve and Youden-index statistical procedures. TAp63high patients displayed significantly shorter time-to-first-treatment (TTFT) (TAp63highmedian TTFT: 1.58 years; TAp63lowmedian TTFT: 4.07 years;p=0.03) and shorter overall survival (OS) (TAp63highmedian OS: 7.825 years; TAp63lowmedian OS: undefined due to the large number of censored cases;p=0.046). Expression analysis in longitudinal samples showed that TAp63 levels significantly increased at disease relapse compared to diagnosis (FD=3.47,p=0.02). We found statistically significant correlation of BCL2 mRNA levels with the corresponding TAp63 mRNA levels (spearman rho=0.31,p=0.01) in the 56 U-CLL cases. In Tet-on-dCas9-KRAB-sgRNA-TAp63 MEC1 cells inducible downregulation of TAp63 expression (gRNA1:FD=1.7,gRNA2:FD=1.53) resulted in downregulation of BCL2 expression (gRNA1:FD=1.34,gRNA2:FD=1.12) with strong correlation (rho=0.97,p<0.0001) between TP63 and BCL2 mRNA levels. We also observed correlation between TAp63 and BCL2 protein expression in primary cells of one representative TP63high CLL case (rho=0.94,p=0.01), in which TAp63 was silenced by RNA interference with 3 different siRNAs. Finally,TAp63high cases were more resistant to treatment with Venetoclax as they showed no statistically significant reduction in cell viability compared to DMSO-treated controls, in contrast to TAp63lowcases (24h:FD=3.63,p=0.004; 48h:FD=7.17,p=0.005).

CONCLUSION: We provide evidence suggesting that up-regulated TAp63 expression represents a novel resistance mechanism to chemoimmunotherapy in CLL. The pro-survival role of TAp63 is supported by its strong association with BCL2. TAp63 appears to act as a positive modulator of BCL2 in CLL cells, rendering them less responsive to apoptosis induction with the BCL2 inhibitor Venetoclax.

13. ROR1 EXPRESSION IN B CELL LYMPHOMAS

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OBJECTIVE: The receptor tyrosine-likeorphan receptor one (ROR1) is an embryonic glycoprotein involved in several developmental processes. In Cll (chronic lymphoid leukemia) ROR1 was shown to be expressed on the cell surface uniformly and independently of any prognostic features. Newly there are hints that ROR1 is not only expressed in CLL, but also in other B-cell Non Hodgkin Lymphomas (NHL). It was shown to be highly and specifically expressed on circulating B lymphoma cells, but not on normal B cells. Hence, it can be suggested that ROR1 is a suitable marker to distinguish abnormal from normal lymphocyte populations.
METHODS: We retrospectively analyzed 271 patient's records who were examined for B cell lymphomas due to lymphocytosis (lymphocyte >5000 cells/µl). Eight color flow cytometric analyses were performed on peripheral blood specimens.
RESULTS: 271 patients with lymphocytosis on blood count including 117 cases of CLL, 12 cases of MCL (mantle cell lymphoma), 21 cases of MZL (marginal zone lymphoma), 9 cases of HCL (hairy cell leukemia), 6 cases of SLVL (splenic lymphoma with villous lymphocytes), 106 cases of healthy population (but lymphocyte count > 5000 cells/µl) were analyzed by 8 color flow cytometry (Males/Females: 145: 126; median age 65.3±13.6 years, range: 44-88 years). Median peripheral blood lymphocyte counts at diagnosis were of $20.4x10^9$ lymphocytes/L (range: $5–274x10^9$ lymphocytes/L). ROR1 expression was detected in less than 5% of lymphocytes in healthy populations If the cut-off value of ROR1 is 20% sensitivity and specificity 0.991, 0.849 respectively; when cut-off 48% sensitivity and specificity 0.924, 0.980 respectively and specificity for Cll is increasing.
CONCLUSION: ROR1 was shown to be expressed at high levels in several hematological malignancies such as CLL, MCL, CML, t (1: 19) B-ALL, as well as many other tumors. ROR1 ligand Wnt5a shares a similar expression pattern in blood malignancies, notably with high levels in B-cell lymphomas compared with no expression on healthy lymphocytes. As in the study Barna Gabor et. Al., in our study ROR1 expression was the highest in CLL samples, followed by MCL and MZL samples. Uhrmacher et.al. showed that ROR1 is high and uniformly expressed on CLL and significant lower on healthy cells. Detecting ROR1 expression on less than 10% in the measured samples can be considered a strong indicator for the absence of any B-NHL diseases. İn our study (as you can see in Table 1) we can't detect
ROR1 expression on normal lymphocyte but high levels in B cell lymphoma.

14. DEFINING THE ONGOING SPONTANEOUS DNA DAMAGE PROFILE IN MULTIPLE MYELOMA

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OBJECTIVE: Genomic integrity is constantly challenged by both endogenous and environmental factors, with double-strand breaks (DSBs) being the most deleterious form of DNA damage. Spontaneous DNA damage represents an important effect of endogenous factors; therefore, we evaluated its type and extent in multiple myeloma (MM) to better understand the disease biology and perturbation of the genome.

METHODS: Bone marrow plasma cells (BMPCs) and peripheral blood mononuclear cells (PBMCs) from 64 individuals were analyzed in this study: 16 patients with monoclonal gammopathy of undetermined significance (MGUS), 18 with smoldering myeloma (SMM), 15 with MM and 15 healthy controls. Moreover, the human cells lines MM1S, RPMI8226, OPM2 and U266 (myeloma), T47D and MCF7 (breast cancer), FLO-1 and OE-19 (esophageal adenocarcinoma), as well as BJ, HEEC and GM05756 (normal) were also examined. Comet assay under alkaline (measurement of both single- and double-strand breaks) or neutral conditions (only DSBs), polyacrylamide gel electrophoresis (PAGE) and Western blotting (for detection of critical markers of DNA damage repair), immunofluorescence antigen staining and confocal laser scanning microscope analysis of γH2AX, Rad51 and Ku70/80, measurement of glutathione (GSH) and oxidized glutathione (GSSG), detection of abasic (AP) sites, the activities of homologous recombination (HR) and non-homologous end-joining (NHEJ) using plasmids kindly gifted by Dr. Vera Gorbunova, chromatin immunoprecipitation (ChIP) and sequential Chip were all performed according to standard protocols.

RESULTS: MM cell lines showed significantly higher levels of endogenous DNA damage, oxidative stress, abasic sites and DSBs repair efficiency compared with normal cells (all $P<0.01$). Interestingly, we found that progressive, significant increase in the endogenous DSBs, the oxidative stress, the abasic sites and the DSBs repair efficiency occur in BMPCs during the transformation process of myelomagenesis and that these changes are also reflected in PBMCs from the same patients (all $P<0.05$). Moreover, in both BMPCs and PBMCs, substantial changes in the DSBs repair efficiency were observed during myelomagenesis, with the repair efficiency being higher in MM than in SMM and lowest in MGUS patients. PBMCs from healthy controls showed the least repair capacity. These data suggest that the accumulation of the endogenous DNA damage in myeloma cells is possibly attributed to increased damage formation rather than delayed repair efficiency. In addition, significant recruitment of the H4K20me2 epigenetic mark was observed in DNA damage sites, in proximity to γH2AX DNA damage-indicative variant. Interestingly, similar results were also obtained in other malignancies, suggesting that these molecular changes may be prevalent across various cancer types.

CONCLUSION: Increased levels of endogenously generated DNA damage, which are correlated with increased DNA damage formation and significant epigenetic changes, may be implicated in the transformation process of myelomagenesis. Since increased levels of endogenously generated DNA damage drive oncogenesis, sustain malignant progression and increase therapy resistance, these results can be exploited for both understanding the mechanistic basis of the pathogenesis and progression of the disease, as well as the development of novel therapeutic approaches.

15. THE COMPARISON OF MELPHALAN ADMINISTRATION ON DAY-3 WITH ADMINISTRATION ON DAY -1 ON NEUTROPHIL AND PLATELET ENGRAFTMENT IN MULTIPLE MYELOMA PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION

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OBJECTIVE: High dose chemotherapy followed by ASCT is the most important step of MM treatment. Melphalan, an alkylating agent, is the most preferable drug for conditioning regimens and dosage and timing is important with regards to side effects or engraftment timing. Engraftment time is determinative on infections and hospitalization duration.
METHODS: We compared the neutrophil and thrombocyte engraftments retrospectively in patients with multiple myeloma who received melphalan 200 mg/m2 single dose on day -3 and day -1 as conditioning regimen. There were 29 patient receiving melphalan on day -1 and 42 patient on day -3.
RESULTS: The mean neutrophil engraftment times for day -1 group and day -3 group were 12,8 ±2.4 days and 10,4±1.3 days, respectively ($p < 0,001$). The mean thrombocyte engraftment times for day -1 group and day -3 group were 13,48 ±3,7 days and 12,7±3,3 days, respectively (p: 0,36). In day -3 group, there was no failure neither in neutrophil nor in thrombocyte engraftment but 1 patient could just get thrombocyte engraftment on day 33. In day -1 group, 2 patients could not get engraftment failure.
CONCLUSION: Administration of melphalan on day -3 is better than on day -1 in terms of neutrophil engraftment and hence in terms of hospitalization duration.

16. NEXT GENERATION FLOW CYTOMETRY FOR MINIMAL RESIDUAL DISEASE EVALUATION IN MULTIPLE MYELOMA PATIENTS WITH SUSTAINED COMPLETE RESPONSE AFTER FRONTLINE THERAPY: RESULTS OF A PROSPECTIVE SINGLE-CENTER ANALYSIS

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OBJECTIVE: The evaluation of minimal residual disease (MRD) in newly diagnosed multiple myeloma (MM) patients after treatment provides valuable information for the depth of response and it correlates with clinical outcome. However, there is high demand for utilizing highly sensitive approaches for MRD detection to distinguish patients who reach complete remission (CR) but are at high risk for disease progression. In the present study we assessed the novel next-generation flow (NGF) approach (sensitivity level of 10-6) to evaluate the frequency of MRD in patients with sustained CR (>2 years) after front-line therapy.
METHODS: The primary endpoint of the study was the estimation of MRD negativity in myeloma patients who were in sustained CR after initial therapy with a sensitivity of 10-6. Secondary endpoints included: i) the concordance between two independent experts who analyzed the results of NGF in a blinded process; ii) the evaluation of differences regarding clinical and laboratory characteristics between MRD negative and MRD positive patients; iii) evaluation of major BM populations for each MM patient and correlation of these data with the presence of MRD as well as with other prognostic factors at the time of diagnosis. The process followed for MRD assessment is consistent with Euroflow guidelines in terms of sample preparation, antibodies used, appropriate cytometer settings and analysis of data obtained. MRD was evaluated by two independent experts in a blinded process to confirm the reproducibility of the results.
RESULTS: A total of 52 MM patients fulfilled the inclusion criteria and had BM aspirates collected for MRD evaluation. To date, with a median period of 58 months in CR (range: 24-197 months) after first-line treatment, 25/52 (48.1%) of MM patients were found MRD positive using NGF: 10/25 (40%) were positive at the level of 10-5 and 5/25 (20%) at the level of 10-6. These five MRD+ patients would have been falsely considered MRD negative if a method of a lower sensitivity (10-5) had been applied. In clinical terms, six cases have relapsed to date, five with MRD positivity. One of these cases was MRD negative at initial testing and reversed to MRD positive status at follow-up testing, 5 months before the disease progression, which imply that all relapses went through an MRD positive phase. The presence of MRD was not correlated with any prognostic parameters at diagnosis; however, it was associated with unique immune signatures characterized by a higher predominance of erythroblasts and monocytes in the bone marrow niche.
CONCLUSION: The NGF methodology recommended by EuroFlow provides a useful and standardized tool that enables the detection of MRD at levels reaching 10-6 and can distinguish those MM patients at higher risk of relapse, who would benefit from early MRD identification. Moreover, the 8-color NGF allows the assembly of a detailed personalized hematopoietic profile for each patient, which may highlight important aspects in the biology of disease progression. It may also eventually reveal unique immune cell signatures that, complementary to MRD, will offer prognostic information associated with MM patients' outcome.

17. DETECTION OF MYD88 AND CXCR4 MUTATIONS IN CELL-FREE DNA OF PATIENTS WITH IGM MONOCLONAL GAMMOPATHIES

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OBJECTIVE: Mutational characterization of Waldenström's macroglobulinemia (WM) currently relies on DNA from CD19-selected cells derived from bone marrow (BM) aspirates, which is an invasive technique, associated with significant patient discomfort. Liquid biopsy is being integrated into cancer diagnostics with profound therapeutic implications. Peripheral blood cell-free tumor DNA (cfDNA), also known as liquid biopsy, has been recently shown to be a powerful non-invasive molecular biomarker in monitoring tumor status in several cancers. In this study, we aimed to characterize the mutational status of WM/IgM MGUS patients by using peripheral blood plasma-derived cfDNA and using matched tumor DNA (tDNA) from BM-CD19+ selected cells, in order to determine whether cfDNA, can be used as an additional diagnostic tool in identifying the mutational profile of WM.

METHODS: A total of 68 consecutive patients with IgM monoclonal gammopathies and 10 controls were included in this study. Peripheral blood (10-12mL) was collected in EDTA tubes and DNA was extracted using the MagMax cell free DNA isolation kit. BM aspirates were collected at the same time with peripheral blood, and were processed for CD19 enrichment, using CD19 magnetic beads, followed by DNA extraction. tDNA and cfDNA samples were analyzed for the MYD88 L265P and for CXCR4 mutations. The presence of L265P mutation was initially assessed by Allele-Specific PCR and then confirmed with direct sequencing. The presence of CXCR4 mutations was assessed with direct sequencing.

RESULTS: Among the 68 patients, 54 patients had both tDNA and cfDNA informative samples. MYD88L265P mutation was detected in 40 out of 54 patients (74%) in both tDNA and cfDNA; in 4 out of 54 (7%) the mutation was seen in tDNA but not in cfDNA and 10 out of 54 (19%) patients harbored the MYD88WT genotype both in tDNA and cfDNA. Thus, the overall concordance between tDNA and cfDNA for MYD88 genotype was 93% (50 out of 54 patients). Among patients with IgM MGUS, WM in remission and sWM/NDWM/relapsed (RR) WM, the concordance rates were 100% (3 out of 3 patients), 96% (25 out 26 patients) and 88% (22 out of 25 patients), respectively. The assessment of CXCR4 mutations in both tDNA and cfDNA was feasible in 47 out of 68 patients (69%). In seven patients (7 out of 47, 19%), mutations were detected in both paired samples. CXCR4 mutations by either cfDNA or tDNA were present in 2 out of 5 MGUS (40%) patients, 7 out of 35 (20%) patients with disease in remission and in 5 out of 26 (19%) patients with sWM/NDWM/RRWM. The pathogenic mutation S338X was present in one patient with sWM and one with NDWM. The F29L, P27T, P31T and I53L mutations were also found 2-4% of patients. In 3 out of 47 patients the E343D, H228Q and L50X truncating mutations were detected in tDNA but not in cfDNA. Overall, the concordance rate between tDNA and cfDNA was 92% (43 out of 47 patients).

CONCLUSION: PB cfDNA is a useful, minimally invasive, cost-effective tool for the detection of MYD88 and CXCR4 mutations in patients with IgM monoclonal gammopathies avoiding unnecessary BM assessment.

18. CHARACTERISTICS AND MANAGEMENT OF AUTOIMMUNE HEMOLYTIC ANEMIA: A SINGLE CENTER STUDY WITH 32 CASES

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OBJECTIVE: Autoimmune hemolytic anemia (AIHA) is characterized by red blood cell destruction mediated with autoantibodies against to RBC antigens. Most common type is warm AIHA which can be either idiopathic or secondary to underlying disorders with immune disturbance. Determining the optimal therapy is a challenge because of insufficient data from prospective controlled trials. The aim of the study was to evaluate the clinical characteristics, treatment responses and outcomes of our AIHA patients.

METHODS: The clinical data of 32 patients with AIHA diagnosed and treated in our center between 2008 and 2016 were retrospectively analyzed.

RESULTS: Median age at diagnosis of AIHA patients was 45 years (range: 20-74 years). Male/female ratio was 1/1.3. Twenty-four of 32 patients (75%) had primary AIHA and 8 (25%) had secondary AIHA with underlying disorders as systemic lupus (SLE) in 2 patients, mixed connection tissue disease (MCTD) in 2, psoriatic arthritis in one, chronic lymphocytic leukemia (CLL) in one, marginal zone lymphoma in one and chronic HCV infection in 1. Median Hemoglobin (Hb) level was 7.4 gr/dl and 5 patients also had thrombocytopenia (<150000) beside hemolytic anemia. Mean LDH level was 544, indirect bilirubin was 2.7, reticulocyte was 11.3%. Eighteen of these 32 patients (56%) required transfusion. In all patients who required treatment (94%) corticosteroids were the first-line therapy with an initial response rate of 93%. Median steroid duration was 3 months range between 1.5 to 96 months. Relapse was occurred in 15 of 30 patients who received steroid (50%) with the median time to relapse (TTR) of 12 months (range: 5-72 months). Eleven of 3 patients (37%) required second-line therapy; seven had undergone splenectomy, three received rituximab, and one received danasin. All of the patients who undergone splenectomy had CR in first month and relapse after splenectomy was seen in 5/7 patients (71%) with a median duration of 60 months. Of three patients who were treated with standard dose of Rituximab; two achieved CR and one did not achieve any response. One of two rituximab-responded patients relapsed at 26. and 60. months and re-treated by rituximab; still following with CR for 16 months.

CONCLUSION: Although corticosteroids are the first choice of initial treatment of AIHA, most of the patients relapse at follow up. Steroid dependency and intolerance are also challenging. Splenectomy is still a considerable option for second-line therapy because of its high response rates and long remission durations. Rituximab is the other effective second-line therapy option with similar response rates to splenectomy. Until prospective studies will be performed, retrospective data would help the clinicians to choose best treatment algorithm for AIHA.

ABSTRACTS
POSTER PRESENTATIONS

P01. THERAPEUTIC RESPONSE OF MYELODYSPLASTIC SYNDROMES TO EPIGENETIC DRUGS INDEPENDENTLY OF ENDOGENOUS RETROELEMENT MODULATION

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OBJECTIVE: Hypomethylating agents (HMA) such as azacytidine and decitabine are the mainstay of treatment for higher risk myelodysplastic syndromes (MDS). Being cytidine analogues, they are incorporated into DNA of highly proliferating cells leading to genome-wide decrease of methylation levels. Although several putative modes of action have been suggested, the precise mechanism underlying treatment success or failure remains incompletely understood. One possible mechanism of HMA action is through 'viral mimicry' of transcriptionally repressed endogenous retroelements (EREs), which is thought to trigger innate immune pathways. EREs comprise nearly half of the human genome and their transcriptional activity is repressed by diverse mechanisms including DNA methylation. According to the 'viral mimicry' hypothesis, HMA induce unphysiological levels of ERE transcription in transformed cells, which in turn generated nucleic acid species, such as double-stranded RNAs from complementary ERE transcripts, activating innate immune sensors. Although support and a mechanistic basis for this hypothesis is provided from a number of in vitro studies, in vivo evidence from the clinical use of HMA is currently lacking.

METHODS: To explore the possible involvement of EREs in the HMA mode of action, we have compared the transcriptional profiles of CD34+ HSCs isolated from bone marrow samples of healthy donors (n=9) and patients diagnosed with AML (n=9), chronic myelomonocytic leukemia–II (CMML-II, n=9) or high-risk MDS (n=11). For MDS and CMML, samples were obtained before, 15 days (D15) after the initiation of azacytidine and/or after cycle 6.

RESULTS: Our analysis revealed that ERE transcription, measured as a proportion of the total polyA-selected transcriptome, is globally repressed in untreated MDS and CMML, in line with the proposed epigenetic repression that characterizes these conditions. Treatment with azacytidine had measureable effect in overall ERE transcription in HSCs from MDS and CMML patients, which by the 6th cycle was raised to levels equivalent to those seen in HSCs healthy controls. Comparable results were also obtained following analysis of a publicly available dataset from CD34+ HSCs isolated from MDS and CMML patients prior to and after the 6th cycle of azacytidine treatment (GSE76203). However, despite noticeable upregulation of overall ERE transcription relative to gene transcription by azacytidine, the therapeutic response was not correlated with or predicted by ERE activity. Indeed, ERE transcriptional activation was frequently observed in azacytidine-treated patients who failed to respond to treatment, whereas it was frequently low in or absent from patients with complete remission.

CONCLUSION: It remained theoretically possible that a therapeutic response to azacytidine depended on the transcriptional activation of a select few ERE loci with innate immune stimulatory properties, which might have been masked by the analysis of global ERE activity. However, few individual ERE loci differed in their activity between patients who responded or not to azacytidine treatment. Moreover, our analysis failed to detect induction of either interferon-inducible genes or interferon-inducible EREs, irrespective of treatment outcome. Together, our results do not support a role for transcriptional activation of EREs or for innate sensing of their nucleic acid products in the therapeutic response of MDS and CMML patients to azacytidine. Investigation of alternative potential mechanisms of azacytidine is therefore warranted.

P02. INCREASED NATURAL KILLER CELL CYTOTOXICITY IS A BIOMARKER PREDICTIVE FOR IMPROVED OVERALL SURVIVAL FOR PATIENTS WITH HIGH RISK MYELODYSPLASTIC SYNDROME AND OLIGOBLASTIC ACUTE MYELOID LEUKEMIA TREATED WITH AZACYTIDINE

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OBJECTIVE: Although it has been suggested, that hypomethylating agents (HMA) act through re-activation of tumor-suppressor genes, the exact mechanism of their action has not been completely elucidated yet. The aim of our study was to identify predictors of outcome for patients treated with azacytidine (AZA).

METHODS: Eighteen patients with high risk MDS (RAEB-II) and oligoblastic AML were included in this study. Patients were not eligible for intensive induction chemotherapy due to age, or presence of comorbidities. NK-cytotoxicity against K562, Rajii, and the percentage of various immune regulatory subpopulations in the peripheral blood (PB) was measured after 3-6 cycles of AZA. NK-cytotoxicity against K562 and Rajii was tested by using a flow cytometry based assay as previously described (1). Quantitative estimation of immune regulatory subpopulations in PB of patients was performed by using flow cytometry. The number of myeloid derived suppressor cells (MDSCs) (CD33+/CD11b+/CD14-/HLADRlow/-) was expressed as percentage among CD45+ PB monocytes. The number of Tregs (CD3+/CD4+/CD25+/Foxp3+) was expressed as percentage among CD3+/CD4+ PB lymphocytes. Comparison between groups was performed by using Mann-Whitney test for continuous variables. Receiver operating curve analysis (ROC) was performed with the aim to determine the ability of various parameters for the prediction of 2-year survival. Kaplan-Meier and log rank test were used for estimating the probability of overall survival (OS).

RESULTS: NK cytotoxicity: Patients with MDS/AML after a short exposure to azacytidine had a significantly reduced NK-cytotoxicity against K562 as compared with NK cytotoxic activity of healthy donors (21.8% v.s 41.8%, p=0.01). NK cytotoxic activity against Rajii of both patients and healthy donors was very low to absent (data not shown). Immune regulatory cells: There was no difference between MDSCs and Tregs in the PB of patients and healthy donors. (data not shown). NK cytotoxicity is a predictor of overall survival in patients treated with AZA: The ability of various parameters including the PB percentage of MDSCs, Tregs, as well as of NK cytotoxic activity to predict survival at 2 years after treatment with AZA was examined by using ROC analysis. NK cytotoxic activity was the only predictor of OS for patients treated with AZA (AUC=0.875, 95% CI, 0.635 - 0.979, p<0.0001). NK-cytotoxicity above 20.85% was estimated as the best cut off with sensitivity and specificity 80% and 87% in predicting 2-year survival. In multivariate analysis, NK cytotoxicity above 20.85% (high NK-activity) was the only parameter statistically associated with significantly improved OS [HR=0.10, 95% CI, 0.01 - 0.90, p=0.041].

CONCLUSION: Our study showed that NK cytotoxicity is a predictor of the outcome for patients treated with AZA. Patients with high NK-activity after a short course of AZA had significantly improved OS as compared with patients with low NK-activity. The results of our study suggest that the therapeutic activity of AZA is at least partly mediated by an immunomodulatory effect. References: 1) Irradiated mononuclear cells express significant in vitro cytotoxic activity: promise for in vivo clinical efficacy of irradiated mismatched donor lymphocytes infusion. Immunotherapy (2014) 6 (4), 409-417

P03. CHARACTERISTICS OF LONG-TERM SURVIVAL OF PATIENTS WITH MDS TREATED WITH 5-AZACYTIDINE. RESULTS FROM THE HELLENIC 5-AZACYTIDINE REGISTRY

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OBJECTIVE: The prognosis of patients with myelodysplastic syndromes (MDS) depends on several inherent disease characteristics but the use of hypomethylating agents (HMAs) has altered the prognosis of patients with higher risk MDS offering a median survival of around 24 months. Nevertheless, some patients achieve long remissions and high survival rates irrespective of their initial prognostic characteristics. Long-term survivors after 5-azacytidine administration constitute a large group of patients with potentially special characteristics and needs. We analyzed data from a large registry of patients with MDS treated with 5-azacytidine to describe the characteristics of long-term survivors and compare them to those of patients with shorter survival.

METHODS: We retrospectively recorded through the Hellenic 5-azacytidine registry the characteristics of adult patients with MDS treated with 5-azacytidine in 28 centers. We defined two groups of long-term survivors based on their survival after initiation of treatment (OST). The first group comprised patients with OST above the third quartile (Q3 or 75th percentile) of the whole group (Q3 group) and the second patients with OST above the 90th percentile (P90 group). Correlations were made between long- and short-term survivors for both groups.

RESULTS: Data from 626 patients were recorded. The Q3 group comprised 157 patients with an OST longer than 24.5 months and the P90 group 63 patients with an OST longer than 36.4 months. Variables such as sex, age, type of MDS (2008/2016 WHO classification), presence of excess (≥5%) marrow blasts, cytopenias, hemoglobin, neutrophil and platelet count, and transfusion needs were not predictive of long-term survival in neither of the groups, while the presence of peripheral blood blasts, the karyotype risk, the IPSS, IPSS-R and WPSS classification and response to treatment

were predictive of long-term survival in both groups. Multivariate analysis revealed that response to 5-azacytidine was the strongest determinant of long-term survival (Log Rank, $p<0.0001$) in a model comprising IPSS, IPSS-R, WPSS and response to treatment. Nevertheless, patients with stable disease were almost equally distributed in the groups of long- and short-term survivors [$p=0.795$ (Q3 group), $p=0.310$ (P90 group)].

CONCLUSION: The use of HMAs in MDS has increased survival rates, hence long-term survival is now a feasible treatment target. One fourth of the patients of this registry achieved an OST over 24.5 months and 10% over 36.4 months. Among the prognostic components of IPSS and IPSS-R at diagnosis, the karyotype risk seems to be the stronger determinant of survival. Nevertheless, among long-term survivors, there are patients with adverse prognostic characteristics at diagnosis, whose prognosis is altered by the administration of HMAs. Failure to respond to 5-azacytidine was a major determinant of OST, but stable disease was not correlated to shorter survival. This result highlights the importance of continuing treatment with HMAs in patients not achieving an optimal response (PR, CR, HI), since many of them may achieve long survival rates. Further search for new prognostic markers is warranted to identify the prognosis of patients with MDS and define those who would benefit from the use of HMAs or other upcoming treatment choices.

P04. HLA-G GENE AND PROTEIN EXPRESSION IN PATIENTS WITH PRIMARY MYELODYSPLASTIC SYNDROMES

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OBJECTIVE: In myelodysplastic syndromes (MDS) deregulation of immune effectors pathogenetically drives abnormal haemopoiesis and enhanced leukemic propensity. Human leukocyte antigen G (HLA-G) is a nonclassical MHC class I antigen, regularly not expressed in normal tissues except in trophoblasts from early gestation placentas and other immune-privileged tissues. Evidence for HLA-G silencing by a DNA methylation process has been reported. Interestingly, the use of demethylating agents such as 5-azacytidine further demonstrated that the repression of HLA-G gene activity in cultured various cell lines is reversed by demethylating treatment. HLA-G primary transcript generates seven alternative mRNAs that encode membrane-bound HLA-G1, -G2, -G3, -G4 and soluble HLA-G5, -G6 and –G7 isoforms. HLA-G has a direct inhibitory effect on the cytolytic activity of NK cells, cytotoxic T lymphocytes and is implicated in the induction of Foxp3-regulatory T cells. Therefore, HLA-G possesses immune tolerogenic activity with potential implications in antitumor immune responses. In order to investigate potential implication of HLA-G in immune deregulation underlying MDS pathogenesis and evolution we studied HLA-G gene and protein expression in CD34 cells of patients with primary MDS.

METHODS: Real Time PCR for HLA-G mRNA expression was performed in CD34 bone marrow (BM) cells derived from 35 untreated primary MDS patients of all subtypes, 7 patients with high-grade Non-Hodgkin Lymphoma without BM involvement, as well as first trimester trophoblasts from 2 donors served as controls. CD34 BM HLA-G protein levels were evaluated by Western blot in 10 study participants and plasma HLA-G protein levels were evaluated by ELISA in 22 MDS samples and 17 apparently healthy age/sex matched controls. Canonical variate analysis (CVA) was used to discriminate MDS subtypes using a set of several well-established laboratory parameters, as well as the HLA-G expression in CD34+ cells. The Kaplan–Meier method was used for calculation of survival probabilities and the Log-rank test for comparison of survival curves between expression levels of HLA-G. Cox regression was used for Overall Survival (OS).

RESULTS: Increased HLA-G mRNA and protein expression was observed in CD34 cells from MDS patients compared to controls ($p=0.04$ and $p=0.0095$, respectively). Plasma HLA-G levels were significantly higher in MDS patients compared to controls ($p=0.0008$). A distinct pattern of expression of various HLA-G mRNA isoforms was noted between MDS patients and controls. HLA G1/G5 was the commonest isoform expressed in CD34 cells, but other less common isoforms namely HLA-G2/-G4, -G3, and –G6 were also expressed. Interestingly, the CVA revealed that the major parameters that play a role in discrimination of the three major MDS subtypes (RCUD, RCMD and RAEBs), were not only the expected parameters of WPSS and percentage of blasts, but also the HLA-G mRNA expression in CD34 cells. OS curves of MDS with HLA-G mRNA over expression differed significantly during follow-up, compared to MDS patients without expression ($p = 0.05$).

CONCLUSION: Given the immune inhibitory properties of the HLA-G molecule, its increased expression in MDS CD34 cells may implicate the mechanisms of resistance or escape to immune surveillance contributing to MDS evolution and prognosis.

P05. AUTOPHAGY IN MYELODYSPLASTIC SYNDROMES: THE ROLE OF HIF-1A/REDD1 MOLECULAR PATHWAY

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OBJECTIVE: Hypoxia is a prominent feature of the bone marrow (BM) microenvironment, influencing both normal and malignant hematopoiesis. HIF-1α, a key regulator of hypoxia responses, mediates the transition to glycolytic metabolism and serves as a cell cycle checkpoint of HSC quiescence and function. Differential HIF-1α protein expression between hypoxic endosteal and less hypoxic vascular niche finely regulates normal hematopoiesis. REDD1 is a direct transcriptional target of HIF-1α linking hypoxia to energy regulation, metabolism and autophagy. We investigated the implication of HIF-1α/REDD1/autophagy/metabolism axis in differentiation/maturation of hematopoietic BM cells of MDS patients.

METHODS: BM aspiration and biopsy samples were collected from 15 untreated MDS patients (all subtypes except MDS-RARS) and 7 age-matched controls. BM biopsies were immunohistochemicallly stained by fluorescent-labeled 2-nitroimidazole to assess hypoxic areas in BM. CD34+ and myeloid lineage cells were isolated using magnetic beads and ficoll double-layer protocol, respectively. BM cell populations were determined by FACS analysis using standard gating strategies. HIF-1α and REDD1 gene and protein expression was evaluated by qRT-PCR and FACS analysis, respectively. Autophagy was determined by immunofluorescence for LAMP-1/LC3B and immunoblotting for LC3B/p62 (SQSTM1), whereas mitophagy by immunofluorescence for LC3B/TOMM20. Mitochondrial membrane potential (ΔΨ) and mass were analyzed by FACS analysis using mitotrackers. Metabolomic analysis of myeloid cells was performed by liquid chromatography mass spectrometry (LC-MSn). Raw data files were processed using several chemo-informatics tools.

RESULTS: We found a preferential strong accumulation of 2-nitroimidazole in intrasinusoidal regions of MDS BM, indicating that hypoxia is a fundamental feature of BM in MDS. We demonstrated a statistically significant REDD1 gene over expression and an increased intracellular protein co-expression of HIF-1α and REDD1 protein levels in both CD34+ and myeloid cells from MDS compared to controls, determined by RT-qPCR and FACS analysis, respectively. Higher REDD1 protein expression was shown in patients with high grade dysplasia as assessed by the Ogata score. Both CD34+ and myeloid cells from MDS demonstrated increased LC3B puncta compared to controls with concurrent staining for CD34 and MPO. The quantitative evaluation of LC3B by Western blot revealed high level of expression of LC3B-II in the MDS myeloid cells compared to controls indicating increased autophagic activity. The observed p62/SQSTM1 degradation along with the colocalization pattern of LC3B/LAMP-1 suggest increased autophagic flux. Metabolomic analysis of MDS myeloid lineage cells compared to controls revealed excessive glycolysis, defective oxidative phosphory-

lation and increased reductive carboxylation glutaminolysis associated with elevated level of intracellular 2-hydroxyglutarate, all indicative of HIF-1α driven metabolism. The co-localization between TOMM20 marker and autophagosomes in MDS myeloid cells was compatible with increased mitophagy whereas, MDS myeloid cells, were characterized by a reduction of mitochondrial mass and membrane potential in comparison to controls, as determined by FACS analysis.
CONCLUSION: Our results provide evidence for the first time of the hypoxia-driven HIF-1α/REDD1/autophagy axis in the pathophysiology of MDS, suggesting that this deregulated pathway is responsible for the production of 2-hydroxyglutarate, an oncometabolite, implicated in dysregulated epigenetic homeostasis. All the above may lead to the dysregulated metabolism and differentiation potential of the myeloid cells, thus unraveling a new pathogenetic mechanism for the MDS development.

P06. CLUSTERING OF SPLICEOSOME MUTATIONS WITH MARKERS OF IMMUNE ACTIVATION AND OTHER CHARACTERISTICS IN MDS PATIENTS: EXPLORATORY PILOT STUDY

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OBJECTIVE: Mutations of key spliceosome proteins are the most frequent molecular aberrations in MDS, followed by abnormalities of epigenetic regulators. SF3B1 mutations are the most common (20-28%), followed by SRSF2 (12%) and U2AF1 (8%). Several immune aberrations have been described in MDS and often the clinical picture is reminiscent of an inflammatory disorder. Trisomy 8 in particular has been linked with CD8+ T cell expansions and Behcet-like syndrome. Since aberrant RNA maturation may result in the production of neoantigens, we asked whether an inflammatory phenotype could be linked to the patient mutational status. Aim: We explored associations of spliceosome mutations with other disease characteristics.

METHODS: Sixteen patients with MDS with complete cytogenetic and molecular data were included. Median age was 73.5 years (range 40-80 years) and 12/16 were male (75%). Four patients had grade 2 BM fibrosis. Karyotype was normal in 10/16. Revised IPSS category was very low in 2, low in 6, intermediate in 3, high in 2, and very high in 3 cases. None of the patients had clinical evidence of coexisting autoimmune diseases, HIV, viral hepatitis or cirrhosis, other primary malignancies or active infections at diagnosis. All patients underwent targeted HRM-based analysis of sequencing of panel of myeloid genes on peripheral blood or bone marrow samples. Gamma globulins were quantified by serum protein electrophoresis densitometry and ratios of concentration over upper limit of normal range were calculated. Hypergammaglobulinaemia (HG) was defined as a ratio >1.2.

RESULTS: Nine patients (56.25%) had mutations in spliceosome genes (SRSF2: n=7, SF3B1: n=1, and U2AF1: n=1). The patient with SF3B1 had MDS-RS and was also JAK2 V617F positive. Four of seven SRSF2 cases carried ASXL1 mutations but only one spliceosome-negative case was ASXL1 positive. IDH1 mutations were detected in two SRSF2 cases and SETBP1 in one SRSF2 case. The most common cytogenetic abnormality was trisomy 8 in 4 patients (25%) all of whom where also SRSF2 positive. Three of these 4 also carried ASXL1 mutations. No gross imbalances in age, ECOG PS, comorbidities, cytopenias, WHO diagnosis, ISS, R-ISS or WPSS scores, marrow blast infiltration, fibrosis or cellularity, LDH, creatinine, ferritin were observed between spliceosome mutated and unmutated cases. WBC counts appeared higher in spliceosome mutated cases while all spliceosome cases were male. Median gamma ratio was 1.19, IQR 1.08-1.285. Fifty percent of patients had HG. Gamma ratios were marginally higher in spliceosome mutated cases (Mann-Whitney p=0.08), significantly higher among SRSF2 mutated cases versus all others (p=0.002) and higher in trisomy 8 cases (p=0.05). We performed kmedian cluster analysis and were able to separate our cases in two groups significantly differing in gamma ratios, SRSF2, ASXL1, trisomy (8), and male sex.

CONCLUSION: In this small pilot study we observed an association of spliceosome mutations (SRSF2) with polyclonal gammopathy and trisomy 8, supporting our hypothesis of neo-antigen mediated immune response. These associations need to be confirmed in larger cohorts. However, our observation may be relevant to MDS pathophysiology and novel targeted treatment pathways.

P07. THERAPEUTIC APPROACH OF ELDERLY PATIENTS WITH ALL: CLINICAL CHARACTERISTICS, PROGNOSTIC FACTORS, OUTCOME

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OBJECTIVE: Acute lymphoblastic leukemia (ALL) in elderly patients is a rare disease with unique characteristics and different biological behavior compared to younger individuals. Despite the progress that has been achieved in the survival of younger patients with ALL during the last decade, the prognosis of ALL in older patients remains poor. Complete remission and disease free survival (DFS) in older patients is minimal thus creating treatment challenges for this group of patients. Impaired functional capacity and comorbidities limit the possibility of administering intensive treatment regimens or transplantation to these patients which present with worse prognostic biological characteristics.

METHODS: We studied, retrospectively, 193 patients diagnosed with ALL in our center of which 33 patients (9 male, 24 female) were aged≥ 55 yrs (55-58, Δm= 66). There were 30 patients with B-ALL (pre-ALL: 12, pro-B: 5, common ALL: 8) and 3 with T-ALL. 17 patients demonstrated poor cytogenetic characteristics: 13 with Ph (+), 12 with hypotriploid karyotype, 3 patients with 11q23 abnormalities.

RESULTS: Co-expression of myeloid markers was found in 12 patients, 2 of which were diagnosed with MPAL leukemia. 16 patients had a high leucocyte count (>30.000), 10 showed grade IV thrombocytopenia and 10 presented with anemia (Hb< 8.5gr/dl). Seven patients had organomegaly and 2 presented with CNS infiltration. 21 patients completed treatment protocol (induction, consolidation, maintenance). Dosing reduction was made In 11 patients. Patients with Ph (+) received additionally a TKI inhibitor, either Imatinib or Dasatinib, while 2 patients received new TKI inhibitors (Bosutinib 1, Ponatinib 1) due to relapse. Intrathecal chemotherapy prophylaxis was administered to all patients. Complete remission was achieved in 23 patients (70%). 13 patients relapsed (56%). Treatment related deaths occurred in 6 patients (13%), the majority over the age of 60. 2 patients developed secondary malignancies (pancreatic cancer, colon cancer). 10/33 (30%) patients survived and remain in complete remission. None of these patients was offered stem cell transplantation as a treatment option because of advanced age, comorbidities or disease relapse. Analysis of patient characteristics in comparison to younger patients showed statistically significant differences in high leucocyte count (p= 0.015), low platelet count (0.045) and the presence of Ph (+) chromosome (39% vs 13%). The estimated 5 year overall survival was 29% (median 20 months) and disease free survival was 22%. Multivariate analysis of the results demonstrated that age over 50 and increased LDH value were statistically significant factors for survival.

CONCLUSION: Compared to younger patients the difference in OS (65% vs 29%, p< 0.001) and DFS (51% vs 22%, p< 0.001) confirms the worse outcome in the group of elderly patients with ALL. Administration of intensive treatment regimens (with or without stem cell transplantation), which remain successful treatment options for younger patients are not well tolerated in older patients. Patient stratification according to performance status, prognostic factors and comorbidities will facilitate the use of more effective and less toxic therapeutic regimens.

P08. SOLITARY CARDIAC RELAPSE OF ACUTE LYMPHOBLASTIC LEUKEMIA AFTER STEM CELL TRANSPLANTATION

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CASE REPORT: A 37-year-old woman was diagnosed with acute lymphoblastic leukemia (pro B acute lymphoblastic leukemia) with a t (4; 11) translocation (AF4/MLL). She achieved complete remission on a standard ALL protocol. She underwent allogeneic peripheral blood progenitor cell transplantation (PBCT) from her HLA-compatible brother. Unfortunately, on day 80, a haematological relapse occurred and the patient received chemotherapy with the IdaFLAG regimen followed by a second transplant. Following this second transplant, the patient entered again a complete haematological remission. RT-PCR was negative for the AF4/MLL fusion gene, so a molecular response was also confirmed. Four months after the second transplant, the patient presented again in our hospital with shortness of breath and chest pain. A chest X-ray revealed pleuritic effusion and an enlarged heart. An echocardiogram revealed pericardial effusion but no signs of anthracycline-associated heart failure. The patient had a normal left ventricular ejection fraction (LVEF=60%). The patient didn't have any signs or symptoms of infection. Blood counts were normal. All symptoms regressed and pericardial and pleuritic effusions disappeared after a short course on corticosteroids. The assumption was that pericardial and pleuritic effusions were possibly a manifestation of polyserositis associated with chronic GvHD. During corticosteroid dose tapering, symptoms reappeared while blood counts remained normal. A bone marrow examination confirmed that the patient remained in complete haematological remission. A bone marrow sample again proved negative for the AF4/MLL fusion gene. Troponin levels and NT-pro-BNP levels were elevated and continued to rise despite treatment with corticosteroids. A cardiac MRI showed marked thickening of the ventricular wall. At the time, it was postulated that the patient had either a cardiac manifestation of GvHD or cardiac infiltration by leukemic cells (solitary relapse of ALL in the heart). Both conditions are very rare. A myocardial biopsy was performed. Histopathology revealed diffuse infiltration of the myocardium by leukemic cells with adjacent necrotic tissue. Unfortunately the patient's condition deteriorated and she passed away shortly after histopathology results were obtained.
DISCUSSION: Very few cases of solitary extramedullary relapse of ALL in the heart have been reported in the literature. Interestingly they all involve patients who relapsed after allogeneic PBCT. This could indicate that the graft versus leukemia effect which keeps leukemia at bay, when it comes to medullary disease, cannot sufficiently suppress extramedullary proliferation of leukemic cells, preferentially allowing extramedullary relapse.

P9. A RARE CASE REPORT: MIXED PHENOTYPIC ACUTE LEUKEMIA

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BACGROUND: Mixed phenotypic acute leukemias (MPALs) are a heterogeneous group of rare leukemias constituting about 1% to 5% of all leukemias. MPAL refers to acute leukemia that displays an ambiguous pattern of antigen expression (ie, reflecting more than one hematopoietic lineage), to a degree that it cannot be unequivocally assigned to one lineage. MPAL, as defined by the World Health Organization (WHO), includes categories that were previously referred to as bilineage leukemia and biphenotypic acute leukemia.
CASE REPORT: A 56-year-old woman presented to the emergency room with fatigue and palpitation. Physical examination was normal except paleness. Blood count revealed pancytopenia (Hemoglobin: 6.9 g/dl, platelet count: 40000/mm3 and wbc: 1100/mm3). Peripheral smear was not diagnostic. Bone marrow smear showed mostly blasts. Flow cytometry revealed the blasts to be positive for CD34, CD117, CD33, cCD3, CD7, CD5, TDT, HLA DR with equivocal myeloperoxidase (Figure 1a and 1b). Conventional cytogenetic was reported as normal karyotype. Fluorescent in situ hybridization was negative for the BCR/ABL gene rearrangement, and quantitative reverse transcription polymerase chain reaction was negative for the t (9; 22)BCR/ABL-1 minor (p190) fusion transcript. ALL like chemotherapy (HyperCVAD (cyclophosphamide, vincristine, dexamethasone, methotrexate, cytarabine)) was started. Hematologic improvement observed with less than 5 percent marrow blastic cells on the third week of cycle. Chemotherapy continues to be applied and the donor is being screened for transplant.
DISCUSSION: The essential feature of MPAL is that cells express lineage – specific myeloid markers as well as lineage – specific T – or B lymphoid markers. MPALs are treate like ALL. The WHO recognizes two distinct categories: MPAL with the t (9; 22)(q34;q11)/ BCR/ABL1 and MPAL with t (v; 11q; 11q23)/MLL rearrangement. One large reported series of MPAL (100 cases) revealed the following breakdown of cases: B+myeloid 58%, T+myeloid 38%, B+T 4%, B+T+myeloid 2%

P10. INITIAL ABSOLUTE OR RELATIVE LYMPHOPENIA AS A PROGNOSTIC FACTOR FOR OUTCOME IN ACUTE MYELOID LEUKEMIA

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OBJECTIVE: White blood cell (WBC) count at diagnosis is considered an important prognostic factor for both acute lymphoblastic (ALL) and acute myeloid leukemia (AML). Absolute lymphocyte count (ALC) after induction treatment has been related to prognosis in acute leukemias, whereas it remains of importance after hematopoetic stem cell transplantation (HSCT) as a marker of immune reconstitution. ALC at diagnosis has been studied as a predictor of survival for non-Hodgkin lymphoma and other cancers. There is little evidence regarding the prognostic significance of ALC and lymphocyte percentage (Ly%) at AML diagnosis.
METHODS: We retrospectively studied ALC in 266 AML patients with median age 50 years (range: 14-75), male/female: 155/111, de novo/secondary: 197/69. Cytogenetic analysis was available in 250/266 cases and was favorable/intermediate/poor prognosis in 31/164/55 respectively. Complete remission (CR) was accomplished in 183/266 (68.8%).
RESULTS: Median ALC at diagnosis was $3.515x10^9$/μl. Elevated ALC was observed in cases of good prognosis karyotype, especially inv (16) (median ALC count: 6900/μl). Low ALC ($<1x10^3$/μl) at diagnosis was observed in 44/266 (16.5%) patients. This patient group correlated with low CR rate (21/44, ie 47.7%, p=0.001), low WBC count, neutropenia and secondary AML, in comparison to patients with an ALC count $>1x10^3$/μl. A trend to shorter disease free survival (DFS) was observed only at the subgroup of intermediate risk cytogenetics (p=0.07). Patients with relative lymphopenia (Ly% ≤20%) were 125/266 (47%) and presented with elevated WBC count, LDH, blast count and neutrophil count at diagnosis and had the same trend for a shorter DFS (p=0.07) in comparison to patients with ≥ 20% lymphocytes at diagnosis. Combining the above findings, a group of 147 patients presenting with relative or absolute lymphopenia could be identified. These patients had a lower CR rate (64% vs 74.8%, p=0.05). Outcome was significantly worse in this group compared with non lymphopenic patients: 5-year DFS was 24% vs 41% (p=0.01) and 5-year OS was 31% vs 46% (p=0.015). Lymphopenia did not influence survival when studied separately in the 3 cytogenetics risk groups. In multivariate analysis lymphopenia defined as ALC $<1x10^3$/μl was an independent prognostic factor affecting OS (p=0.016) but not DFS. Other independent prognostic factors for OS were karyotype, age, CR achievement post induction therapy (all p<0.001) as well as LDH levels at diagnosis (p=0.005).
CONCLUSION: In conclusion, patient's lymphocytes at diagnosis may have some assistive role in AML and the prognostic significance of lymphopenia in this setting needs further investigation. It is possible that differential signatures of lymphocyte subsets (eg NK cells) are more important than total lymphocyte counts.

P11. PROGNOSTIC SIGNIFICANCE OF TIME FROM DIAGNOSIS TO TREATMENT INITIATION IN ACUTE MYELOID LEUKEMIA PATIENTS

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OBJECTIVE: According to published data, delays in induction chemotherapy initiation in acute myeloid leukemia (AML) patients were associated with a poor outcome, especially in younger (<60 years old) patients and in case of delays concerning more than 5 days. On the contrary, a subsequent French survey did not validate this observation.
METHODS: We retrospectively studied 375 newly diagnosed AML patients who were treated with chemotherapy. Patients with secondary AML (92) were excluded from the study due to generally slower AML progression rates. Finally, 244 de novo AML patients were included and studied according to time to treatment initiation (TTTI). Patient group consisted of 134 men and 110 women, of median age 57 (14-73) years with non-M3 AML.
RESULTS: Median TTTI in the whole group was 3 (0-52) days. In 172 patients the TTTI was ≤ 5 days and in 72 >5 days. No statistically significant difference in TTTI was observed regarding sex, white blood cell count (WBC) at diagnosis (cut-off 50x109/l), LDH and good, intermediate or poor karyotype. On the contrary, older patients and patients with poor performance status (PS) were correlated with significant delays in TTTI (p=0.00011 and p=0.00015 for age >60 years and ECOG PS ≥ 2 respectively). Complete response rates did not differ in the two groups, achieving 75% vs 83% for TTTI ≤ 5 and > 5 days respectively. Also relapse rates were not affected by TTTI (54% vs 48%, p=ns). Early mortality (TRMe) was the same in both groups (5% vs 3%, p=ns). Overall survival (OS) and disease-free survival (DFS) ware also not significantly different in the two groups, with 10-year OS achieving 36% vs 30% and median OS 15 vs 16 months respectively, and 10-year DFS 28% vs 12% respectively, all p=ns. The same factors were studied in a subgroup of younger patients <60 years old and no significant difference was observed. In multivariate analysis only karyotype was a significant prognostic factor for OS and DFS, while age, WBC at diagnosis, PS and TTTI were not.
CONCLUSION: In conclusion, TTTI in AML has no impact on final outcome, therefore treatment delays are acceptable if required in order to obtain important test results or to ameliorate patient status and control infection.

P12. STUDYING NPM1 EXON12 MUTATIONS IN ACUTE MYELOID LEUKEMIA

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OBJECTIVE: Acute Myeloid Leukemia (AML) is clinically and biologically, a heterogeneous group of diseases, as a result of genetic and epigenetic events. The importance of genetic events to diagnose and monitor the effectiveness of the treatment, especially for "standard risk" patients, as well as to determine patients who will benefit from bone marrow transplantation has become widely accepted. Thus, the discovery of many molecular abnormalities in myeloid neoplasms has led to the publication of another revision of the classification of hematologic neoplasms by the World Health Organization (WHO). Almost 45% of AMLs show a Normal Karyotype (NK) by conventional cytogenetics, yet the clinical and biologic features of this large cytogenetic subgroup remain poorly understood. Mutations, occurring in exon 12 of nucleophosmin1 (NPM1) gene, are the most frequent genetic abnormality in de novo AML-NK patients (60%) and almost always involve a 4-bp insertion. They represent a stable molecular marker for MRD monitoring, as shown by follow-up studies that have confirmed consistent presence at the time of relapse.

METHODS: Bone marrow or peripheral blood samples from 366 AML patients were analyzed for the detection of NPM1 mutation from 7/2008 to 7/2018. Genomic DNA and tRNA were extracted from mononuclear cells, isolated by gradient centrifugation. DNA was amplified with labeled primers that span exon 12 of NPM1 and products were run on ABI3130 Genetic Analyzer. Mutated samples were recognized by the presence of a second peak different than the peak that corresponds to the wild type (169bp) after analysis with GeneMapperv4.0. Initially NPM1mut samples were further analyzed using MutaScreen kit (Qiagen) on ABI7500, in order to identify mutation A,B,D. On 2016 a Pyrosequencing method was set up on PyromarkQ24 (Qiagen) platform and an algorithm was created in order to easily identify the most common and rarer mutations. Then pyrosequencing was applied in order to verify the results of the mutation identification retrospectively.

RESULTS: 146/366 (39.9%) patients were found carrying the mutation, that is in accordance with bibliography. In 112 (78.3%) out of them mutA was identified, 8 (5.6%) were mutB and 12 (8,4%) mutD. 14 patients were carrying a different type of NPM1 mutation. The percentage of mutA is consistent with the literature as opposed to the percentages of B and D being inversely. MRD monitoring of NPM1 mutA,B and D patients is performed using the MutaQuant kits (QIAGEN) on LightCycler2 and LightCycler480, after reverse transcription of tRNA according the EAC (Europe Against Cancer), and RT-qPCR results are analyzed using absolute quantification fit point module in LightCyclerSW4.1.1 and LightCycler480SW1.5.1 respectively.

CONCLUSION: In this work we present the preliminary data of the NPM1 mutation study conducted in our laboratory. We plan to study the reduction rate of NPM1 transcripts after induction and consolidation therapy retroactively, the presence of additional molecular markers and their correlation to disease outcome given that 66 patients are being monitored for a median time of 27 months. We believe that incorporating the study of the data on new patients and assessing the results according to the international standards, will lead to early evaluation of the effectiveness of the treatment, to possibly provide pre-emptive therapy.

P13. SAFETY AND EFFICACY OF DIFFERENT ARA-C REGIMENS DURING THE CLINICAL COURSE OF ACUTE MYELOID LEUKEMIA

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OBJECTIVE: Cytosine arabinoside (Ara-C) is the key chemotherapeutic agent for the management of acute myeloid leukemia (AML). Ara-C in consolidation therapy played an important role for preventing relapses of AML patients that achieved complete remission. However, the optimum dose of Ara-C is still not certain. We retrospectively evaluated patients with AML who were treated in a single center to identify certain variables that could have affected the outcomes, including the role of two different doses of Ara-C administered during the consolidation phase of the treatment schema. To compare the modified dose cytosine arabinoside (mDAC; Ara-C at a dose of less than 1.5 g/m^2 every 12 hours on days 1, 3 and 5) versus high dose cytosine arabinoside (HDAC; Ara-C at a dose of 1.5 g/m^2 and higher every 12 hours on days 1, 3 and 5) in post-remission therapy for acute myeloid leukemia to confirm the post-remission antileukemic efficacy, reliability, tolerability and toxicity of mDAC.

METHODS: The five hundred and five patients who admitted to our Hematology clinic and were diagnosed as acute myeloid leukemia, between 1999 and 2018, were evaluated. Following the detailed interim analyses 66 patients were included in to this study. The patients who received 3 consolidation chemotherapy after induction were selected for further analyses. The Ara-c doses were similar for every 3 consolidation chemotherapy received for each of the patients. The patients who received different numbers and different doses of consolidation chemotherapy were excluded from the analyses. The 66 patients who received cytosine arabinoside consolidation were assigned to two groups based on their cytarabine dose protocol. HDAC group (n=30) received, ≥1.5 g/m2 every 12 h on days 1, 3 and 5 and mDAC group (n=36) received < 1.5 gr/m2 every 12 h on days 1, 3 and 5.

RESULTS: We analyzed survival outcomes HDAC group versus mDAC group among the 66 adult patients with AML. Median following for all patients was 12 months (range 1-76 months). The median duration of overall survival was 31 months (95% C.I. 12.6–50.8 months) with HDAC and 22 months (95% C.I. 22.5–35.4 months) with mDAC (p=0.24). The estimated median duration of PFS was 23 months (95% C.I. 13.1–34.5 months) with HDAC and 16 months (95% C.I. 5.8–26.0 months) with mDAC (p=0.05). There were no statistically significant differences between HDAC and mDAC group in the incidence of hematologic and non-hematologic toxicity (p>0.05).

CONCLUSION: The results of our present study suggests that mDAC may have an equivalent post-remission antileukemic efficacy in comparison to HDAC for the management of AML patients. Likewise, there were no significant differences between HDAC and mDAC group in the incidence of hematologic and non-hematologic toxicity. Thus, mDAC seems to be is appropriate with higher reliability for AML patients.

P14. A NEW CASE OF AML T (4; 12)(Q12;P13) WITH LITERATURE REVIEW

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BACKGROUND: Acute leukaemia with t (4; 12) is a rare, non-random event with an estimated incidence of 0.6% among adults (mean age 66yrs) and male to female predominance of 1.2-1.5: 1, with peculiar clinical and morphological features. This translocation is mostly seen in adult AML but less frequent in pediatric ALL. We present the case of a patient with this rare leukaemia.
CASE REPORT: A 60-year-old lady was referred to our Department due to leukocytosis and anaemia. Additionally, she reported anorexia, loss of approximately 17kg within 3 months and drenching night sweets. Physical examination: mild painless hepatomegaly. Complete blood count and peripheral blood smear: Hct 25.6%, Hb 8 g/dL, MCV 81.3fL, MCH 25.4pg, WBC 229.600x109/L (neutrophils 6%, lymphocytes 13%, monocytes 2%, baseophils 1%, myelocytes/metamyelocytes 5%, promyelocytes 3%, blasts 70%) with dysplastic changes of myeloid lineage and PLT 252x103/μL. Biochemistry: LDH 1035 IU/L and the rest within normal limits. Coagulation assays: within normal limits. Bone marrow aspiration: Cellularity 100%. Granulocytic and erythroid lineages markedly depressed. Megakaryocytes were adequate in number, with dysplastic morphology. Infiltration 58% by blasts of medium to large size with prominent nucleoli and agranular cytoplasm was detected. Flow Cytometric Analysis: CD7 (+),CD13 (+),CD33 (+),CD34 (+),CD38 (+),CD117 (+),HLA-DR (+),MPO (+). Cytogenetic analysis: 46,XX, t (4; 12)(q12;p13)[24]. The patient received induction therapy according to '7+3' protocol (AraC+Idarubicin). As disease was refractory, re-induction with Ida-FLAG was administered. Bone marrow aspiration on day +26 was indicative of extremely hypocellular bone marrow, with absence of blast cells or haemophagocytosis. Blood counts never recovered. Patient passed away on day +30 due to septic shock.
DISCUSSION: Within the few cases (n=40) published so far, several similarities are evident: trilineage dysplasia, low/absent myeloperoxidase activity, retention of platelets in peripheral blood and megakaryocytes in the bone marrow, basophilia and a pseudo-lymphoid morphology. The surface markers of the blasts show positivity for CD7, CD13, CD33, CD34, HLA DR, suggesting the immature myeloid stem cell origin of the leukemic cells. Less than 50% of the patients achieve remission with intensive induction chemotherapy with persistent bone marrow dysplasia being frequently observed. None of the patients who do not achieve morphologic remission survive beyond 6 months. The breakpoint at 12p13 in t (4; 12) AML is located within or near the ETV6 gene locus. The ETV6 gene has been implicated in both myeloid and lymphoid malignancies. ETV6 belongs to the ETS family of transcription factors and has two important domains: HLH and an ETS DNA binding domain. Cools et al. identified the genes involved in this translocation to be ETV6 (12p13) and BTL (4q12). A number of genes have been mapped to the band 4q12 including mac25, PDGFRA, AFP, and a beta-sarcoglycan gene. AML t (4; 12)(q12;p13), is a rare but recurrent entity. Our patient shared several characteristics with patients described elsewhere, such as basophilia, retention of platelets and megakaryocytes, trilineage dysplasia and blast positivity for CD7, CD13, CD33, CD34 and HLA-DR. Of note, MPO was positive. In addition, our patient experienced disease resistance to multiagent chemotherapy, resulting to prolonged cytopenias and severe infections and managed to survive only 60 days after diagnosis, indicating the dismal prognosis of this abnormality.

P15. LONG NEAT1 REDUCTION BY USING CRISPR-CAS9 GENOM EDITING SYSTEM COULD BE A CRITICAL REGULATOR IN TYROSINE KINASE INHIBITOR-BASED RESISTANCE IN CHRONIC MYLEOID LEUKEMIA CELL MODEL

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OBJECTIVE: Chronic myeloid leukemia (CML) is a malignant disorder of the haematopoietic stem cell characterized by BCR/ABL oncogene. BCR/ABL forms hematopoietic cells independent from exogenous growth-stimulatory signals by engaging signaling pathways such as JAK-STAT signaling. First line therapy for CML is Imatinib, a tyrosine kinase inhibitor (TKI) that targets BCR-ABL and greatly improves prognosis for CML. However, the emergence of TKI resistance, largely due to mutation of the ABL kinase domain is a major problem. Long non-coding RNAs (lncRNAs) are non-coding RNAs that are > 200 nucleotides in length and are involved in several diseases. Aberrant expression of the lncRNA Nuclear paraspeckle assembly transcript 1 (NEAT1) has been linked to many different cancer types. Two isoforms of NEAT1: NEAT1_1 (3.7 kb) and NEAT1_2 (23 kb) originate from the same promoter. In mammalian nuclei the expression of NEAT1_2 results in the formation of nuclear RNA-protein bodies named paraspeckles, wheras NEAT1_1 produces many smaller nuclear microspeckles. The function of NEAT1, microspeckles and paraspeckles still remains largely unknown in BCR-ABL mediated CML and TKI resistance mechanism. In this study we aimed to evaluate the cytotoxic effect of imatinib in CML cells by altering NEAT1 expression levels and gene expression of NEAT1 targets which are associated with CML progression and TKI resistance mechanism. In this study we aimed to evaluate the cytotoxic effect of imatinib in CML cells by altering NEAT1 expression levels and gene expression of NEAT1 targets which are associated with CML progression and TKI resistance mechanism.

METHODS: K562, a human chronic myeloid cell line, was treated with imatinib in a time and dose dependent manner and cytotoxicity and NEAT1 levels were evaluated. In order to modulate NEAT1 expression levels, CRISPR-Cas9 genome editing was also performed in K562 cells.

RESULTS: Our findings showed that decreased expression of NEAT1_2/paraspeckles effects the imatinib response in K562 cells.

CONCLUSION: One possibility we are investigating is that NEAT1 might target resistance-associated genes downstream of the BCR-ABL pathways that leads to imatinib resistance in CML. These results suggest that NEAT1 may be a therapeutic candidate in CML.

P16. PRIAPISM IS A RARE PRESENTATION OF CHRONIC MYELOID LEUKEMIA

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OBJECTIVE: Priapism is prolonged, painful and abnormal erection of penis unassociated any sexual desire. It is traditional to consider priapism as idiopathic and secondary. Idiopathic priapism accounts for 64% of all cases of priapism and may be due to thrombosis occurring in the venous plexus. About 20% of cases of seconder priapism are caused by hematological disorders including sickle cell disease, leukemia, various thromboembolic disease. Sickle cell disease is the most common cause of hematologic disorder to priapism in children. The incidence of priapism in adult leukemic patients is about 1-5 percent and leukemia is frequently associated with painful priapism. Chronic myeloid leukemia (CML) causes 50% of cases of priapism in patients with leukemia. In this report, we aim to present a CML patient admitted to our hospital with priapism.

METHODS:

RESULTS: Eighteen years old male patient was admitted to emergency with the painful, swelling and erected penis since about five days. Patient diagnosed with priapism by urology had splenomegaly (spleen was palpable 10 cm below the left costal margin) but no fever and lymphadenopathy, on physical examination. Total blood count showed a decreased hemoglobin 6,9 g/dL, increased leukocyte 215x109/L, (lymphocyte 7.1x109/L, neutrophil 192x109/L, monocyte 3.8x109/L, basophil 16x109/L, eosinophil 9.1x109/L), and platelets 470x109/L counts. Peripheral blood smear revealed nearly 62% neutrophils, 8% metamyelocytes, 13% myelocytes, 5% promyelocytes, 6% basophils, 7% eosinophils and there were no blast cells. Coagulation profiles were normal and serum chemistries were unremarkable except that lactate dehydrogenase was 681 u/l. Sufficient hydration and allopurinol 300 mg/day was started for potential tumor lysis syndrome. After bone marrowb iopsy, we also started cytoreductive therapy with hyroxyurea tablets at 3 g/day and leukapheresis. At the end of five sessions of leukapheresis, leukocyte decreased to 24x109/L and the penil erection, swelling and ecchymoses was relieved. Cytogenetic analysis revealed the presence of Philadelphia chromosome and molecular studies confirmed the presence of BCR-ABL transcript, so the patient was commenced on imatinib 400 mg/day.

CONCLUSION: Hyperleukocytos is caused by blasts or leukocytes is the most important cause in the pathogenesis of priapism. This is usually seen in acute leukemias, CML or chronic lymphocytic leukemia. In such emergencies where hyperleukocytosis is the cause, cytoreductive treatment should be started quickly with tumor lysis measures, if necessary; the patient should be treated with leukopheresis.

P17. A STUDY OF HEMOSTATIC PARAMETERS IN PATIENTS WITH PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS: CORRELATION WITH CLINICAL, LABORATORY, MOLECULAR AND TREATMENT CHARACTERISTICS

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OBJECTIVE: Patients with Philadelphia-negative myeloproliferative neoplasms (PN-MPN) namely polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (MF) are at a higher risk for arterial and venous thrombosis. Global coagulation assays such as thromboelastography, may be more efficient to evaluate the patient`s thrombotic risk. The aim of the present study was to examine the hemostatic profile of patients with PN-MPN and correlate it with clinical, laboratory, treatment, and mutational analysis of JAK2, MPL, CALR, and polymorphisms of poly (ADP ribose) polymerase (PARP1).
METHODS: The study included adult patients with a confirmed diagnosis of PN-MPN according to the revised 2016 WHO classification. The presence of splenomegaly, vascular events, PN-MPN specific therapy, and anticoagulation treatment were recorded. All the patients were assessed with complete blood count, routine coagulation tests [PT, INR, aPTT, fibrinogen and D-Dimers], platelet function performed with PFA-100 (COL, EPI, ADP), and global hemostatic potential assessed with ROTEM Tromboelastometry (EXTEM), recording clotting time (CT), clot formation time (CFT), maximum clot firmness (MCF), lysis index at 30 (LI30) and 60 (LI60) minutes, and α angle. Mutational profiles of JAK2,MPL and CALR were defined using peripheral blood DNA.The rs1136410/PARP1 (V762A) single nucleotide polymorphism (SNP), was detected with an RFLP method.
RESULTS: Seventy-four patients were included in the study (22 PV, 47 ET, 5 MF) with a median age of 63 years (25-87) and 68 healthy controls for the SNP/PARP1 study. At the time of sample collection, 71 (95.9%) patients were under treatment [hydroxyurea (HU), 57 (77.0%); anagrelide, 19 (25.7%); ruxolitinib, 9 (12.2%); interferon alpha, 2 (2.7%); an alkylating agent, 4 (5.4%)]. Forty-seven (63.5%) patients were on aspirin, 4 (5.4%) on clopidogrel, 7 (9.5%) on a combination of the two, 3 (4.1%) on a vitamin K antagonist, and 2 (2.7%) on a Xa-inhibitor. Nineteen (25.7%) patients had developed thrombosis after diagnosis. Among 69 patients who were not receiving anticoagulation, 5 (7.2%) had an abnormal CT, 4 (5.7%) had abnormal CFT, 3 (4.3%) had an increased α angle, and 18 (26%) had an increased MCF value. Women had shorter CFT, higher α angle, and higher MCF ($p<0.05$ for all parameters). Patients with ET had higher MCF compared to PV and MF. Patients with mutated JAK2, CALR, or MPL had higher WBC and shorter CFT ($p<0.05$). Patients receiving anagrelide or alkylating agents, had shorter CFTs, higher α angles, and higher MCFs compared to those receiving HU. COL-EPI was normal in 10/54 patients on aspirin and COL-ADP was normal in 5/11 patients on clopidogrel, implying that the antiplatelet treatment may not be sufficient in certain cases. No correlations were found between PARP1 polymorphic status and any of the studied parameters, nor between patients and healthy controls.
CONCLUSION: Global assays such as thromboelastography are more useful than conventional hemostatic laboratory tests in depicting the hypercoagulable state in MPN and in identifying patients at higher risk for thrombosis to guide clinicians for the type of treatment. Tests of platelet function assessment may help the clinicians adjust the type and dose of antiplatelet therapy.

P18. A CHORT STUDY INVESTIGATING THE RELATIONSHIP BETWEEN JAK2 MUTATION AND THROMBOSIS FREQUENCY

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OBJECTIVE: Essential thrombocytosis is a myeloproliferative disease which is usually associated with arterial and venous thrombotic complications. Thrombosis is a major cause of morbidity and mortality in myeloproliferative disease.
METHODS: In this retrospective study we record 95 patients who met the World Health Organisation (WHO) criteria for Essential Thrombosis (ET) and was followed up Hematology outpatient clinic between 2004-2018 years. Patients were classified according to Janus Kinase 2 (JAK2) mutation and thrombosis history as well as laboratory findings, demographic datas.
RESULTS: Our patient's average age is 53.9± 38, wbc count: 11.400 mm3, Hb: 13.4g/dl, plt count: $926x10^9$/L at diagnosis. 36% (35) of the patients were male, 64% (60) were female; 17.5% (16) of the patients had a diabetes mellitus (DM) and 36.8% (35) had hypertension (HT) history. Venous thrombosis history was 5.2% (5) and arterial thrombosis was 26.3% (25) of the patients. In our data DM increased the likelihood of arterial thrombosis (OR: 1.083) and venous thrombosis (OR: 1.35). Hypertension increased the likelihood of arterial thrombosis (OR: 1.114 but not venous thrombosis (OR: 0.14) In patients with JAK2 mutation, the incidence of arterial thrombosis was 17.5% (16) and venous thrombosis was 8.4% (8). There was no statistically significant difference in thrombosis between JAK2 positive and negative group (p: 0.578).
CONCLUSION: Already we know that DM and HT are risk factors for thrombosis especially in arterial thrombosis and according to studies traditional cardiovascular risk factors may also play a role in venous thromboembolism. There are some studies in the literature show thrombotic events are more common in JAK2 mutation positive individuals, but not in other studies. In our study there was no increase in the frequency of thrombosis in the presence of the JAK2 mutation. Some molecular studies have shown that JAK2 influences megakaryocytes via thrombopoetin at the molecular level and triggers thrombosis through numerical and structural changes in platelets. The differences between our study and literature especially retrospective studies may be related with inhomogen distribution of patient groups and the inadequacy of patient numbers. Establishing the precise risk of thrombosis in ET is difficult. But thrombotic risk factors must be well defined due to their impact on mortality and morbidity.

P19. ISCHEMIC COLITIS DESPITE ANTICOAGULANT THEARPY IN A PATIENT WITH POLYCYTHEMIA VERA

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BACKGROUND: Chronic myeloproliferative disorders (MPDs) share a stem cell-derived clonal myeloproliferation. These disorders can be associated with genetic mutations affecting protein tyrosine kinases, resulting in different configurations of abnormal signal transduction. The Janus tyrosine kinase 2 mutation can be used as a key diagnostic tool for diagnosing MPDs. Patients with ET and PV are at an increased risk for thromboembolic and hemorrhagic events. We present a case of PV causing arterial thromboembolism, despite being on adequate warfarin.
CASE REPORT: A 76-year-old woman was diagnosed with polycythemia vera and concomitant thrombocytosis after development of deep vein thrombosis after pubis fracture. Following this event, the anticoagulant therapy was started warfarine monotherapy. One and half years later, she was admitted to our institution for abdominal pain. Abdominal computer tomography was performed that showed mesenteric ischemia related ileus. An urgent laparotomy was planned, because bowel necrosis was strongly suspected. But septic shock and disseminated intravascular coagulation were developed and the patient passed away.
DISCUSSION: This case report illustrates that anticoagulant therapy alone was not sufficient to prevent arterial thrombosis, especially polycythemia vera accompanied by thrombocytosis.

P20. CONTRIBUTION OF MULTIPARAMETRIC FLOW CYTOMETRIC ANALYSIS IN THE DIAGNOSIS OF MAST CELL DISORDERS: REPORT OF TWO CASES

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BACKGROUND: Systemic Mastocytosis (SM) consists of a heterogenous group of rare disorders that are mainly characterized by abnormal accumulation of mast cells in ≥1 tissues, leading to systemic and local manifestations, as a result of increased mast cells (MC) burden and/or release of MC-associated mediators. Disease's rarity and diversity of clinical manifestations result in delayed diagnosis. Multiparameter flow cytometry (MFC) is a valuable tool for prompt diagnosis. Main findings are the aberrant expression of CD25 and/or CD2, the upregulation of membrane expression of proteins of MC activation, the degranulation-related and complement-associated molecules and the altered expression of adhesion molecules. Here, we describe two cases of SM, focusing on the contribution of MFC in diagnosis and follow-up of patients with SM.
CASE REPORTS: First case: A 33-years-old man was referred to our Department due to flushing episodes. Physical examination was positive for hepatosplenomegaly. Complete blood count (CBC) and biochemical assays were within normal limits. Coagulation tests were slightly prolonged. Bone marrow aspiration (BMA) showed cellularity 100% and marked infiltration by morphologically abnormal MC, which expressed CD25 in MFC analysis. Serum tryptase was markedly elevated. Cytogenetic analysis was normal and c-KIT gene was unmutated. The diagnosis of Mast Cell Leukemia was established. Second case: A 63-years-old man was referred to our Department due to anorexia and weight loss. Physical examination was positive for cafe-au-lait skin lesions. CBC showed mild neutropenia with dysplastic features, monocytosis and thrombocytopenia. BMA and biopsy showed trilineage dysplasia, 5% blasts infiltration, as well as infiltration 50% by abnormal MC. MFC identified the presence of abnormal MC (CD117+CD25+). Additionally, serum tryptase was elevated; FISH for D816V mutation of c-KIT was positive and biopsy of large intestine revealed infiltration by abnormal mast cells. Overall, the diagnosis of SM with Associated Myelodysplastic/Myeloproliferative Neoplasm was confirmed. Interestingly, biopsy of skin lesions was negative.
DISCUSSION: MFC is a valuable tool, which may contribute in diagnosis, classification and prognosis of SM rapidly, in considerably shorter time than other assays, such as immunohistochemistry on tissue biopsies. The above is mediated by distinction of three MC immunophenotypic profiles: mature phenotype (i.e. CD34- CD117+FcεRIhigh, aberrant expression of CD2/CD25, abnormal/increased expression of MC-activation, e.g. CD69, FcγRI, HLA-DR, HLA-DQ, and degranulation-related molecules, e.g. CD63, CD203c, usually seen in cases of indolent systemic mastocytosis), immature phenotype (increased expression of CD25, CD123, HLA-DR, lower expression of CD117, HLA-I, FcεRI, cytoplasmic tryptase, decreased light-scatter features, usually in the absence of CD2, often seen in cases of mast cell leukemia and aggressive systemic mastocytosis), and mature-resting phenotype (CD117strFcεRIstr, usually CD2-CD25-, normal expression of CD59, CD63, CD69, CD203c). Increased awareness by MFC specialists is required for accurate identification of abnormal MC even if they represent a rare event in the specimen, especially in the context of another concomitant hematological neoplasm. Furthermore, MFC may reveal positivity for CD30 antigen, which is expressed abundantly in the cytoplasm of neoplastic mast cells (MCs) in patients with advanced SM and has certain therapeutic implications. In the future, detection of minimal residual disease may be feasible by means of FC.

P21. REMARKABLE FUNCTIONAL CONSTRAINTS ON THE ANTIGEN RECEPTORS OF CLL STEREOTYPED SUBSET #2: HIGH-THROUGHPUT IMMUNOGENETIC EVIDENCE

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OBJECTIVE: Subset #2 is the largest stereotyped subset in chronic lymphocytic leukemia (CLL). This clonotypic B cell receptor immunoglobulin (BcR IG) is composed of the IGHV3-21/IGLV3-21 gene combination with the IGHV3-21 genes displaying a variable load of somatic hypermutation (SHM), including mostly mutated (M-CLL) but also unmutated (U-CLL) cases. Subset #2 cases, independently of the SHM status, have a particularly dismal clinical outcome similar to that of patients with TP53 aberrations, although lacking such aberrations. Subset #2 BcR IGs display recurrent SHMs and a cell-autonomous signaling capacity that is critically dependent on a Glycine (G) to Arginine (R) substitution at the lambda VL-CL linker region. Altogether, these features strongly implicate antigen selection in CLL subset #2 ontogeny. However, all available molecular evidence to date derived from low-throughput immunogenetic analysis. **METHODS:** Here, we sought to overcome this limitation by performing next-generation sequencing (NGS) of HC and LC gene rearrangements of 20 subset #2 patients on the MiSeq Illumina Platform. Rearrangements with identical CDR3 amino acid (aa) sequences were defined as clonotypes, whereas clonotypes with different aa substitutions within the V-domain were defined as subclones. **RESULTS:** Considering HCs, we obtained 3,340,508 (mean: 291,751, range: 101,231-186,055) productive reads. On average, each sample carried 92 distinct clonotypes (range: 71-152) with the dominant clonotype having a mean frequency of 96% (range: 67-99%) and displaying considerable intraclonal heterogeneity (mean: 5,082 subclones/sample, range: 2,946-11,041). Turning to LCs, we obtained 5,094,045 (mean: 231,547, range: 38,036-507,949) productive reads. LCs carried a higher number of distinct clonotypes/sample compared to their partner HCs (mean 222, range: 156-306). The dominant clonotype had a mean frequency of 96% (range: 74-98%), whereas intraclonal heterogeneity was more pronounced with a mean of 7,382 subclones/sample (range: 1,946-11,866). The analysis of the entire subset #2 VH or VL CDR3 dataset (i.e. the CDR3 aa sequences from all clonotypes of all cases) as a single entity branching through diversification enabled the identification of 2 distinct VH and 3 distinct VL CDR3 sequences present at varying frequencies in the majority of cases (range: 13-20). These results allude to important constraints in the composition of the antigen binding site. Focusing on SHM, the following notable observations could be made. (i) The G-to-R substitution at the VL-CL linker was a clonal event in all cases underscoring the seminal role of this recurrent SHM, likely due to mediating self-association. (ii) A recurrent 3-nucleotide deletion was detected in the VH CDR2 of all cases, strongly supporting functional pressure. This change, previously identified by Sanger sequencing in subset #2 at a frequency of 25%, was clonal in 4 cases and subclonal in the remainder. (iii) Recurrent aa substitutions in both the VH and VL domain, mostly at subclonal level: the prime example concerned the G for Serine (S) substitution within the VL CDR3, detected in all samples at a mean frequency of 44.2% (range: 6.3-87%). **Conclusion:** In conclusion, we provide compelling immunogenetic evidence for functional pressure in the ontogeny of CLL subset #2 rendering subset #2 as perhaps the most striking example of antigen-driven leukemogenesis reported thus far.

P22. LONGITUDINAL HIGH-THROUGHPUT T-CELL REPERTOIRE PROFILING OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS UNDER DIFFERENT TYPES OF TREATMENT: IMPLICATIONS FOR COMBINATION STRATEGIES

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OBJECTIVE: Prompted by our previous next-generation sequencing (NGS) study in treatment-naive chronic lymphocytic leukemia (CLL) indicating T-cell clonal selection by restricted antigens, we here sought to comprehensively assess CLL T-cell repertoire changes over treatment in relation to both treatment type and clinical response by combining NGS immunoprofiling, flow cytometry and functional assays. **METHODS:** We performed NGS profiling of the T-cell receptor (TR) gene repertoire in 28 CLL patients who received FCR (n=9), ibrutinib (IB, n=15) and/or rituximab-idelalisib (R-ID, n=10) at successive timepoints (pre, +3mo, +9mo and at deepest clinical response, total samples: n=113). For repertoire analysis, clonotypes (i.e. rearrangements with identical TRBV gene usage and amino acid CDR3 sequence) were considered. Also, we evaluated activation markers on CLL T-cell subpopulations for 8 CLL patients (R-ID, n=4; IB, n=4) pre- and +3mo post-treatment by flow cytometry. Finally, we investigated the ability of T cells, purified from 13 patients pre- and +3mo post-treatment (FCR, n=3; R-ID, n=5; IB, n=5), to form immune synapses with autologous CLL cells through quantitative relative recruitment index (RRI) analysis. **RESULTS:** We analyzed 20,347,768 filtered-in TRB sequences (Δμ=155,479/sample). All cases displayed significant clonal T-cell expansions both pre- and post-treatment [median clonality, measured as the cumulative frequency of the 10 most expanded (major) clonotypes/sample: 30.3% and 39.6%, respectively]. Median clonality significantly increased at +3mo in the FCR (29.0% to 46.9%, p<.001) and R-ID group (33.0% to 39.1%, p<.001), but not in the IB group (33.3% to 31.2%, p>.05). Overtime analysis revealed a gradual increase of clonality over deepening clinical response (pre-, +3mo, +9mo, deepest response) in the R-ID group (33.0% to 39.1% to 46.0% to 46.1%, respectively; p<.001), but only a trend in this respect for IB (33.3% to 31.2% to 33.8% to 42.0%; p>.05). Importantly, FCR resulted in T-cell repertoire reconstitution whereas BcRis retained pre-treatment clones. Cross-comparison across all CLL patients and against 767,438 unique TRB sequences retrieved from multiple public databases revealed 23/563 major clonotypes shared exclusively among CLL patients, alluding to selection by conserved CLL-related antigens. Further to these, we found statistically significant upregulation of T cell activation markers for R-ID compared to IB, particularly for: (i) CD69 in CD4+ effector memory (EM) T cells (p<.01); (ii) CD25 in CD8+ TEMRA T cells (p=0.006); and, (iii) CD38 in CD8+ EM T cells (p<.05) and CD8+ TEMRA cells (p<.05). Finally, RRI analysis for F-actin showed that both R-ID (p<.01) and IB (p<.05) treated T cells form polarized immune synapses in contrast to FCR (p>.05). **CONCLUSION:** Taken together, NGS immunoprofiling suggests that BcRis retain T cell clones that may have developed in response to CLL-related antigens, which in the case of R-ID expand and peak at +3mo. Phenotypic and immune synapse bioassays support a concurrent restoration of functionality, mostly evident for R-ID, arguably contributing to clinical response. Overall, this data provides rationale for designing combination strategies, e.g. of R-ID with immunomodulating drugs, aiming to boost cytotoxic anti-tumor responses. Moreover, identifying the relevant neoepitopes may eventually pave the way for stratified cellular treatments, especially if these epitopes are conserved among CLL.

P23. TREATMENT STRATEGIES AND PROGNOSTIC FACTORS FOR THE OUTCOME OF VERY LATE RELAPSES (VLRS) OCCURRING ≥5 YEARS AFTER INITIAL TREATMENT WITH CHEMOTHERAPY ± RADIOTHERAPY (CT±RT) IN HODGKIN LYMPHOMA (HL): A JOINT STUDY FROM TWO REFERRAL CENTERS

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OBJECTIVE: To evaluate the treatment strategies adopted for patients with VLRs in 2 referral Centers in Greece and Northern Italy as well as their outcome and prognostic factors for Freedom From Second Progression (FF2P) and overall survival after failure (O2S).
METHODS: Patients with HL, who experienced VLRs after CT±RT were identified retrospectively from the databases of the participating centers. Statistical endpoints were the estimation of FF2P and O2S and the identification of relevant prognostic factors.
RESULTS: 87 patients with VLRs after CT±RT were identified. Relapse occurred >15 years after the initial diagnosis in 21% of the patients, 64% of the patients were males and 16% were ≥65 years old. Reinduction with the same regimen was given in 28% of the cases, 5 patients (6%) received salvage RT only and 22% proceeded to high-dose therapy and autologous stem cell transplantation (HDT/ASCT). The 5- and 10-year FF2P was 56% and 51%, while the 10-year O2S was 56%. Among 32 deaths, only 19 were due to HL; 13 deaths were purely attributed to 2nd malignancies (n=9) and unrelated causes (n=4). Reinduction or HDT/ASCT did not significantly affect FF2P and O2S; 4/5 patients selected for salvage RT only had long-term remissions. A clear numerical difference was observed regarding 5-year FF2P for patients <65 years old who received HDT/ASCT (88% vs 55%), but this was just 59% vs 55% at 10 years and did not reach statistical significance (p=0.42). In multivariate analysis only anemia at relapse was independent predictor of FF2P; both anemia and age ≥65 years at relapse independently predicted for worse O2S. However, B-symptoms at relapse and relapse after >15 years were borderline in univariate analysis and deserve further consideration.
CONCLUSION: The prognosis of VLRs after CT±RT for HL does not appear very favorable; however, a considerable proportion of patients succumb to 2nd malignancies and unrelated diseases. Treatment approaches are heterogenous and HDT/ASCT is underused. Advanced age and anemia at

relapse were the most important prognostic factors in this series.

P24. PROGNOSTIC VALUE OF ABSOLUTE LYMPHOCYTE/MONOCYTE RATIO IN PERIPHERAL BLOOD AT DIAGNOSIS OF HODGKIN'S LYMPHOMA

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OBJECTIVE: Decreased absolute lymphocyte/monocyte ratio (LMR) in peripheral blood has been reported as an unfavorable prognostic marker in Hodgkin lymphoma (HL). Latest literature data several biological markers may play a role in the prognosis of HL. Some groups investigated the prognostic value of absolute lymphocyte /monocyte ratio of peripheral blood in HL. We studied the peripheral blood absolute lymphocyte/absolute monocyte count ratıo at diagnosis in Hodgkin Lymphoma and examined the effect on survival.
METHODS: We retrospectively analyzed 44 patient's records with Hodgkin's lymphoma in Ankara Numune Training and research hospital, Hematology department.
RESULTS: The median age at diagnosis was 39 years (range, 17-69 years). Of the 44 patients, 11 were females and 33 were males. According to the ann arbor staging system, 19 patients were stage 4, 13 patients were stage 3, 11 patients were stage 2, and 1 patient was stage 1. Thirty-two patients were advanced and 12 were early stage. The mean follow-up period was 45 months and 12 patients were relapsed and 1 patient was accepted as refractory disease. We found that patients with ALC/AMC ratio >1.1 group had superior overall survival than ALC/AMC ratıo <1.1 group ($p<0.005$).
CONCLUSION: The pathological biomarkers tumor-infiltrating lymphocytes and tumor associated macrophages are associated with clinical outcomes in classical Hodgkin's Lymphoma. Elevated tumor associated macrophage (TAM) ratıo in lesional tissues is associated with a worse overall survival. These macrophages are originated from circulating monocytes and migrate to the lymph nodes due to the effect of soluble chemotactic factors derived from the tumor. While absolute monocyte count may provide information about tumor microenvironment, absolute lymphocyte count of peripheral blood can be related to the immunity of patients. Combined the absolute lymphocytes count and absolute monocyte at diagnosis, as representative biomarkers of these. Koh et al. İdentified low ALC/AMC (<2.9) as an independent prognostic factor for overall survival. Porrata et al. İndicated that those with ALC/AMC had a superior survival, lymphoma specific survival and time to progression compared to patients with ALC/AMC<1.1. In our study we found that patients with ALC/AMC ratio >1.1 group had superior overall survival and combination with IPS ıt could be additional prognostic value in Hodgkin Lymphoma.

P25. MORPHOLOGIC, IMMYNOPHENOTYPIC AND GENETIC FINDINGS IN 3 UNIQUE CASES OF BURKITT LYMPHOMA COMPLICATING B-CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL): UNRELATED EVENT OR TRANSFORMATION?

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OBJECTIVE: Richter transformation refers to the transformation of B-CLL to diffuse large B-cell (DLBCL) or Hodgkin lymphoma (HL). We report 3 unique cases of B-CLL with "Richter transformation" to Burkitt lymphoma and attempt to investigate their clonal relationship.

METHODS: The following cases were retrospectively retrieved from our database. Case 1: A 65-year-old male patient was diagnosed 12 years ago with B-CLL. He was under "watch and wait" until the development of progressive lymphocytosis and massive abdnominal/pelvic lymphadenopathy. He received successively rituximab-chlorambucilx3, FCRx1, R-CHOPx3 and ibrutinib always due to treatment failure/disease progression. An excisional biopsy of the right pelvic lymph mass revealed a typical Burkitt lymphoma. The disease remained unresponsive to GMALL chemotherapy and was transiently controlled with local radiotherapy. Currently he receives DHAP. Case 2: A 76-year-old male patient with B-CLL under "watch and wait" for ~8 years, presented with rapidly growing right axillary lymphadenopathy. An axillary node biopsy revealed typical Burkitt lymphoma. The patient was not fully staged due to rapid clinical deterioration and death. Case 3: A 81-year-old male patient presented with acute back pain, leukocytosis-lymphocytosis, deteriorating severe thrombocytopenia, rapidly rising serum LDH and ferritin levels, but no palpable lymphadenopathy or organomegaly. Peripheral blood morphology and immunophenotyping were diagnostic of B-CLL with a small component (1%) of aggressive B-cell lymphoma. Bone marrow (BM) morphology and immunophenotype revealed 2 separate lymphoid populations; one compatible with B-CLL and another compatible with Burkitt lymphoma. The patient is currently in complete remission under R-CHOP.

RESULTS: Case 1: The morphologic examination of the pelvic mass specimen was typical of Burkitt lymphoma CD20+,CD79α+,CD10+,bcl6+,c-myc+(80%),ki-67 100% on immunophenotype. FISH revealed MYC rearrangements. Molecular analysis of the B-CLL-affected bone marrow and Burkitt-affected node revealed 2 distinct clones with different VH gene usage. Case 2: Lymph node biopsy revealed typical Burkitt lymphoma with the following immunophenotype: CD20+,CD79α+,CD10+,bcl6+,ki67>95%,CD5-,CD23-,bcl2-. FISH revealed MYC rearrangements. A simultaneous BM bi-

opsy demonstrated 10-12% nodular B-CLL infiltration. Genetic analysis is still in progress. Case 3: BM aspiration was typical of Burkitt lymphoma predominantly demonstrating a population of medium-sized immature cells with basophilic cytoplasm and numerous vacuoles. BM immunophenotype demonstrated two abnormal B-cell populations, both with ∂-light chain restriction: 1) 50% typical B-CLL lymphocytes; CD5+,CD23+,CD43+,CD200+,CD20+(weak),CD79α+(weak); 2) 45% lymphocytes consistent with Burkitt lymphoma CD10+,CD38+,∂+(moderately),CD20+,CD79α+. BM FISH analysis demonstrated MYC but not BCL2 rearrangements, while the BM karyotype was: 47,XY,+7,t (8; 14)(q24;q32)[18]/47,idem,+1,der (1; 15)(q10;q10). The BM biopsy demonstrated infiltration by small and medium-sized B-cells, reportedly CD5+,CD23+,bcl2+,CD10+, but it was unclear whether the high-grade B-cells or the B-CLL cells expressed bcl2. Thus, the diagnosis was either Burkitt lymphoma or Burkitt-like high-grade B-cell lymphoma not otherwise specified (HG-BCL-NOS) with MYC but not BCL2 rearrangements. Genetic analysis is suggestive of 2 underlying clones, but definite sequencing study is pending.

CONCLUSION: We showed for the first time that, apart from DLBCL and HL, B-CLL can be rarely complicated by other forms of aggressive lymphoma, such as Burkitt lymphoma. At least in a proportion of these cases the implicated clones are distinct, as also is the case in a considerable minority of patients with Richter Syndrome.

P26. PRIMARY BONE NON-HODGKIN'S LYMPHOMA: A SPECIFIC CLINICAL ENTITY WITH AGGRESSIVE CLINICAL COURSE – RETROSPECTIVE ANALYSIS OF 31 PATIENTS FROM WESTERN GREECE.

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OBJECTIVE: Primary Bone non-Hodgkin's Lymphoma (PB-NHL) is rare and citutes about 3% of the total NHL patient population. We retrospectively analyzed all cases with PB-NHL, diagnosed in the area of Western Greece between 1.1.1990 and 30.6.2018 in an effort to describe the clinical, histological and prognostic features of this tumor.
METHODS: Among 1225 patients with all types of NHL, diagnosed the above-mentioned period, 31 (2.5%) had PB-NHL. Patients were 20 males and 11 females (♂/♂ratio 1.81) with a median age of 63 years (range 20-85). In 21 of them (67.7%) the disease was presented with local symptoms only, in 3 (9.7%) with systemic/constitutional symptoms, and in the remaining 7 (22.6%), with both, local and constitutional symptoms. The mainly affected bones were thoracic vertebrae (6 cases), iliac/sacral area (5), lumbar vertebrae (4), humerus (4), tibia (3), femur (2), clavicle (2), ribs (2), skull, mandible and ankle (1 each). Treatment was offered in 26 patients, but two elderly accepted only radiotherapy. Anthracycline-based chemotherapy was given in 24 patients, and in 13 of them was accompanied by local adjuvant radiotherapy. For 5 patients treatment information was unavailable.
RESULTS: The main histological type was Diffuse Large B-cell Lymphoma Not Otherwise Specified (DLBCL-NOS: 19 cases), diffuse large B-cell immunoblastic (4), Ki-1+ anaplastic (2), small lymphocytic (2), mantle cell (2), diffuse small-cleaved cell, centrocytic (1) and pleiomorphic immunocytoma (1). A B-cell phenotype was revealed in 30 cases and non-B/non-T cell CD30+ in 1. Lymphomatous cells were found CD20+ (27/27 cases), CD45RO+ (25/26), CD79α+ (19/20), CD23 (7/17), CD10+ (9/24) at low proportion and/or intensity, CD30+ in 5/30 cases, LMP1- (18/18), CyclinD1- (16/18), bcl-6+ (11/14), and bcl-2+ (12/17) at various proportions. Among 24 patients screened one was HBsAg (+), one anti-HCV (+) and another one HIV (+). Disease stage at diagnosis was early (I-II) in 12 patients and advanced in 19 (61.3%), whereas B symptoms were present in 10 (32.2%). Seven patients (22.6%) had one additional involved extranodal site (bone marrow in 6) and 5 (16.1%) had >1 such site. 99Tc bone scan was positive in all 15 cases and PET/CT in all 5 cases performed. Anemia was present in 11 patients (35.5%), leukocytosis-neutrophilia in 5, and thrombocytosis in 5. Elevated serum LDH was found in 20/30 patients (66.7%), CRP in 19/30 (63.3%), β2-microglobulin in 5/21 (23.8%) and alkaline-phosphatase in 12/29 patients (41.4%). Among 22 evaluable patients CR was obtained by 12 (54.5%) and PR by 6 (27.3%) with a median DFS of 59.3 months. After a median follow-up of 42.2 months 11 patients are alive 10 of them disease-free and the remaining is under treatment. Median overall survival is 43.5 months and among responded patients 61.5 months. No survival benefit was found among patients who received adjuvant radiotherapy (median survival 46.5 versus 57.5 months, p:n.s.).
CONCLUSION: PB-NHL is an aggressive lymphoma subtype, classified mainly as DLBCL-NOS and response rates are similar to other nodal and extranodal aggressive lymphomas. Radiotherapy appears not to add on survival and should only be used for local palliation.

P27. UPDATED RESULTS ON CORRELATION OF SERUM TRANSFORMING GROWTH FACTOR-BETA1 (TGF-BETA1) LEVELS WITH SURVIVAL AND TIME TO TREATMENT IN WALDENSTROM'S MACROGLOBULINEMIA (WM)

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OBJECTIVE: Serum transforming growth factor-beta1 (TGF-beta1) is a pleiotropic cytokine involved in normal hematopoiesis as negative regulator of B-cell proliferation and immunoglobulin production. It was found to play a role in the pathogenesis of many hematologic malignancies, including Multiple Myeloma, but its' role has not been extensively investigated in WM. The purpose of this study was to update and expand former results from our group on correlations between serum TGF-beta1 levels and disease charateristics, time to first treatment (TFT) and overall survival (OS) in a series of WM patients with a long follow-up.

METHODS: We studied 77 WM patients from diagnosis to last follow-up or death; their files were reviewed after patients' informed consent was obtained, and clinical and laboratory characteristics and treatment details were collected. Patients' sera, drawn at diagnosis and kept frozen, were retrospectively analyzed. Serum TGF-beta1 measurements were done by ELISA according to the manufacturer's instructions. Statistical analysis was performed by SPSS software, v 22.0.

RESULTS: Patients' median age was 70 years, 67% were males and 95% were symptomatic or became symptomatic during follow-up time. At the time of diagnosis 27% of patients presented severe anemia (Hb<10g/L), 12% blood lymphocytosis, 17% thrombocytopenia, 41% increased beta2-microglobulin (b2M> 3,5mg/L), 18% elevated LDH, while median IgM levels were 2000 mg/dl and median bone marrow lymphoplasmacytic infiltration 45%. Seventeen percent of patients presented lymphadenopathy and 12% splenomegaly. Median survival of the whole series was 106 months and median follow-up time was 143 months. It should be noted that all over their disease course patients received new agents (80% rituximab, 30% DRC, 30% BDR, 18% ibrutinib). Median serum TGF-beta1 levels were 34420 pg/ml (range 1665-615000) in WM patients and 32902 pg/ml in 20 healthy individuals (range 1941 – 58123). Patients with TGF-beta1 levels above median had a longer TFT than the others (p=0,017) and an significantly improved OS (p=0,002). Among clinical and laboratory findings at diagnosis, serum TGF-beta1 correlated only inversely with platelet counts (p=0,01).

CONCLUSION: In conclusion, although serum TGF-beta1 levels did not correlated with clinical and laboratory findings at diagnosis, they were highly prognostic of outcome and patients with increased serum TGF-beta1 had a longer TFT and OS than the others. New agent treatment did not obcured serum TGF-beta1 prognostication in WM.

P28. PRELIMINARY RESULTS ON CORRELATIONS OF SERUM VON WILLEBRAND FACTOR LEVELS WITH ANGIOGENETIC CHARACTERISTICS IN NEWLY DIAGNOSTED PATIENTS WITH WALDENSTROM'S MACROGLOBULINEMIA (WM).

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OBJECTIVE: Von Willebrand factor (vWF) is a glycoprotein produced by the endothelial cells and megakaryocytes, that performs critical functions in primary hemostasis;it is also a marker of endothelial "stimulation". It was shown that high levels of vWF are associated with adverse prognosis in patients with symptomatic WM and it was suggested that vWF levels may reflect interactions between lymphoplasmacytic cells and other cells of their microenvironment such as mast cells and endothelial cells (Hivert et al; Blood 2012; 120: 3214-21). On the other hand, angiogenesis also is very important in the pathophysiology and course of several hematological malignancies including WM. Bone marrow microvascular characteristics, such as microvessel caliber (area, perimeter, axis major and axis minor length), microvascular network (total vascular area-TVA, microvascular density – MVD) and microvessel shape (compactness) correlate with clinical stage and outcome of WM (ref). The purpose of this study was to investigate any possible correlation between vWF levels and various microvascular characteristics in a series of WM patients at diagnosis.

METHODS: We studied 15 WM patients at diagnosis; their files were reviewed after patients' informed consent was obtained, while clinical and laboratory characteristics as well as treatment details were collected. Patients' sera, drawn at diagnosis and kept frozen, were retrospectively analyzed. Serum von Willebrand Factor measurements were performed by ELISA according to the manufacturer's instructions and microvascular characteristics were evaluated on CD34-stained slides in bone marrow trephine biopsy specimens. Statistical analysis was performed by SPSS software, v 22.0.

RESULTS: Patients' median age was 67 (51-80) years, 87% were males and 93% needed treatment. At the time of diagnosis of WM, median serum vWF levels were 464 pg/ml (range 39-1289), median MVD 34 μm2 (range15-64), median TVA 32,046 μm2 (range 13,143-69,160), median axis major length 42 μm (range 32,8-50,28), median axis minor length 16,9 μm (range 13,7-21,3), median area 915,6 μm2 (range 690,4-2034,13), median perimeter 147,1 μm (range 117,8-196,3) and median compactness 30,15 (range 21,3-39,2). The results of our analysis showed that serum vWF levels correlated only with TVA (p=0,014) and axis major (p=0,009) and axis minor lenght (p=0,027), while they did not correlated with MVD (p=0,056), area (p=0,954), perimeter (p=0,528) and compactness (p=0,488).

CONCLUSION: In conclusion, we confirm that serum vWF levels correlated with some of the microvascular characteristics but indeed, further research in larger series is needed.

P29. IBRUTINIB AS SALVAGE TREATMENT IN MANTLE CELL LYMPHOMA (MCL) PATIENTS WITH CENTRAL NERVOUS SYSTEM INVOLVEMENT (CNSI); PRESENTATION OF TWO CASES AND REVIEW OF THE LITERATURE

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BACKGROUND: CNS involvement is extremely rare at MCL diagnosis but more frequent at relapse (4-8%) with very poor prognosis. Leptomeningeal infiltration is more frequent. Treatment includes CNS directed chemotherapy crossing the blood-brain barrier (BBB) and consolidation with HDT/ASCT for fit patients. Retreatment with chemotherapy, irradiation and palliative care are options in relapsed/refractory (R/R) or unfit cases. Recently in a few case reports and small case series ibrutinib has shown significant single agent activity in R/R MCL with CNSi (Bernard et al, Blood. 2015; Mannina et al, Case Rep Hematol. 2017; Vitagliano et al, Leuk Lymphoma 2017; Tucker et al, BJH 2017). We present two cases of MCL patients with secondary CNSi, successfully treated with Ibrutinib in our center.

CASE REPORTS: Case 1: A 58-year-old male was diagnosed with high risk stage IV MCL in November 2011. He achieved CR with R-CHOP (8 cycles) followed by R maintenance. Three years after initial diagnosis he developed eye proptosis with pain and periorbital oedema. MRI scan showed intra-orbital mass infiltrating the rectus muscles and extending into the maxillary sinuses. Further imaging only showed cervical lymphadenopathy. CSF examination was negative. He was treated with high dose Methotrexate and Cytarabine and achieved partial response. Additional treatment with Bendamustine-Rituximab (6 cycles) led to further radiological improvement. He suffered second disease recurrence in the same area in September 2016. Whole body imaging with PET/CT scan revealed hypermetabolic foci inside the right frontal lobe with high uptake compared to brain parenchyma (SUV 16.7). CSF examination was negative. MRI confirmed the presence of intraparenchymal solid lesion. He was commenced on Ibrutinib 540 mg/d in November 2016. Follow-up imaging in March 2017 showed significant shrinkage of the intracranial tumor and complete response was seen in July 2017. Latest MRI imaging in July 2018 showed continuous CR on ibrutinib. Case 2: A 58 year-old male was diagnosed with stage IV, MIPI high MCL in November 2013. He achieved good PR after 6 cycles of R-CHOP. He was not considered eligible for transplant consolidation. He relapsed six months later with Waldeyer ring involvement (confirmed by biopsy) and leptomeningeal CNSi. He was treated with three cycles of HyperCVAD/MA and IT chemo to CR. In June 2015, there was systemic relapse with CNSi. He received salvage R-DHAP with responding systemic disease but only partial CNS response. There was CNS progression in October 2015. He started Ibrutininb 560 mg/day in November 2015. There was immediate response of CNS disease with CR on imaging 10 months later. Systemic disease remained in remission throughout, however he developed isolated CNS relapse in March 2017 (leptomeningeal and parenchymal). Disease responded (PR) to Bendamustine, Cytarabine, Dexamethasone and Rituximab plus IT chemo (4 cycles). There was progression of CNS disease (parenchymal only) again in February 2018. He was started on Rituximab- Ifosfamide-Etoposide without response and he passed away 4 months later.

DISCUSSION: Ibrutinib has been reported to have impressive clinical results in cases with CNS MCL relapse. Our experience described herein has also been positive. Salvage of MCL patients progressing on Ibrutinib treatment is difficult and outcomes are generally poor.

P30. A COMPLEX KARYOTYPE IN A SMALL LYMPHOCYTIC LYMPHOMA CASE THAT SHOWS TRANSLOCATION (1,6) AND ISOCROMOSOME 17 Q POSITIVITY

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BACKGROUND: Isochromosome 17q [i (17q)] and t (1; 6) are rare mutations in lymphoproliferative diseases. [i (17q)] positive CLL/SLL patients often have complex karyotype and it shows poor prognosis. Translocation (1; 6) can be together with unmutated IgHv cases and heralds an aggressive course because it accompanies high risk chromosomal aberrations such as del 17p and del 11q. Here we present a SLL case which shows both mutations simultaneously.
CASE REPORT: A 56-year-old male patient came to our clinic with a mass complaint at his neck. Blood counts and biochemistry revealed: WBC: 8460/mcrL, lymphocyte: 4580/mcrL, Hb: 12.5 gr/dl, PLT: 342,000/mcrL, LDH: 155 U/L. Physical examination showed extensive lymphadenopathy and abdomen BT also showed multiple enlarged lymphnodes (maximum size 17x10 mm, paraaortic localization). For diagnosis, supraclaviculary LAP and bone marrow aspiration/biopsy were performed. Both biopsies showed CD20, CD5, CD23 positivity and cycline D1, CD10, CD3, MUM1 negativity. Dıffuse lymphocytic infiltration was dominant at the bone marrow. Flow-cytometric analysis from the marrow showed CD38: 5%, CD5: 84%, CD19: 73%, CD 5/19 common zone: 64%, CD23: 56%, ZAP70: 2% positivity. Based on CLL/SLL working group criteria, the patient was diagnosed as SLL stage 4B and standart R-CHOP treatment was started. The conventional chromosomal analysis from the bone marrow detected a translocation between 1st and 6th chromosomes, chromosomal excess on 9 th chromosome's short arm and isochromosome 17q (it was evaluated as complex karyotype); del11q, trisomy 12, del 13q and de 17 p were all negative by FISH analysis.
DISCUSSION: Isochromosome 17 q mutation means the duplication of the long arm and deletion of the short arm of 17 th chromosome.It's an unexpected finding in lyphoproliferative diseases unlike myeloid type neoplasms or carcinomas. This mutation also heralds blastic crisis at CML. [i (17q)] positivity seen at CLL/SLL is usually detected with complex karyotype and heralds poor prognosis. On the other hand, t (1; 6) translocation is detected with IGHv unmutated cases and targets MUM1/IRF4 gene which is responsible of B cell production and differantiation. It can be detected in high risk, 11 q or 17p positive CLL cases. All of these information can express t (1; 6) and [i (17q)] are both related with short survey and dismal prognosis. The conventional or FISH based techniques are very crucial at hematological diseases and offer import information about the prognosis and response to treatment. More detailed studies are needed to illuminate the mutations which are usually seen in myeloid malignancies but not lymphoid neoplasms.

P31. PLASMABLASTIC LYMPHOMA IN A HIV NEGATIVE PATIENT WITH MDS/MPN WITH RING SIDEROBLASTS AND THROMBOCYTOSIS - A CASE REPORT

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BACKGROUND: Plasmablastic lymphoma (PBL) is a rare, aggressive type of B cell lymphoma with the vast majority of patients responding poorly to therapy or progressing shortly thereafter. Cyclophosphamide-Doxorubicin-Vincristine-Prednisolone, (CHOP) or CHOP-like regimens have disappointing results in this setting. We report the case of PBL arising in a previously diagnosed Myelodysplastic/Myeloproliferative Neoplasm (MDS/MPN) with ring sideroblasts and thrombocytopenia (RS-T) HIV negative patient treated with the combination of CHOP and bortezomib.

CASE REPORT: On November 2016 a 74- year old Caucasian male presented with transfusion dependent anemia and was diagnosed with MDS/MPN with RS-T according to WHO 2016. He was started on erythropoetin alpha, 40.000 units per week administered subcutaneous (s.c) and acetylsalicylic acid 100 mg per day. His transfusion need was compromised. On August 2017, he sought medical advice for a fast growing right submandicular mass. A CT scan of the neck showed a right submandicular lymph node block measuring 5.5 x 3.2cm with focal cystic degeneration with peripheral contrast media attenuation. An ultrasound guided fine needle biopsy revealed a diffuse infiltrate of large neoplastic lymphoid cells in a cohesive pattern with plasmablastic and plasmacytic features. Some small mature tumor cells with plasmacytic differentiation were identified. Concomitant neoplastic necrosis with both histiocytic and neutrophilic infiltration was noted. Immunohistochemical study revealed negativity for B-cell markers CD20, Pax-5 and only weak, focal expression of CD79a. Plasmacytoid differentiation markers CD38, CD138, MUM-1, EMA were uniformly, intensely positive. The proliferation index demonstrated by Ki-67 expression was approximately 90%. Epstein-Barr virus was not detected by the means of EBER in situ hybridization. Testing for HIV1, 2 antibodies were negative. A diagnosis of PBL was made. A CT scan staging approach revealed no lymph enlargement besides the right submandicular lymph node block, however an FDG PET/CT scan revealed an increased uptake in the base of the tongue (SUV max=5.8), in addition to an increased uptake in the submandicular lymph node block (SUV max= 4.5) and in a right cervical lymph node (SUV max= 3.1). A repeat bone marrow biopsy was negative for bone marrow infiltration and CSF analysis was normal. He was appointed stage IIE disease IPI score= 2. He was started on CHOP every 21 days plus bortezomib 1.3 mg/m^2 administered subcutaneously (s.c.) on days 1, 4, 8 and 11 of every 21-day cycle and central nervous system (CNS) prophylaxis with intrathecal methotrexate (IT) at dose 12.5mg on day 1. Significant clinical improvement was noted by the completion of the first cycle with minimal palpable residual mass. After 6 cycles of therapy, he was on complete metabolic remission with negative PET/CT scan and at 6 months' follow up he was still on remission, with negative CT scans.

DISCUSSION: Our case is remarkable for the coexistence of PBL with MDS/MPN with RS-T. Furthermore, the combination of CHOP plus bortezomib seems to have benefited the patient far more than what would have been expected with the sole use of CHOP, as shown by his long (almost one year) event-free survival.

P32. AN ATYPICAL PRESENTATION: FOLLICULAR LYMPHOMA WITH PERIPHERAL BLOOD INVOLVEMENT

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BACKGROUND: Follicular lymphoma (FL) is the second most common non-Hodgkin lymphoma (NHL) subtype and has an indolent clinical course. At the time of diagnosis, widespread lymphadenopathies, splenomegaly and considerable bone marrow involvement is present but it is mostly asymptomatic despite advanced disease. Cytopenia is frequently observed in the presence of bone marrow involvement whereas the presence of lymphocytes is rarely seen in the peripheral blood at the time of diagnosis. The incidence of FL with PB involvement at initial diagnosis is reported as 7-11%. Although its rarity, a patient with lymphocytosis who was diagnosed with follicular lymphoma is presented in this report.

CASE REPORT: A 67-year-old male, who had a medical history of mine work and chronic obstructive pulmonary disease, had a mass in the lower lobe of the left lung 2 years ago. The biopsy result of the mass had been reported as mesenchymal tumor. The patient had been treated with chemotherapy. Cure had been obtained after treatment and had been followed up. The patient was referred to hematology due to leukocytosis/lymphocytosis were detected in his follow-up. Bilateral cervical, axillary, inguinal multiple lymph nodes and splenomegaly (3 cm below the costal) were detected in physical examination. The patient did not have any of B symptoms. In laboratory examination we found: leukocyte counts: $52x10^9$/L, lymphocyte counts: $38.1x10^9$/L, Hb: 12.3 g/dL, platelet counts: $173x10^9$/L, while the biochemical tests including LDH and sedimentation were normal. Atypical small- to medium-sized lymphocytes (some with notched or cleft nuclei) were observed in the peripheral spread and thereafter bone marrow aspiration and biopsy were performed. The bone marrow biopsy was diagnosed as low-grade b cell lymphoma. CD19-20-23 was positive and CD5 was negative in flow cytometry. An excisional cervical LAP biopsy was performed and was resulted as the follicular lymphoma in grade 2. PET/CT was stained for staging. Bilateral cervical, axillary, mediastinal and bilateral inguinal lymph nodes with slightly increased FDG uptake and multiple lymph nodes that were smaller than 3 cm in size and slightly FDG uptake in the spleen and bone marrow and heterogeneous density with minimal FDG uptake in the left lower lobe of the lung lesion (residual malignancy?) was detected in PET/CT. A biopsy was planned for the lesion in the lung and the treatment will be planned according to the result of lung biopsy.

DISCUSSION: FL with PB involvement at the time of initial diagnosis is rarely observed and usually associated with nodal and / or extranodal involvement whereas pure leukemic form has been reported very rarely. Peripheral involvement is usually associated with a moderate and high Follicular Lymphoma International Prognostic Index (FLIPI) score. The presence of peripheral blood involvement in the follicular lymphoma is associated with poor overall survival and progression-free survival. However, according to literature, the presence of a pure leukemic form without nodal or extranodal involvement is more indolent than with nodal and extranodal involvement.

P33. PRIMARY BONE DIFFUSE LARGE B-CELL LYMPHOMA WITH MULTIFOCAL OSTEOLYTIC LESIONS: A RARE ENTITY

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BACKGROUND: Bone lymphomas can be classified as primary (PBL), or secondary (SBL). PBL is a rare entity, accounting for approximately 7% of malignant bone tumors, 5% of extra – nodal lymphomas and < 1% of all non-Hodgkin lymphomas. We present a case of multifocal bone lymphoma since it is a rare entity and on the other hand in many cases it is difficult to distinguish the primary site of lymphoma and to categorize it as primary or secondary.
CASE REPORT: A 72-year-old female patient, without any notable medical or family history presented to her general practitioner with persistent middle back pain and failure of common analgesics to ease the pain. Anorexia, weight loss of about 6 kg in the last 3 months and fatigue were also reported. An outpatient MRI of thoracic and lumbar spine revealed a complete fracture of the T10 vertebra and multiple, high signal vertebral lesions indicative of secondary origin. In our Dept, her laboratory tests showed a moderate elevation of LDH (380 U/L, normal range: 135-214 U/L), ESR (60 mm) and beta-2 microglobulin (4.1 mg/L, normal range: 1.42-3.21 mg/L). In CT and MRI scan a complete fracture of the left hip and multiple vertebral lesions were revealed. Bone biopsy of the left hip showed a Diffuse Large B-cell Lymphoma. She was treated with combined chemotherapy (R-CHOP regimen) plus radiation, followed by bracing with cervical immobilization and cimentoplasty of lumbar vertebrae. She achieved complete response and she is alive at last follow up.
DISCUSSION: The survival of patients with primary bone DLBCL is related to disease stage, while it is independently associated with age, performance status and serum LDH levels. Prognosis seems to be better in patients with multifocal DLBCL compared to disseminated nodal lymphoma; MUM 1 expression >10%, low CD10 expression and a nongerminal centre signature are associated with poorer outcome. Relapses are sign of poor prognosis, while presentation at the initial onset of the disease with pathological fracture is associated with worst outcome. First-line treatment should be based on R-CHOP regimens followed when indicated by involved-field radiotherapy and CNS prophylaxis in high risk patients. With this strategy the overall response rate (ORR) is over 90% and the 5-year overall survival is 84%. However there still remain several issues with regard the sequence of treatment, the choice of radiation volume and the role of surgery for fixation of pathological fractures.

P34. PREGNANT AND NEWBORN WITH NON-HODGKIN`S LYMPHOMA: CASE REPORT

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BACKGROUND: Lymphomas are heterogeneous group disorders resulting in malign proliferation of lymphocytes. According to the main histological classification, two groups are distinguished: Hodgkin`s Lymphoma and Non-Hodgkin`s Lymphoma (NHL). Although Hodgkin`s disease is the most common type of lymphoma in pregnancy, Hodgkin`s Lymphoma is rarely seen in pregnancy. In this case report; it is aimed to present a pregnant woman with a diagnosis of diffuse large B-cell lymphoma (DLBCL) and midwife care given to her and her newborn. The necessary permits were obtained from the pregnant and the Hematology Clinic where she was followed. Republic of Turkey Ministry of Health`s Prenatal Care Guide, Postnatal Care Guide, perinatology and midwifery literature were reviewed and she was monitored in line with her individual needs, and midwifery care was provided.

CASE REPORT: A 31-year-old woman has been married for 10 years and has complained for B-symptoms during the 27th week of pregnancy. She was diagnosed with DLBCL at the 31st gestational week and two doses of chemotherapy were administered during the follow-up period. With individualized care given to the patient who delivered with cesarean section during the 34th gestational week; complications that developed during her treatment were minimized, acceptance of the disease process and participation in self care, and psychological support had a positive effect on the prognosis.

DISCUSSION: Data regarding NHL occurring during pregnancy are limited and are mainly derived from anecdotal reports and small retrospective series. With this presentation, we think that midwifery care in a rare disease will contribute to the better management of such cases.

P35. MOLECULAR MECHANISMS OF CARFILZOMIB-INDUCED CARDIOTOXICITY IN MICE AND THE EMERGING CARDIOPROTECTIVE ROLE OF METFORMIN

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OBJECTIVE: Carfilzomib (Cfz) is an irreversible proteasome inhibitor, which is used for the treatment of relapsed/refractory multiple myeloma (RRMM). In phase III trials, Cfz has been associated with higher cardiotoxicity and heart failure rates compared to the control treatment. Due to the severity of these adverse events and the lacking data regarding the induced cardiotoxicity, there is an imperative need for the elucidation and abrogation of the underlying mechanisms of Cfz-induced cardiotoxicity. The aim of this study was to investigate the molecular mechanisms of Cfz-induced cardiotoxicity and to evaluate possible cardioprotective effects of concomitant medications based on our initial results.

METHODS: Protocol 1: Male C57BL/6 mice, were randomized into: Control (N/S 0.9%, n=7) and Cfz group (n=8). Based on the results showing below we also developed a second protocol using metformin (met); Protocol 2: Male C57BL/6 mice were randomized into: Control (N/S 0.9%, n=8); Cfz (n=8) and Cfz+Met (n=10). Cfz (8 mg/kg ip) was administered every 48 hours in both protocols and Met (140 mg/kg po) every 24 hours for 6 days. Fastening glucose levels were monitored. At baseline and at the end of treatments mice underwent echocardiographic assessment. Animals were sacrificed and blood and myocardial tissue samples were obtained for the analysis of proteasome peptidases activity, protein phosphatase 2A (PP2A) activity and molecular signaling mechanisms. Protein kinase Akt, along with its downstream NO synthases; endothelial (eNOS) and inducible (iNOS), were identified as targets of possible endothelial dysfunction and inflammation. Moreover, the transcription factor FOXO1, downstream target of Akt and AMPKα, was identified in order to investigate possible changes in the expression of apoptotic factors. Finally, AMPKα was identified since – besides phosphorylating eNOS and FOXO1 – it functions as a regulator of autophagy.

RESULTS: Administration of Cfz resulted in significant reduction of the chymotrypsin-like (CT-L) proteasome activity in myocardial tissue and peripheral blood mononuclear cells of Cfz-treated mice vs controls ($p<0.01$). Protocol 1: Reduction in fractional shortening (FS%) was observed in

the Cfz group vs Control at Day 6 (39.87±0.47% vs 42.05±0.64% respectively, $p<0.05$). Cfz increased PP2A activity vs Control ($p<0.05$), without altering PP2A expression. A decrease in pAkt/tAkt ($p<0.05$), peNOS/teNOS ($p<0.05$), pAMPKα/tAMPKα ($p<0.001$) and an increase in the expression of iNOS ($p<0.01$) was observed in the Cfz group vs Control. Protocol 2: Met did not reduce fastening glucose levels at day 6 in Cfz+Met compared to Control and Cfz groups. Echocardiographic assessment at day 6 revealed that Met reversed Cfz-induced reduction in the FS% in Cfz+Met vs Cfz group (43.4±0.5% vs 41.5±0.4% respectively, $p<0.05$). AMPKα phosphorylation was significantly increased in the same group compared to Cfz group ($p<0.01$).

CONCLUSION: Cfz induces cardiac dysfunction via increasing PP2A activity, leading to decreased phosphorylation of Akt, eNOS and AMPKα. The disturbance of Akt/AMPKα/eNOS axis and the increase of iNOS, suggests that Cfz might intervene with oxidative stress, apoptosis and myocardial energetic pathways. Thus, Cfz-induced increase in PP2A activity seems to be essential in the mechanism of cardiotoxicity. Met restored AMPKα phosphorylation and reversed Cfz-induced contractile dysfunction, emerging to be a potent pharmacological intervention for the management of Cfz-induced cardiotoxicity.

P36. NATURAL HISTORY AND OUTCOMES OF ASYMPTOMATIC IGM MONOCLONAL GAMMOPATHY

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OBJECTIVE: The aim of this study was to report the outcomes of IgM MGUS and asymptomatic Waldenström's Macroglobulinemia (aWM).

METHODS: We studied 103 patients who were diagnosed and followed in our center since 1995 and had BM biopsies available. According to the definition by Kyle et al, 62 (61%) subjects had aWM and 41 (39%) IgM MGUS.

RESULTS: At the time of diagnosis of the IgM gammopathy, median age of all subjects was 71 years (range 25-86), 76% had IgMκ and 24% IgMλ; only one had a biclonal gammopathy (IgM and IgG). Median IgM was 1024 mg/dL (range 104 - 5320) and the monoclonal spike in the SPEP was 0.84 g/dL (ranging <0.1-4.35) and only 3% had M-monoclonal protein ≥3 g/dl. Median hemoglobin was 12.8 gr/dl and median β2-microglobulin (β2M) was 2 mg/L (range 1.1-16.8). Among 71 patients with available free light chains at the time of initial diagnosis, 44 (63%) had abnormal FLC ratio. The median clonal cell BM infiltration was 20% (range 0-90%) and there was an association of serum IgM levels and extent of clonal cell infiltration (r=0.416, p<0.001). Immunoparesis was present in 32% of the subjects; 22% hag low IgG, 28% low IgA and 18% had both IgG and IgA suppressed. Patients with aWM had higher M-protein (median 1.24 vs 0.55 g/dL) and more often monoclonal protein ≥1 g/dl (58% vs 13%, p<0.001) so that 42% of patients with <1 g/dL of monoclonal IgM had ≥10% clonal lymphoplasmacytic cells. Median follow up of the whole cohort is 5 years (range 1-21); 15 (14%) patients have progressed to develop symptomatic disease and 8 (7%) have died without developing symptomatic disease. For patients with aWM, the respective rate of PD to symptomatic disease and of unrelated death was 20% and 3% at 2 years and 39% and 8% at 5 years respectively. For those with IgM MGUS the PD rate and unrelated death rates were 0% and 3% at 2 years and 3% and 7% at 5 years. Thus, aWM was associated with a 7-fold increase of risk of progression to symptomatic disease compared to IgM MGUS (95% CI 3-27, p=0.001). Symptoms defining progression included peripheral neuropathy in 4 patients, B-symptoms in 5, anemia (<10 g/dl) in 8 and organomegaly (splenomegaly and lymphadenopathy) in two. The overall survival for all patients is 91% at 5 years, similar for IgM MGUS and aWM. In patients with aWM, hemoglobin <12 g/dl (p=0.004) and BM clonal cells ≥50% (p=0.04) were associated with higher risk of progression but not IgM levels or abnormal FLCs ratio or the presence of immunoparesis. In multivariate analysis, hemoglobin <12 g/dl (p=0.016) and BM clonal cells ≥50% (p=0.051) were independently associated with shorter time to PD.

CONCLUSION: Patients with aWM should be followed without treatment unless symptoms develop. Bone marrow infiltration ≥50% and a hemoglobin <12 g/dL are the major risk factors for progression to symptomatic disease. Because 42% of patients with monoclonal IgM <1 g/dL had ≥10% lymphoplasmacytic cells, bone marrow biopsy is indicated in all patients.

P37. EVALUATION OF THE PREDICTIVE VALUE OF HEAVY/LIGHT CHAIN RATIO IN INTACT IMMUNOGLOBULIN MULTIPLE MYELOMA PATIENTS SURVIVAL

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OBJECTIVE: During 2009, Bradwell et al presented a new technique for intact Immunoglobulin Multiple Myeloma (IIMM) and Waldenstrom's Macroglobulinemia (WM) monitoring. They developed and validated a method for the separate quantification of the kappa and lambda bounded amounts of circulating IgG, IgA and IgM (Heavy/Light Chain-HLC assay). This was achieved by developing antisera with specificity for unique epitopes present at the junction between the heavy and light chains constant regions of each immunoglobulin molecule. This assay allows the quantification of the absolute value of the involved IgGκ, IgGλ, IgAκ, IgAλ, IgMκ and IgMλ along with their deriving ratios (IgGκ/IgGλ etc, Heavy/Light Chain ratio, HLC ratio). According to the literature, these measurements have been proven sensitive and specific for the monitoring of patients with IIMM. Additionally, the prognostic significance of HLC measurements for symptomatic IIMM patients (before treatment initiation) has been investigated. According to the results of two relatively recent studies (Bradwell 2013, Ludwig 2013), extreme low or high HLC ratios (<0.01 or >200) were associated with decreased overall survival of symptomatic IIMM patients. The aim of this study was the investigation for existence of any prognostic significance of HLC measurements for symptomatic IIMM patients (before treatment initiation) diagnosed and treated in our Hospital's Hematology and Lymphoma Department.

METHODS: Forty-one newly diagnosed symptomatic IIMM patients were studied. Twenty-five of them were men and 16 women. Their median age was 68 years (range: 43-83). The isotype of paraprotein was in 31 cases IgG and in 10 cases IgA. Twenty-four patients were ISS stage I, 13 stage II and four stage III. Patients median follow-up was 16 months (range: 6-24). HLC ratio was determined in all patients before treatment initiation. HLC measurements were performed by using the HevyliteTM assays (The Binding Site Group Ltd, UK) on a SPA PLUS turbidometer.

RESULTS: Statistical analysis was done by using the x2 test. At the time of last evaluation, 36 patients were alive. Five patients had died due to disease progression and their median survival was seven months (range: 2-14). Extreme HLC ratios (<0.01 or >200) emerged in 14 patients (7/31 IgG and 7/10 IgA, p <0.05). Two out of five deceased patients were IgG and three IgA. Also, four out of the five deceased patients had extreme HLC ratios (p <0.05). It is noted that all three IgA deceased patients emerged extreme HLC ratios (p <0.01). **Conclusion:** Despite the limited number of patients in our study, it is clear from the above-mentioned that there is a statistically significant correlation between IgA isotype of paraprotein and HLC ratio extreme values (<0.01 or >200). Also, there is a statistically significant correlation between mortality and HLC ratio extreme values, especially for IgA patients.

P38. PROGNOSTIC SIGNIFICANCE OF THE REVISED INTERNATIONAL STAGING SYSTEM IN TRANSPLANT-ELIGIBLE PATIENTS WITH MULTIPLE MYELOMA

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OBJECTIVE: In an attempt to define different prognostic groups, with a final goal to personalize optimal approach in treatment of patients with multiple myeloma (MM), Revised International Staging System (R-ISS) was established. The aim of the study was to analyze the prognostic significance of the R-ISS score in MM patients eligible for autologous stem cell transplantation (ASCT).
METHODS: A total of 112 newly diagnosed MM patients (median age 54 years, range 22-65years; 63 male/49 female), were analyzed in the study, with following distribution: IgG myeloma had 72 patients (64.2%), IgA 19 (17.0%), IgD 3 patients (2.7%), light chains 14 (12.5%), and non-secretory 4 (3.6%), and. According to the clinical stage (CS, DurieG-Salmon), advanced III CS was found in 87 patients (77.7%), II in 15 (13.4%), and symptomatic I CS in 10 (8.9%) patients. The ISS score 1 had 56 (50.0%) patients, 21 (18.7%) ISS 2, and 35 patients (31.3%) had ISS 3. Renal impairment existed in 14 patients (12.5%). According to the R-ISS score, the distribution was as follows: RSS I 58 patients (51.8%), II 39 (34.8%), and III 15 patients (13.4%). Patients were treated with induction therapy based on triple combinations with thalidomide and/or bortezomib, followed with high-doses of Melphalan (HDT, 200mg/m2), and supported with ASCT.
RESULTS: The overall treatment response (CR/VGPR/PR, IMWG criteria), analyzed on +100. day after HDT+ASCT, was achieved in 108 patients (96.4%). According to the R-ISS score, overall treatment response was achieved in all of 58 patients with R-ISS I; in 38/39 (97.4%) with R-ISS II; and in 32/35 (91.4%) with R-ISS III. The median follow up of analyzed group was 52 months (range 12-143 months). The R-ISS was highly statistically relevant regarding both EFS (Log Rank=13.729, p=0.001) and OS (Log Rank=10.486, p= 0.001). Cox regression analysis confirmed that R-ISS was the most important prognostic parameter that influenced OS. (95% CI, 1.056-7.120; p=0.038).
CONCLUSION: The R-ISS score is highly significant prognostic factor in transplant eligible myeloma patients. It represents currently most sensitive prognostic tool in multiple myeloma with consequent implications to personalized treatment approach.

P39. ABSENCE OF BONE DISEASE IS AN INDEPENDENT FAVORABLE PROGNOSTIC FACTOR OF OVERALL SURVIVAL IN SYMPTOMATIC MULTIPLE MYELOMA PATIENTS TREATED WITH NOVEL AGENTS

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OBJECTIVE: Bone disease is a common clinical manifestation in Multiple Myeloma (MM). However, a remarkable number of patients are diagnosed with symptomatic MM without evidence of bone disease. The prognostic role of absence of bone disease has not been investigated. We evaluated the clinical characteristics of patients presenting without bone lesions and we examined the possible prognostic significance of bone disease absence in symptomatic MM patients treated with novel agents.
METHODS: We studied the medical records of patients with symptomatic MM defined by conventional criteria (i.e. anemia and/or hypercalcemia/and or renal insufficiency and/or lytic bone lesions) diagnosed and treated upfront with novel agent combinations. We compared those with symptomatic disease excluding bone lesions (group A) with those who had documented bone disease by conventional X-ray imaging (group B); computer tomography was performed per protocol, according to clinical indications.
RESULTS: Three-hundred thirty-eight consecutive symptomatic MM patients were evaluated (M/F: 179/159, median age 67, range: 34-88, IgG: 184, IgA: 89, light-chain: 54, non-secretory: 11, ISS1: 101, ISS2: 105, ISS3: 132); 96/338 (28%) presented at diagnosis without bone disease. Patients characteristics such as median age, β2 microglobulin, lactate dehydrogenase (LDH), hemoglobin, platelets, albumin, creatinine, calcium, International staging system (ISS), revised-ISS (R-ISS), type of MM and high-risk cytogenetics were well balanced between groups. Patients with bone disease had more frequent worse performance status according to Eastern Cooperative Oncology Group (ECOG scale 3/4: 40% vs. 22%, respectively; p=0.003). All patients were treated with novel agents in 1st line, and 20% underwent autologous transplantation. Type of first or second line treatment did not differ between the 2 groups; no difference in response rates or progression-free survival (PFS) after induction therapy was observed; PFS2 was marginally longer in patients without bone disease (49 months vs. 36 months; p=0.058). After a median follow up of 71 months (95% CI: 61-80), the median OS for patients without bone disease was 65 months (95% CI: 46-84) vs. 45 (95% CI: 37-53) for those with documented bone disease (log rank p<0.001). In the univariate analysis, ISS, R-ISS, high risk cytogenetics, estimated glomerular filtration rate (eGFR) <40ml/min/1.73m2, LDH ≥300U/L and absence of bone lesions were predicted for OS (p<0.05 for all parameters); absence of bone lesions, and R-ISS were significant predictors for OS in the multivariate analysis (HzR: 0.64, p= 0.03, R-ISS1-3: HzR: 0.28, p<0.001,R-ISS2-3 HzR: 0.48, p=0.007, respectively).
CONCLUSION: Based on our analysis the absence of bone disease at diagnosis of symptomatic MM patients is one of the most powerful prognostic factors for OS, leading to a 36% reduction of death probability; of note, patients without bone disease exhibited marginally longer PFS2, suggesting that they may display a more sensitive first relapse. Further investigation is needed to confirm this finding, using modern imaging for the definition of bone disease in order to integrate this marker, in possible future prognostic models.

P40. PROGNOSTIC SIGNIFICANCE OF THE CHROMOSOME 1 ABNORMALITIES IN PATIENTS WITH MULTIPLE MYELOMA

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OBJECTIVE: For the past two decades, several chromosomal abnormalities (CA) of high-risk significance on the course of multiple myeloma (MM) were defined, aiming to establish different prognostic groups. The aim of this study was to analyze the prognostic significance of the chromosome 1 molecular abnormalities in MM patients.

METHODS: A total of 104 newly diagnosed MM transplant-ineligible (median age 68 years, range 65-80 years; 56 male/48 female), were analyzed in the study, with following distribution: IgG myeloma had 68 patients (65.4%), IgA 17 (16.3%), IgD 1 patients (1%), light chains 16 (15.4%), and non-secretory 2 (1.9%). According to the clinical stage (CS, Durie and Salmon), advanced III CS was found in 82 patients (78.8%), II in 14 (13.5%), and symptomatic I CS in 8 (7.7%) patients. The ISS score 1 had 30 (28.8%) patients, 40 (38.5%) ISS 2, and 34 patients (32.7%) had ISS 3. Renal impairment existed in 29 patients (27.9%). According to the Revised ISS core (R-ISS), the distribution was as follows: RSS I 43 patients (41.3%), II 37 (35.6%), and III 24 patients (23.1%). Applying interphase fluorescent in-situ hybridization (iFISH) with probe 1p32/1q21 (CDKN2C/CKS1B), chromosome 1 abnormalities were identified in 54 (59.3%) patients: del1p32 in 16 patients (29.6%); and +1q21 in 38 patients (70.4%). Thalidomide based combinations were applied in 86 patients (82.7%), while bortezomib based combinations were applied in 18 patients (17.3%) with high-risk features.

RESULTS: The overall treatment response (CR/VGPR/PR, IMWG criteria) with chr1 abnormalities, was achieved in 32 patients (51.9%): in 10/32 patients (31.3%) with del1p32; and 22/32 patients with +1q21 (68.7%). The median follow up of analyzed group was 22 months (range 6-100 months). Patients with chromosome 1 abnormalities had shorter PFS, still without statistical significance (Breslow 2.499; p=0.114), and statistically significant shortness of OS (Breslow 5.344; p=0.016). However, statistical analysis did not confirm the prognostic impact of +1q21 (PFS: Breslow 2.123, p=0.145; OS: Breslow 1.833, p=0.176), or del1p32 (PFS: Breslow 0.366, p=0.545; OS: Breslow 0.505, p=0.477). In addition, Cox regression analysis indicated R-ISS3 as the most important prognostic parameter that influenced OS (95% CI, 1.760-7.1429; p=0.006).

CONCLUSION: Molecular abnormalities of the chromosome 1 are of the negative prognostic significance in the MM patients. Still, it seems that R-ISS score 3 is of major impact on the course of disease with subsequent implications on the treatment approach.

P41. EARLY RELAPSE IS A POWERFUL INDEPENDENT NEGATIVE PREDICTOR FOR OVERALL SURVIVAL IN MULTIPLE MYELOMA PATIENTS TREATED WITH NOVEL AGENTS

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OBJECTIVE: Novel agents have improved outcomes of Multiple Myeloma (MM) patients yet some patients display early relapse (ER), defined as relapse occurred within 12 months from starting initial therapy. The aim of the study was to examine the clinical characteristics related to ER, the impact of ER on survival and possible factors predicting ER, in patients treated upfront with novel agents.
METHODS: We reviewed the medical files of patients with symptomatic MM treated with novel agents; eligible for analysis were considered patients who achieved at least partial response (PR) after initial therapy and lived for >12 months. Patients with ER were compared with those who either progressed or continued to response beyond 12 months.
RESULTS: Two hundred and fifty-six consecutive symptomatic MM patients were evaluated (M/F: 129/127, median age 66, range: 37-88, IgG: 138, IgA: 68, light-chain: 41, non-secretory: 9, ISS1: 81, ISS2: 79, ISS3: 96); ER occurred in 22/256 patients (8%). Median age at diagnosis did not differ between the 2 groups; ER patients had more frequently lower platelets, higher β2 microglobulin (β2M), higher bone marrow plasma cell (BMPC) infiltration and advanced ISS stage, compared to others ($p<0.05$); The number of patients with high risk cytogenetics did not differ between groups ($p>0.05$); 45% of patients received upfront IMiD-based therapies and 55% bortezomib-based therapies. There was no significant difference in treatments applied in each group either initially or at first relapse; 60/256 (23%) patients underwent autologous stem cell transplantation after induction; patients with ER displayed less frequent complete response (CR) compared to others (18% VS. 47%; $p=0.01$). The median time to progression for ER patients was 7 months (95% CI: 5-8.7 months) vs. 35 months (95% CI: 30-39 months) for the remaining patients ($p<0.001$); PFS2 was significantly shorter in patients with ER compared with the rest (49 months vs. 19 months; $p<0.001$). After a median follow up of 76 months (95% CI: 69-82), the median OS for patients with ER was 25 months (95% CI: 12-37) vs. 57 months (95% CI: 51-63) for those who continued to respond beyond 12 months ($p<0.001$); ER and high-risk cytogenetics were the most powerful negative predictors of OS in the multivariate cox regression analysis ($p<0.001$; HzR: 3.7 and $p=0.009$; HzR: 1.9). In the univariate logistic regression analysis, platelets <100.000/μL, hemoglobin <10g/dL, β2M>5.5mg/dL and bone marrow (BMPC) plasma cells >50%, were independent predictors for ER ($p<0.05$); platelets <100.000/μL and BMPC >50% were significant predictors for ER in the multivariate logistic regression analysis (OR: 4.9, $p= 0.01$ and 3.6, $p=0.03$, respectively).
CONCLUSION: Our data demonstrate that despite the use of novel agents, ER remains a clinical issue and correlates significantly with parameters related to high tumor burden. Platelets <100.000/μL and BMPC>50% were independent predictors for ER; ER led to significantly shorter PFS2 and a 4-fold increase of the probability of death, suggesting that patients displaying ER should be treated with an aggressive therapeutic approach in the early lines of myeloma therapy.

P42. EXTERNAL VALIDATION OF THE MULTIPLE MYELOMA RISK-STRATIFICATION ALGORITHM IN A REAL-WORLD GREEK DATA SET

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OBJECTIVE: Multiple myeloma (MM) is heterogeneous, with varying drivers of progression, prognosis and response. There is a need for tools to help physicians evaluate MM prognosis at first relapse. A novel risk-stratification algorithm (RSA) was developed using real-world data from the Czech Registry of Monoclonal Gammopathies (RMG – Development Cohort (DC)) for patients with relapsed MM initiating second-line (2L) therapy. The RSA used 16 predictors from routine clinical practice to estimate risk scores: age; ECOG performance status; extramedullary disease; new bone lesions; refractory status; severe toxicities at first line; time to next treatment; serum beta2-microglobulin and LDH at diagnosis and at relapse; bone marrow plasma cell count, thrombocyte count, calcium and albumin at relapse; cytogenetic abnormalities at diagnosis. Scores were used to stratify patients into four risk groups (1 (lowest risk) – 4 (highest risk)), based on overall survival (OS) expectations. The RSA also provides a frailty and aggressiveness score to define drivers of risk. This analysis aimed to assess validity of the RSA in a real-world data set in Greece.
METHODS: To assess RSA validity, data from a single center registry between June 2000 to October 2017 were analyzed. All patients with MM initiating 2L treatment during this period were included. Data for one of the RSA predictors (new bone lesions at 1st relapse) were not available; bone lesion data were randomly assigned by applying the incidence of bone lesions observed in the DC. A sensitivity analysis of five imputed data sets, pooled using Rubin's rules, and scenario analyses for the bone lesion data were performed.
RESULTS: Data from 232 patients were analyzed. Patient characteristics showed differences from the DC which resulted in different distribution of risk groups in the two data sets. The median OS from initiation of 2L was 62.3 months. Median OS was not reached in group 1 and median OS in groups 2–4 was 74.9, 33.7 and 10.8 months, respectively. There was a clear discrimination of OS between the four risk groups. The frailty and aggressiveness scores across the four groups showed similar distribution to the DC, with a trend towards lower frailty-driven risk and similar aggressiveness-driven risk. The c-index was 0.77 (95% CI: 0.72–0.82); a value in the range 0.7–0.8 had been predetermined to identify accurate discriminative power. The R2 value for the Greek data was 0.43.
CONCLUSION: The RSA was effective in quantifying risk and stratifying patients with relapsed MM, using long-term follow-up real-world data from a single center. Given the uncertainty in all analyses comparing risk groups, the HRs estimated by Rubin's rules gave results consistent with the base-case analysis. The RSA could be used as stratification criteria in clinical trials for patients with relapsed MM. It may also help identify patient severity and drivers of risk to tailor management strategies.

P43. SERUM NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN INDEPENDENTLY PREDICTS FOR RENAL RESPONSE IN MYELOMA PATIENTS WITH SEVERE RENAL IMPAIRMENT

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OBJECTIVE: Severe renal impairment (RI) is a common complication of multiple myeloma (MM). Neutrophil gelatinase-associated lipocalin (NGAL) is one of the earliest and most robust markers of acute kidney injury while serum Cystatin C (CysC) reflects renal function more accurately than creatinine and correlates with both tumor burden and renal function in MM. Our aim was to evaluate serum NGAL and CysC in MM patients with severe RI (eGFR<30 ml/min/1.73m2, by CKD-EPI formula), including those on dialysis, as biomarkers for prediction of renal responses.

METHODS: NGAL and CysC were measured in the same frozen serum sample collected before any therapy was given. Serum NGAL was measured using ELISA (BioPorto Diagnostics A/S, Gentofte, Denmark), while CysC was measured using a latex particle-enhanced nephelometric immunoassay (Dade Behring-Siemens Healthcare Diagnostics, Liederbach, Germany). IMWG renal response criteria were used (Dimopoulos et al, JCO 2016).

RESULTS: The analysis included 82 newly diagnosed MM patients with severe RI. Median creatinine was 5 mg/dl (range 2->10), median eGFR (ml/min/1.73 m2) was 11.3 (1.3-29.8) and dialysis was required in 32 (39%) patients. The median age was 71 years, median involved FLC was 5225 mg/L, hypercalcemia was found in 24%, LDH ≥ULN in 41% and high-risk cytogenetics in 27%, while 98% were ISS-3 and 56% were R-ISS-3. Treatment was bortezomib-based in all patients (in 23% VD and in 77% a triplet). Median NGAL levels were 191.5 ng/mL (range 20-550 ng/ml) and of CysC were 3.42 mg/L (1.1-7.8 mg/L) and were strongly correlated (R2=0.421, p<0.001); also both correlated with eGFR (for CysC: R2=0.43, p<0.001 and for NGAL: R2=0.225, p<0.001) and both were higher in patients requiring dialysis (median NGAL: 308 vs 153 ng/mL, p<0.001 and median CysC: 4.99 vs 2.73 mg/L, p=0.001). Renal response (Rrenal), was achieved by 60%, including 50% major Rrenal, including 34% that discontinued dialysis. Median time to Rrenal was one month and median time to dialysis independence was two months. Lower levels of NGAL (p=0.009) and CysC (p=0.014) were associated with higher probability of major Rrenal among patients with severe RI but not on dialysis, but baseline eGFR was not (p=0.346). None was associated with probability of dialysis independence among those requiring dialysis. Based on ROC analysis, in patients with severe RI but not on dialysis, NGAL <130 ng/ml was strongly associated with major Rrenal (86% vs 24% at 3 months, p<0.001). Regarding CysC, levels <2.6 mg/L were associated with higher probability and shorter time to major Rrenal (p=0.012). Both NGAL and CysC had no predictive value for patients under dialysis. In multivariate analysis performed in patients not on dialysis, that included age, NGAL, CysC and eGFR, only NGAL <130 ng/ml remained a significant factor associated with major Rrenal (HR 5, 95% CI 2-18, p=0.01).

CONCLUSION: Serum levels of NGAL were strong predictors of major renal response in MM patients with severe RI, but not on dialysis. Thus, serum NGAL could identify MM patients with severe RI who should be treated with more aggressive therapies, that could include high cut-off dialysis membranes and more effective and rapidly acting antimyeloma regimens.

P44. PROCOAGULANT PHOSPHOLIPID DEPENDENT CLOTTING TIME IS A SIGNIFICANT PREDICTOR OF POOR TREATMENT RESPONSE

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OBJECTIVE: Research has long focused on the implications of platelet, endothelial cell and blood coagulation activation in the risk of venous thromboembolism in patients with multiple myeloma (MM). Using data from the prospective, longitudinal observational study ROADMAP-MM CAT (PROspective Risk Assessment anD bioMArkers of hyPercoagulability for the identification of patients with Multiple Myeloma at risk for Cancer Associated Thrombosis) we aimed to identify biomarkers of cellular and plasma hypercoagulability that play a role in determining response to the anti-myeloma treatment.
METHODS: Newly diagnosed patients with MM were enrolled. Patients on anticoagulant treatment were excluded. A standardized clinical research form (CRF) was completed at enrollment (T0) and at 3 months post treatment initiation (T1). Procoagulant phospholipid-dependent clotting time (Procoag-PPL), tissue factor activity (TFa), thrombomodulin activity (TMa), factor VIIa, factor V (FV), antithrombin (AT), fibrin monomers (FM) and D-Dimers were measured with respective assays from Diagnostica Stago (Asnieres, France). Plasma levels of P-Selectin and heparanase were measured with ELISA Kits from Cusabio Biotech (CliniSciencies, France) and R&D Systems (Lille France), respectively. Samples of platelet-poor plasma (PPP) were assessed for thrombin generation (TG) with the TF 5pMPPP-Reagent on Calibrated Automated Thrombogram (Stago, France). The control group (CG) consisted of 30 healthy age and sex-matched individuals.
RESULTS: A total of 100 treatment naïve MM patients were enrolled. Median age was 66 (37-88) years (54% male patients). Disease stage was distributed as follows: 24.3% ISS-I, 20.4% ISS II and 55.3% ISS-III. Bortezomib-based therapy was given to 64.1% of the patients, thalidomide-based in 6.8% and lenalidomide-based in 24.3%. Median time to follow-up was 11.5 months. The mortality rate was 162/1000 person-years (median time of death since diagnosis 4.5 months, range 1-9 months). At T1, patients with stable disease (n=8) and progressive disease (n=14) were classified as non-responders. A total of 78 patients achieved at least a partial response and were classified as responders. Compared to controls at T0, MM patients showed significantly increased levels of TFa, D-Dimers and FM and significantly shorter Procoag-PPL clotting time. P-selectin levels were significantly decreased and heparanase levels significantly increased. FVIIa, FV and AT were not significantly different between patients and controls. MM patients showed significantly increased lag-time and ttPeak and significantly lower Peak, ETP and MRI as compared to the control group. TFa and D-dimer levels decreased significantly 3 months post treatment initiation (T1) whereas TMa and AT significantly decreased. At T1 thrombin generation was further attenuated (lower ETP and Peak, prolongation of ttPeak and lower MRI). Univariate analysis demonstrated that among the studied biomarkers only short PPL-ct was associated with a significantly increased risk of no response to treatment. The area under the curve (AUC) in a plot of PPL-ct against no response to treatment (receiver operator curve analysis) was 0.7 (p=0.01).
CONCLUSION: The prospective ROADMAP-CAT multiple myeloma study demonstrates for the first time that among a large number of hypercoagulability biomarkers assessed in newly diagnosed treatment-naïve MM patients, PPL-ct, that reflects the amount of procoagulant microparticles present in plasma, is a significant predictor of poor treatment response.

P45. EVALUATION OF PULMONARY FUNCTION TESTS IN PATIENTS WITH MULTIPLE MYELOMA REVEALS THAT PULMONARY ABNORMALITIES ARE COMMON AND ARE INDEPENDENTLY ASSOCIATED WITH WORSE OUTCOME

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OBJECTIVE: Pre-existing pulmonary disease may affect the treatment choices, toxicity and the survival of patients with multiple myeloma (MM). However, data on the prognostic value of Pulmonary Function Tests (PFTs) in myeloma patients' outcome, at the time of initial assessment of newly-diagnosed patients, are scarce. The aim of this study was to evaluate the incidence and prognostics importance of lung function abnormalities in patients with symptomatic MM.
METHODS: We prospectively performed PFTs in 121 consecutive newly-diagnosed MM patients, before initiation of treatment and we evaluated possible associations of baseline lung function with their outcomes.
RESULTS: Pulmonary function evaluation with PFTs revealed that 54 patients (44.63%) had either obstructive or restrictive pulmonary function defects, even among patients that did not report a history of lung disease. The survival was significantly worse in patients with obstructive pulmonary defect (median OS: 32.8 months) vs those with restrictive (median OS: 52.5 months) or normal lung function (median not reached, 3-years survival 76%) (p=0.013). In the univariate analysis specific indices of lung function that were associated with survival included Forced Vital Capacity (FVC)(lt)(p=0.012), FVC (%)(p=0.006), Forced Expiratory Volume in 1 sec (FEV1)(lt)(p=0.018), FEV1 (%)(p=0.013), Peak Expiratory Flow (PEF)(lt/min)(p=0.008), PEF (%)(p=0.005), carbon monoxide diffusion capacity corrected for hemoglobin (DLCO)(p=0.012), maximal expiratory (Pe)(kPa)(p=0.032) and Pe (%)(p=0.024) and inspiratory pressures (Pi)(kPa)(p=0.023) and Pi (%)(p=0.027). Other baseline factors associated with survival included ISS stage (p=0.008), hypercalcemia (p=0.064), and the presence of high risk cytogenetics (any of t (4; 14), t (14; 16) or del17p) (p=0.004). Abnormal PFTs were associated with early mortality (<1 year from initiation of therapy). Low PEF was strongly associated with early death (p<0.001); other indices included FVC (p=0.001), FEV1 (p=0.001) and DLCO (p=0.005). Abnormal breathing pattern was also associated with early death, especially obstructive pattern (HR: 8, 95%CI 2.1-30, p<0.001) and less restrictive (HR: 2.2, 95%CI 0.9-9.7, p=0.068), compared to normal pattern. We identified that PEF <65% of predicted (33 months vs not reached at 3 years, HR: 2.8, 95%CI 1.47-5.5, p=0.001) and a DLCO <65% (median OS of 33 months vs not reached, HR: 2.54, 95%CI 1.3-5.1, p=0.005) were associated with worse survival. There was a strong association of the two indices (p<0.001): 21% of patients had both, 19% only PEF <65%, 6% only DLCO <65% and 53% none of the two. Multivariate analysis indicated that R-ISS-3 and the presence of either or both PEF (%)<65% and DLCO<65% of predicted were the strongest prognostic factors for survival. Thus, we formulated a prognostic score encompassing myeloma-related and myeloma-independent factors that discriminates 3 groups with different survival: 3-year survival probability was 85% and 59% for patients with none or either of the risk factors and 18% (median survival of 7 months) if they had both (p<0.001). Importantly, the prognostic significance of this score was independent of the age of the patients.
CONCLUSION: PEF and DLCO could be useful in the initial assessment of newly-diagnosed MM patients as significant predictors of survival. Respiratory screening should be included in the routine initial evaluation of myeloma patients, despite the presence or absence of respiratory symptoms or abnormal clinical respiratory examination.

P46. DIFFUSE MEDULLARY HYPERDENSITIES OF THE FEMORA AND HUMERI ON WHOLE-BODY LOW-DOSE COMPUTED TOMOGRAPHY IDENTIFY DIFFUSE MRI PATTERN OF INVOLVEMENT AND CORRELATE WITH ADVANCED DISEASE STAGE IN PATIENTS WITH MULTIPLE MYELOMA

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OBJECTIVE: Whole-Body Low-Dose Computed Tomography (WBLDCT) is considered one of the most accurate methods for the detection of osteolytic lesions in multiple myeloma (MM). On WBLDCT, hyperdense tumor deposits in the medullary cavities of the femora and humeri are often observed; these may represent the only evidence of myeloma in patients without osteolyses. These abnormal hyperdensities may be nodular or diffuse and their density is quantitated by Hounsfield Units (HU). The aim of this study was to evaluate the incidence, the type and the value of hyperdensities in newly-diagnosed MM (NDMM) patients with a diffuse MRI pattern who also underwent a WBLDCT at the time of diagnosis.
METHODS: Seventy-four newly-diagnosed MM patients were evaluated. WBLDCT and MRI of the spine and pelvis were performed at diagnosis, before the administration of any kind of therapy. Five types of hyperdensities could be identified in the WBLDCT: 1) Fatty (F) - only negative HUs were recorded throughout the medullary cavity, consistent with the presence of normal fatty (yellow) marrow; 2) Fatty marrow intermingled with indistinct hyperdensities (FIH); 3) Nodular hyperdensities (N); 4) Diffuse hyperdensities (D) – characterized by the presence of a very dense, homogeneous appearance of part or the whole of the medullary cavity, presumably representing diffuse tumoral involvement.
RESULTS: The median age of patients was 70.5 years; per ISS, 37%, 35% and 28% had ISS-1, -2 and -3 disease, respectively. Out of the 29 patients with a diffuse MRI pattern 11 (38%) had no osteolyses on WBLDCT. 28/29 patients (97%) had medullary hyperdensities in the femora and/or humeri: 22 patients (76%) had diffuse hyperdensities (D) in at least one of the four bones (average density 68.7 HU) and 6 patients (21%) had only nodular hyperdensities (average density 76.5 HU). Interestingly, all patients with ISS-3 or R-ISS-3 and a diffuse MRI pattern had diffuse hyperdensities (D) on WBLDCT. Out of the 15 patients with a focal MRI pattern, only one patient (6.7%) displayed a diffuse hyperdensity (D) in at least one long bone; 7/15 patients (46.7%) had nodular hyperdensity lesions (N) in at least one long bone. Finally, out of the 30 patients with normal MRI pattern, there was only one patient (3.3%) with diffuse hyperdensities (D). In most of these patients either a fatty (F) or a mixed fatty with indistinct hyperdensities (FIH) pattern was identified on WBLDCT. The presence of diffuse hyperdensities (D) in the medullary cavity of at least one bone had 96.5% sensitivity and 95.5% specificity for the diagnosis of a diffuse MRI pattern. In 10/29 patients (34.4%) medullary hyperdensities were the only sign of bone involvement, since no osteolyses were detected (1/29 patient had neither osteolyses nor hyperdensities).
CONCLUSION: In about one third of MM patients with a diffuse MRI pattern of involvement, the presence of hyperdensities in the medullary cavities of the femora and humeri may be the only abnormality detected on WBLDCT. Diffuse hyperdensities are the most commonly encountered type in this subset of patients and they seem to be associated with advanced myeloma at initial diagnosis (ISS-3 or R-ISS-3).

P47. UNUSUAL LOCATIONS OF EXTRAMEDULLARY RELAPSE IN MULTIPLE MYELOMA DIAGNOSED BY MULTIPARAMETRIC FLOW CYTOMETRY

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OBJECTIVE: Extramedullary relapse constitutes an uncommon manifestation of multiple myeloma, but central nervous system-involvement as the only manifestation of relapse, as well as abdominal plasmacytomas and ascites seem even less common. Multiparametric flow cytometry has recently been shown to be an essential tool for diagnosis and minimal residual disease detection in multiple myeloma patients. However, data in the literature regarding cerebrospinal and ascitic fluid examination by flow cytometry in myeloma patients are limited, since most of the cases were diagnosed by cytologic assays. **Methods:** Four patients with plasma cell neoplasia and extramedullary relapse are presented. Three patients with multiple myeloma and one patient with de novo plasma cell leukemia achieved complete remission after autologous stem cell transplantation. Few months later, while being in systemic remission, two of the patients with multiple myeloma as well as the patient with plasma cell leukemia presented with neurological manifestations. The third myeloma patient presented with abdominal plasmacytomas and ascites. Cerebrospinal and ascitic fluid samples were studied by 5 and 6-color combinations of monoclonal antibodies
RESULTS: Plasma cells were identified as CD38(+)/CD138(+) events and were further characterized by immunophenotypic aberrations and light chain restriction. Regarding CD56 expression, plasma cells infiltrating cerebrospinal fluid samples were CD56 positive, while those in ascitic fluid, CD56 negative. Plasma cells were CD19 negative in all samples.
CONCLUSION: Flow cytometry is a useful tool providing rapid diagnosis of extramedullary multiple myeloma relapse. The role of CD56 (neural adhesion molecule) in favoring extramedullary localization of multiple myeloma is controversial. Downregulation of CD56 was considered essential in the pathogenesis of malignant plasma cells escape from bone marrow microenvironment. However, more recent reports are not in agreement with such hypothesis, as CD56 was positive on myeloma plasma cells in cases of central nervous system involvement.

P48. BENEFITS AND OBSTACLES OF FISH IN MULTIPLE MYELOMA

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OBJECTIVE: For the past two decades, the most widely used technique for the assessment of molecular genetics in multiple myeloma (MM) was interphase fluorescent in-situ hybridization (iFISH) analysis. However, today we are faced with disadvantages of this technique, mainly due to the time consuming technical procedure, and incompleteness of acquired impression of individual patient's genetic profile due to the limitations of the applied panel of recommended iFISH probes. The aim of the study was to analyse the applicability, precision and accuracy of routine iFISH analysis in MM patients. **Methods:** In this single-center study, during five-year period, December 2012 to December 2017, the iFISH analysis was performed in 234 newly diagnosed MM on the isolated plasma cells from the bone marrow aspirates. The panel of the following iFISH probes was applied: 17p13.1 (TP53), 13q14 (D13S25), p32/1q21 (CDKN2C/CKS1B), 14q32 (IGH breakapart), t (4; 14) (IGH/FGFR3) and t (14; 16) IGH/MAF) (Abbott/Vysis) in accordance to the standard protocol procedure. **Results:** The successful iFISH results were obtained in 222 patients (95%), while it was failed in 12 patients due to insufficient number of plasma cells. The aberrant iFISH signals were recorded in 135 of patients (61%). Rearrangements of the analysed regions were further present in a descending order: 13q14 (60%), 1q21 (32%), 17p13 (16%), t (4; 14) (11%), del1p (10%), IGH break apart (excluding t (4; 14) and t (14; 16)) (9%), and t (14; 16) (2%). When analysing the type of rearrangement for each of the FISH probes, we got the expected aberrant pattern in majority of the patients, eg.: del (13q14), del (17p13), t (4; 14) etc. However, less prevalent types of aberrations, such as accompanied trizomies of the D13S25, TP53, FGFR3, IGH and MAF genes, were recorded as well (7%) indicating hyperdiploidy. On the other hand, the monosomy of MAF gene was present in 4% of the patients, while the rearrangement of the IGH gene (without t (4; 14) and t (14; 16)) was seen in 9% of them, implicationg possibility of hypodiploid karyotype. Furthermore, two groups of patients with 1q rearrangements were recorded: those with a simple gain of 1q (trizomic signals), and those with a real 1q amplification (more than 5 signals) indicating hyperdiploid pattern.
CONCLUSION: Because of the complexity of cytogenetic abnormalities in MM, iFISH provide initial, rather robust, impression of individual prognostic profile of MM patients. However, the other more advanced methods as next generation sequencing, RNA sequencing, or whole-genome sequencing may offer additional answers for genetic doubts unsolved by iFISH method.

P49. REAL-WORLD DATA ON THE TREATMENT OF RELAPSED/REFRACTORY MYELOMA WITH LENALIDOMIDE AND DEXAMETHASONE IN 2ND LINE: THE PROGNOSTIC SIGNIFICANCE OF BIOCHEMICAL VS. CLINICAL RELAPSE. THE "LEGEND" STUDY FROM THE GREEK MYELOMA STUDY GROUP

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OBJECTIVE: The combination of lenalidomide/dexamethasone (LenDex) is an established treatment for relapsed/refractory. Multiple Myeloma (MM) patients; however, apart from clinical trials, there is limited data for the efficacy of this combination as 2nd line treatment. Furthermore, the efficacy of LenDex when administered before evident clinical manifestations, namely in the case of biochemical relapse as compared to clinical relapse, has not yet been assessed.In the current study, we evaluated response rates and progression-free survival (PFS) in patients treated with LenDex in 2nd line and we compared survival parameters for patients treated with LenDex at biochemical relapse vs. those treated at clinical relapse.

METHODS: Medical files of 207 patients with MM diagnosed between 2000-2013 in 18 Greek centers and treated with LenDex as 2nd line treatment from January 1st 2009, up to March 1st 2014, were retrospectively studied. Overall response and PFS were evaluated for all patients. Additionally, PFS was compared in patients treated at either biochemical relapse (group A) or at clinical relapse (group B). The prognostic significance of biochemical relapse adjusted with important patients' characteristics was also evaluated. Classical methods were used for statistical analysis.

RESULTS: Two hundred and seven patient files were recorded and analyzed (M/F: 112/95, median age: 67.2y, range 31-91y, IgG: 115, IgA: 55, Light chain: 22, non-sectretory: 2, IgD: 5, IgM: 1, unknown: 7, ISS I: 54, ISS II: 74, ISS III: 77, high risk: 13%, standard risk: 87%). First line treatment included bortezomib-based regimens (63.3%), immunomodulatory drug-based combinations (34.8%) and chemotherapy (40.1%); 25% of patients underwent autologous stem cell transplantation; 2nd line treatment with LenDex was administered at biochemical relapse in 67.5% (95% CI: 61.1%>73.9%) of patients and at clinical relapse in 32.5% (95% CI: 26.1-38.9) of patients. The overall response rate (ORR) was 73.4%; 23.7% of patients achieved very good partial response (VGPR) and 17.8% complete response (CR). The number of patients that achieved at least VGPR did not differ between the 2 groups ($p>0.05$). The median time to best response was 6.7 months (range 0.6- 51.9). After a median follow-up of 52.8 months, 112 (54.1%) patients are alive and 95 (45.9%) patients are deceased; 131 patients (63.3%) have relapsed (biochemical relapse: 66.4%, clinical relapse: 33.6%). Median PFS and PFS rate at 12 months was 19.2 months (95% CI: 15.6-25.2) and 67.6% respectively. The median PFS was 24 months (95% CI: 18.0-34.8) for patients in group A vs. 13.2 months (95% CI: 8.4-19.2) for patients in group B (HR: 0.63, $p=0.006$). When adjusted for important prognostic patients' characteristics (ISS, age, β2 microglobulin, and LDH), biochemical relapse maintained its prognostic significance for PFS ($p<0.05$).
CONCLUSION: Our data confirm that LenDex combination as 2nd line treatment leads to high overall response rates and prolonged PFS. Additionally, we have shown for the first time in routine clinical practice that MM patients who receive 2nd line therapy with LenDex at biochemical relapse have a significantly longer median PFS compared to patients treated at clinical relapse, underlining the importance of potentially starting treatment before evident clinical manifestations at the first relapse.

P50. CHARACTERISTICS AND MANAGEMENT OF OCTOGENARIAN MYELOMA PATIENTS: A SINGLE-CENTER ANALYSIS IN 110 PATIENTS

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OBJECTIVE: In the era of an aging population an increasing number of patients are diagnosed with multiple myeloma (MM) at an age of ≥80 years. The aim of our study was to analyze the disease characteristics, frailty score and treatment toxicity profile in this group of patients.
METHODS: All newly diagnosed symptomatic MM patients ≥80 years of age who received at least one dose of treatment in our center were included in the study.
RESULTS: Among 827 consecutive, newly diagnosed, symptomatic MM patients who were treated in our department after 01/01/2000, 110 were ≥80 years old (13.3%); their median age at diagnosis was 83 years (range 80-92 years) and 55% were male. Performance status (PS) was 1-2 in 40% and 3-4 in 60% of the patients respectively. Anemia (Hb <10 g/dl) was present in 64.5% of patients, increased serum LDH (>250 IU/L) in 12%, hypercalcemia (≥11 mg/dL) in 19% and osteolytic bone lesions in 73% of patients. Serum creatinine was ≥2 mg/dl in 30% and eGFR (CKD-EPI) was <60 ml/min/1.73m2 in 69% and in 36% of patients was <30 ml/min/1.73m2. Per ISS, 56% had stage-3, 35% had -2 and 9% had disease stage-1, respectively. First line treatment was bortezomib-based in 30% of patients, IMiD-based in 62%, while 8% received conventional chemotherapy. Overall response to first line treatment (≥PR) was 62%. At the time of diagnosis 41% of patients required hospitalization due to disease related complications, while 60% were hospitalized at least once during their disease course. Low eGFR was associated with need for hospitalization at the time of diagnosis (p<0.001). Treatment emerging toxicities were common; 63% of our patients experienced at least one grade ≥3 event and 14% had grade 5 events. Grade ≥3 hematological toxicity was reported in 24% and grade ≥2 neuropathy in 23% of patients; 28% of patients experienced at least one grade ≥3 infectious episode during their disease course. According to IMWG geriatric assessment, due to the age of the patients (≥80), all of them were categorized as frail. Median survival was 21 months and early mortality (at 2 months from treatment initiation) was 20%. Several factors were associated with inferior survival in univariate analysis, including female gender (p=0.042), ISS stage-3 (p=0.015), LDH ≥250 IU/L (p=0.001) and eGFR <30 ml/min/1.73m2 (p<0.001) at the time of diagnosis. Factors such as the need for hospitalization at the time of diagnosis (p<0.001) and the presence of grade 3 or 4 adverse events during the disease course (p=0.003) had a negative impact on survival. In the multivariate analysis, eGFR <30 ml/min/1.73m2 (p=0.003) and LDH ≥250 IU/L (p=0.048) were independently associated with poor OS.
CONCLUSION: Octogenarian MM patient population is a distinct frail subset of MM patients and its management should be individualized considering both disease characteristics and functional status. Renal impairment at diagnosis and high LDH correlated independently with OS in this group of patients. This vulnerable patient population needs detailed assessment, individualized treatment and intensive supportive care to avoid hospitalization due to complications in order to reduce early mortality.

P51. ASSESSMENT OF THE QUALITY OF REPORTING OF RANDOMIZED CLINICAL TRIALS IN REFRACTORY RELAPSED MULTIPLE MYELOMA BASED ON THE CONSORT 2010 STATEMENT

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OBJECTIVE: Refractory/relapsed multiple myeloma (RRMM) is currently under extensive clinical research with several new classes therapeutic agents being under investigation in Randomized Clinical Trials (RCTs). Nevertheless, it is possible a clinical trial may give unreliable results either due to poor design, improper conduct and misrepresentation of the actual results leading to false conclusions. Reporting guidelines to investigators and authors are issued to ensure transparency and accuracy of clinical trial reporting. Our aim was to assess the reporting quality of RCTs published in the last 30 years using the CONSORT 2010 Statement.

METHODS: We searched for RRMM phase II and III RCT publications in PUBMED and EMBASE within January 1st 1987 and December 31st 2017. The overall reporting quality of the RCTs assessed was determined by using the overall quality score (OQS) which consisted of 29 items based on the 2010 CONSORT statement. Each item received a score or "1" if it was clearly reported and "0" if it was not stated or clearly stated. Publication characteristics were analyzed using descriptive statistics. For the identification of factors that are associated with a high reporting quality, we modelled the OQS using univariate regression and then included every covariate associated with $\alpha < 0.10$ in multivariate linear regression. It was hypothesized that publications within the same journal will have correlated OQSs, therefore generalized estimated equations were used.

RESULTS: Forty five RCTs were assessed for their reporting quality. Overall, 72% of the CONSORT items were adequately reported. Mean OQS of RCTs published after 2010 is 22.8 compared to a mean OQS of 17.4 in RCTs published before 2010. This denotes a 30% improvement the reporting quality in RCTs published after 2010 in relation to the RCTs published before 2010. The COSSORT 2010 items that displayed the largest improvements were 'Trial design' [Δ (%) = 66%], followed by 'Registration of the trial in a registry' [Δ (%) = 64%], 'Participant flow exclusions' [Δ (%) = 43%] and 'Outcomes and estimation' [Δ (%) = 40%]. Items describing randomization, blinding and statistical methods had modest improvement in reporting after 2010 with Δ (%) being around 20-25%. Regarding the four randomization items, overall scoring was poor with only the randomization restriction item being adequately reported in 73% of the RCTs, while none of the trials analyzed reported any implementation of randomization. Only five RCT publications included a protocol. In univariate analysis, the following trial characteristics were associated with a higher OQS score ($p<0.05$): publication after 2010, RCTs conducted at an international level, RCTs fully funded by industry, publication in high IF (>10) journal, phase III RCTs, trial outcome being positive and large sample size (>200) being randomized. Out of the above characteristics, publication year, journal IF, trial phase and sample size were independent predictors of the OQS.

CONCLUSION: Our findings show an improvement in the overall quality of reporting RCTs in RRMM based on the CONSORT 2010 Statement. Technical methodological aspects of the RCTs such as randomization and protocol accession still require substantial improvement.

P52. RECENT DATA ON HEALTH RELATED QALITY OF LIFE OF MULTIPLE MYELOMA PATIENTS

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OBJECTIVE: Multiple Myeloma (MM) represents about 1% of all malignancies and about 10% of hematological malignancies. During the last decade, treatment options for MM have expanded significantly, resulting in a significant prolongation in the median survival of these patients (from about four to seven years). Given the survival benefit, improving the quality of life of MM patients remains an important issue. The aim of this study is the evaluation of Health related Quality of Life (HRQOL) of MM patients, the comparison with normative data, the comparative evaluation of the questionnaires used and the investigation of factors which have an impact on these patients HRQOL. **Methods:** The sample consisted of 60 MM patients diagnosed, treated and under monitoring in Hematology and Lymphoma Department of "Evaggelismos" General Hospital (Athens, Greece). HRQOL was assessed by using the EQ-5D, QLQ-C30 and QLQ-MY20 questionnaires. Also, a comparative evaluation of the questionnaires used was performed. In addition, linear regression analyses were performed. The scale scores of the previously referred questionnaires were used as independent variables. Patients characteristics (demographic and other general data, clinical and laboratory features disease related, treatment response and toxicities) were used as dependent variables.

RESULTS: The EQ-5D scale scores of the MM patients included in this study were lower compared with the Greek normative data. Also, a statistically significant correlation between most of the QLQ-C30/QLQ-MY20 scale scores and the EQ-5D scores was found. The following characteristics of the evaluated patients were in a statistically significant correlation with EQ-5D, QLQ-C30 and QLQ-MY20 scale scores, affecting their HRQOL: Female gender, Older age, Marital status, High Body Mass Index, Antidepressant treatment, Extended Salmon-Durie stage, Non-Secretory MM variant, Prolonged time since disease diagnosis, Hospitalization, Anemia, Bone disease, Extramedullary disease, Relapse, Peripheral Neuropathy.

CONCLUSION: Our MM patients had inferior HRQOL compared with general population. Many of their characteristics (demographic and other general data, clinical and laboratory features disease related, treatment response and toxicities) were in a statistically significant correlation with HRQOL.

P53. A BIOBANK FOR PLASMA CELL DYSCRASIAS: PRESENT AND FUTURE

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OBJECTIVE: Biobanks are facilities that collect, process, store and distribute biospecimens and associated data, mainly for biological and medical research. They constitute a crucial resource, supporting cutting-edge investigation in fields such as oncology, genomics and personalised medicine, and the development of diagnostics and therapeutics. Our department is faced with the challenge of storing complex specimens such as plasma cells and B lymphocytes from bone marrow aspirates. Sample organisation throughout the biobank work flow can greatly contribute to the maintenance of operational efficiency and sample traceability.
METHODS: At the moment our biobank still relies on manual loading and retrieval methods. Work flow starts with sample collection and processing which are both crucial when setting up and expanding a biobank. When collecting and processing our samples, we rigorously adhere to Good Clinical Practice (GCP) and Good Laboratory Practice (GLP) in order to avert problems that could jeopardise valuable specimens and compromise years of research.
RESULTS: Our biobank presently consists of peripheral blood (plasma and PBMC's) and bone marrow aspirate (CD138+, CD138-, CD19 and plasma) samples from consenting patients with various types of plasma cell dyscrasias including patients with myeloma, symptomatic or smoldering, MGUS, Waldenström's macroglobulinemia and AL amyloidosis. Whenever possible, samples are taken at various time points in order to obtain sequential samples in the biobank. Future prospects: We are now looking into facilitating the intended retrieval rates and storage capacity through automated tracking technology. In this case the tubes will be labelled with permanent traceable features that enable scanning and tracking through data management software (for example barcode systems). We will also integrate software systems to store all clinical and biological information associated with our samples with the use of Laboratory Information Management Systems (LIMS). The LIMS can be fully integrated with all instruments in the lab so that work flow is improved and more efficient, and all test data will be electronically and securely compiled and stored with each sample. In the future a centralised LIMS will enable us to scale up as demand increases because it can manage all biospecimen locations, online request management, data compliance and security.
CONCLUSION: Biobanks are becoming an essential and increasingly sophisticated resource in biomedical research. Technological advances such as automation and computerisation are transforming the management of biobanks and enabling the implementation of integrated systems to manage samples, data, personnel, policies and procedures for the distribution of biological specimens and other services. The trend is towards larger and more centralised biobanks, which improves the economics of sample processing, storage, distribution and data analysis. The development of evidence-based standard operation procedures (SOPs) and the adoption of technical best practices, in combination with the use of technological innovations in materials and equipment, can support the generation of biobanks holding high quality samples associated with well-characterised, reliable clinical data. The work flow from sample collection to storage in a biobank should accommodate the possibility that the sample will likely be used downstream in an assay that is currently not even imagined.

P54. BACTEREMIA DUE TO ELIZABETHKINGIA MENINGOSEPTICA IN RELAPSED REFRACTORY MULTIPLE MYELOMA PATIENT

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BACKGROUND: Elizabethkingia meningoseptica (E. Meningoseptica) is a nonfermentative gram-negative bacillus that is ubiquitously found in hospital environments and as such, it has been associated with various nosocomial infections. Because of resistance to gram negative anitibotics, treatment is difficult and bakteremia may be mortal. We report a case of bacteremia due to E. Meningoseptica in a patient who necessitated chronic hemodialysis therapy with relapsed refractory multiple myeloma (MM).

CASE REPORT: We report a case of 68-year-old female with recurrent refractory MM and a history of end-stage renal disease followed for 3 years. Autologous stem cell transplantation (ASCT) was performed in response to partial remission after 3 cycles of velcade-cyclophosphamide-decort (VCD) chemotherapy. Second ASCT was performed who relapsed after the first year of transplantation. Velcade-revlimid-decort (VRD) was initiated who developed relapse 1.5 years after the second transplant. Because acute renal insufficiency the patient was hemodialysis dependent. We started empirical tazobaktam because of fever and dyspnea. Posteroanterior chest X-ray showed pleural effusion on the right. Blood sputum and catheter cultures taken during fever; E. Meningoseptica grew. Ciprofloxacin was added to piperacillin tazobactam treatment. Control ECO was done. Nodular lesion on the aortic valve was detected. Transesophageal echocardiography and sampling pleural effusion couldn`t be performed because hemostasis parameters are not appropriate. Despite supportive treatments and antibiotherapy, the patient`s clinic deteriorated and no response to fever was obtained. E. Meningoseptica grew in recurrent blood and catheter cultures. Catheter revision was done. Increased pleural effusion, bleeding diathesis and renal insufficiency after worsening respiratory failure than the patient passed away.

DISCUSSION: Bacteremia due to E. Meningoseptica primarily occurs in immunocompromised individuals, including those with various nephropathy which necessitated hemodialysis therapy. E. Meningosenptica cause pneumonia and multidrug resistance endocarditis infection. Recognition of E. Meningoseptica is of critical importance for clinicians since conventional empirical treatment against gram-negative bacteria may result in unfavorable outcome given its unique antimicrobial susceptible pattern.

P55. BLADDER INVASION IN MULTIPLE MYELOMA

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BACKGROUND: Extramedullar plasmocytomas may involve any organ, either as a solitary lesion or as part of a systemic disease. Bladder plasmocytomas are very rare but bladder invasion with malign plasma cells without formation of a plasmocytoma is quite exceptional. Here we present a case of bladder invasion with malign plasma cells at a patient with multiple myeloma who had no sign of disease at a recent bone marrow biopsy examination.
CASE REPORT: A 50-year-old woman with a history of relapsed refractory multiple myeloma (Ig G-lambda) applied to the hospital with complaints of dyspnea and being unable to urinate. She had history of 4 cycles of chemotherapy (2 cycles of vincristin, adriamycin, dexamethasone-2 cycles of bortezomib and dexamethasone), autologous stem cell transplantation, and disease relapse with pleural effusion. After the relapse, she was given 3 cycles of bortezomib, adriamycin and dexamethasone and her bone marrow biopsy was normal after the last chemotherapy courses. At laboratory evaluation, her creatinine level was high (9.66 mg/dl). She was hospitalized and hemodialysis was performed immediately. On abdominal ultrasound, there was bilateral pelvicalyceal dilatation and both ureters couldn't be visualized. There was no image of any mass inside the bladder except balloon of a Foley catheter. A PET-CT was performed in order to see the extension of the disease and multiple sites of involvement were observed. The patient was consulted with urology department as she couldn't urinate and they decided to perform transurethral resection fo the bladder to find out any disease involvement. The biopsy specimen revealed bladder involvement of multiple myeloma.
DISCUSSION: Although bladder involvement of multiple myeloma is very rare, it should be kept in mind for patients with urination difficulties and postrenal acute renal failure findings. Such an involvement can be present even at patients who are proven to be in remission by bone marrow biopsies and even when there's no sign of bladder plasmocytoma at radiological imaging.

P56. MULTIPLE MYELOMA OF THE CENTRAL NERVOUS SYSTEM

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BACKGROUND: Multiple myelomas (MM) is a malignancy characterized by a monoclonal increase of plasma cells in bone marrow, the presence of paraproteins in plasma and urine. Although neurological symptoms are common due to hyperviscosity, hypercalcemia, medullar compression and paraprotein-related neuropathy, central nervous system (CNS) involvement is a rare complication and occurs in less than 1% of patients with MM. The prognosis of CNS myeloma is poor, with an average overall survival of 3–6 months. Here we present a refractory case of multiple myeloma with central nervous system involvement.
CASE REPORT: A 42-year-old male patient was diagnosed with IgG kappa multiple myeloma. The cytogenetic analysis did not show any high-risk abnormality. He remained in complete remission for 17 months after autologous bone marrow transplantation and received four cycles of bortezomib, cyclophosphamide, and dexamethasone for progressive disease. During follow-up, lenalidomide dexamethasone treatment was started for recurrent plasmacytomas. On the 10th month of treatment, the patient was evaluated for a headache, double vision and grade 2 papilledema was detected. Cranial magnetic resonance imaging (MRI) examination revealed multiple lytic bone lesions of the skull, a hypointense lesion on right occipital gyrus, a hypointense lesion on left frontal gyrus, and 16x13 mm sized cystic lesion with similar signal intensity with cerebrospinal fluid (CSF) in the anterior medial segment of the right frontal lobe. A heterogeneous hyperintense lesion was observed in the D4 vertebra corpus on the whole spinal MRI examination. Opening pressure was 210 cm H 2 O in the lumbar puncture, and plasmacytoid cells were observed in CSF. VTD-PACE chemotherapy was initiated with anti-edema treatment for the CNS involvement of MM. On the 13th day of chemotherapy, he was admitted to the intensive care unit for unconsciousness and low Glasgow Coma Scoring and eventually the patient died on the 23rd day of treatment.
DISCUSSION: Multiple Myeloma is a plasma cell malignancy involving multiple organ systems with several clinical pictures. Extramedullary involvement is more common during the terminal aggressive phase in the clinical course of the disease. Although neurological symptoms due to metabolic changes are common, the involvement of the central nervous system is a rare event and should be considered in patients with neurological symptoms. Risk factors for CNS involvement include high tumor burden, plasmablastic morphology, cytogenetic abnormalities, the absence of the CD 56 surface antigen, circulating plasma cell presence in the peripheral blood, plasma cell leukemia and high LDH levels. The optimal treatment for CNS infiltration is not well defined in the literature. Systemic chemotherapy, in conjunction with intrathecal chemotherapy and/or radiotherapy, has been the mainstay of treatment. Systemic chemotherapy with or without radiation therapy has better survival rates over patients who are treated without systemic chemotherapy. Recently, the efficacy of pomalidomide has been shown in CNS MM. Radiotherapy may be an effective treatment option for localized lesions at low doses. Since intrathecal therapy is mostly combined with systemic therapies, there is not enough data on the efficacy of intrathecal therapy only.

P57. CLINICAL CHARACTERISTICS OF NEWLY DIAGNOSED PATIENTS WITH AL AMYLOIDOSIS WITH "NONMEASURABLE" FREE LIGHT CHAINS: PROGNOSTIC IMPLICATIONS AND RESPONSE EVALUATION

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OBJECTIVE: Current criteria for the assessment of hematologic response in patients with AL amyloidosis are based on the measurement of the difference between the involved and uninvolved serum FLCs (dFLC). These criteria have been validated but require that dFLC should be ≥50 mg/L at baseline in order to assess hematologic responses. However, patients with dFLCs <50 mg/L are excluded from clinical trials as having "non-measurable" disease although the natural history of their disease may not differ from that of patients with "measurable dFLCs". Our aim was to evaluate whether patients with light chain (AL) amyloidosis and "non-measurable" dFLCs (i.e with dFLC <50 mg/dL) have different clinical characteristics or outcome than patients with measurable dFLCs.
METHODS: The analysis included 244 patients with AL amyloidosis treated in a single center. Organ involvement was defined according to the 2005 ISA criteria. Hematologic complete response (hem-CR) was defined as a negative serum and urine immunofixation and a normal FLC ratio. Serum FLC concentration was measured using the FREELITE assay (The Binding Site, Birmingham, UK).
RESULTS: Forty-nine (20%) patients had dFLC <50 mg/L at the time of diagnosis and before initiation of any therapy. These patients had more often renal involvement (84% vs 64%, p=0.031), but had less often cardiac involvement (47% vs 72%, p=0.004), peripheral nerve (8% vs 25%, p=0.021) or soft tissue involvement (8% vs 23%, p=0.032). However, median eGFR was lower in patients with dFLC <50 mg/L (48 vs 77 ml/min/1.73 m2, p=0.005) and median BM infiltration was 10% vs 15% (p<0.001) while distribution per Mayo stage was 29%, 50% & 21% vs 17%, 49% & 34% for stage-1, -2, & -3 respectively (p=0.09). There was no difference in the treatments that were given (bortezomib based in 67% vs 59% respectively) or ASCT (8% vs 6%) (all p>0.5). The median OS was significantly longer for those with dFLC <50 mg/L (1-year OS: 82% vs 61%, 3-year OS: 82% vs 45%, 5-year OS: 70% vs 35%, p=0.001). In multivariate analysis for OS, dFLC <50 mg/L was associated with a 75% reduction in the risk of death (HR: 0.25, 95% CI 0.12-0.56, p=0.001) independently of cardiac involvement and Mayo stage. Regarding renal survival, we found no significant difference in rates of progression to dialysis for the two groups, with and without dFLC <50 mg/L. Among patients with dFLC <50 mgL but with dFLC ≥20 mg/L, the reduction of dFLC at 3 months landmark to <10 mg/L was associated with better OS (3 years OS 100% vs 80%).
CONCLUSION: About one fifth of newly diagnosed patients with AL amyloidosis have low level dFLC ("non-measurable" by current criteria) and this is associated with significantly better outcome, independently of other standard prognostic factors. This parameter should be included in the standard staging and risk assessment of patients with AL amyloidosis. Furthermore, patients with dFC <50 mg/L should be included in clinical trials as they benefit from reduction of their FLCs to <10 mg/L, and this criterion should be accounted for in the hematologic response criteria.

P58. EVALUATION OF MINIMAL RESIDUAL DISEASE USING NEXT GENERATION FLOW CYTOMETRY IN PATIENTS WITH LIGHT CHAIN (AL) AMYLOIDOSIS

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OBJECTIVE: Complete hematologic response (hemCR) is associated with better survival and organ function improvement in patients with AL amyloidosis. Next generation flow cytometry (NGF) is a very sensitive method to evaluate the presence of minimal residual disease (MRD) and is a standard method for the assessment of MRD in patients with myeloma. The aim of the current study was to evaluate feasibility and applicability of MRD by NGF in patients with AL at hemCR.
METHODS: We evaluated MRD in 24 patients with AL amyloidosis in sustained hemCR. MRD was assessed in BM samples according to the Euroflow guidelines. A median number of 5 million events (range 3.9x106-6.1x106) were acquired for each tube in a BD FACSCantoII cytometer and data analysis was conducted with Infinicyt software, that allowed merging of the two panels based on the 6 backbone markers, offering a median sensitivity level of 2.3x10-6 (range 2x10-6-3.1x10-6).
RESULTS: The median age at diagnosis was 59 years (range 42-75), 75% had ∂-light chain, 90% had renal, 15% liver and 35% had cardiac involvement; 40% were Mayo stage-1, 50% stage-2 and 10% stage-3. At diagnosis median dFLC was 94 mg/L (range 17-879) and 15% had negative serum and urine immunofixation. Median clonal plasma cell infiltration was 8%. Primary treatment was bortezomib-based in 88% and Mdex in 11%, while 33% had received ASCT. At the time of MRD testing, 50% of the patients had achieved a renal response, 4/7 (57%) patients with cardiac involvement had a cardiac response, and 1/3 (33%) a liver response. Ten (42%) patients were MRD negative (MRDneg) and 14 (58%) were positive (MRDpos). Notably, 6/14 (43%) MRDpos cases had very low residual tumor load at levels <3x10-5. Median time from CR to MRD testing was 36 months (39 versus 35 months for MRDpos versus MRDneg patients). MRD was positive in ¾ patients with negative baseline serum and urine immunofixation and in 2/3 patients with baseline dFLC <40 mgL. Responses of at least one involved organ were documented in 70% of patients and in particular in 70% of MRDpos patients versus 67% of MRDneg. Among cardiac responders (n=4), 3 were MRDneg. Renal responses occurred in 67% of MRDpos and 75% of MRDneg patients. MRDneg patients had more often response in more than one organ (in all a cardiac and a renal response) while among MRDpos patients all had responses to a single organ. We found no significant differences in baseline characteristics (age, dFLC, BM infiltration, NTproBNP, Mayo stage) among MRDneg and MRDpos patients. Among patients who received ASCT, 2/8 (25%) were MRDneg versus 6/12 (50%) who were MRDneg among patients who did not receive ASCT.
CONCLUSION: Among patients with AL amyloidosis in sustained hemCR, assessed with high sensitivity NGF, 42% were MRDneg and 58% were MRDpos. This finding may have implications in the management of patients with AL who achieve a hemCR, especially among those who fail to achieve an organ response and may also have implication for their management, in an era of expanding treatment options.

P59. A PATIENT DIAGNOSED WITH POEMS SYNDROME WITH ATYPICAL PRESENTATION

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BACKGROUND: POEMS syndrome is a paraneoplastic syndrome due to an underlying plasma cell neoplasm. The major criteria for the syndrome are polyradiculoneuropathy, clonal plasma cell disorder (PCD), sclerotic bone lesions, elevated vascular endothelial growth factor and the presence of Castleman disease. Minor features include organomegaly, endocrinopathy, characteristic skin changes, papilledema, extravascular volume overload and thrombocytosis. Autologous Stem Cell Transplantation (ASCT) might be potential approach of choice in patients that are eligible. İn this paper we reported that a patient which was treated with VAD (Vincristine, Doxorubicin, Dexamethasone 40mg) and ASCT and improved; although the bone marrow biopsy did not observe a plasma cell clone.

CASE REPORT: A 41-year-old Caucasian female patient admitted to the hospital with the loss of sense in her hands and skin hyperpigmentation for 6 months. Electromyography (EMG) was reported as demyelination and secondary axonal sensorimotor polyneuropathy. Abdominal USG confirmed the hepatosplenomegaly. In laboratory test revealed that hypothyroidism. Immunofixation electrophoresis and protein electrophoresis revealed that there was IgA lambda monoclonal gammopathy and beta gamma monoclonal band respectively. Bone marrow aspiration and biopsy revealed as normal with normal percentaged of plasma cells. She was diagnosed with POEMS syndrome because of she had neuropathy, monoclonal gammopathy, endocrinopathy, organomegaly and skin changes and treated with VAD and performed ASCT with high dose melphalan. ASCT was successfully performed and the patient recovered quickly. Now she is under follow up and she is very well.

DISCUSSION: POEMS syndrome is a rare paraneoplastic plasmacell disorder. ASCT might be potential approach of choice in patients that are eligible. As an exception to our case; although the patient had normal plasma cells in the bone marrow biopsy, she responded to the ASCT.

P60. EFFECTIVENESS OF ALLOGENEIC TRANSPLANTATION IN PATIENTS WITH ACTIVE MYELOID DISEASE

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OBJECTIVE: Survival of patients transplanted with acute myeloid leukemia is poor with recent retrospective studies reporting a survival of <10% (Todisco et al). Encouraging results have been reported with the sequential administration of chemotherapy (pre-treatment) and administration of the conditioning regimen in the cytopenia phase (FLAMSA-protocol, Schmidt et al). The purpose of this study was to evaluate the efficacy of a FLAMSA-like protocol in allogeneic transplantation (allo-HCT) in patients with active myeloid disease (sequential chemo-allo).

METHODS: Patients who underwent allo-HCT with active myeloid disease were analyzed. Patients received intensive AML-chemotherapy (pre-treatment) one week before the administration of the conditioning regimen, according to an approved protocol by the Scientific Committee (344/04.08.06). The choice of myeloablative or reduced intensity conditioning regimen was made according to age and co-morbidity. An analysis of overall survival (OS) and relapse free survival (RFS) were made with the Kaplan-Meier method and analysis of cumulative incidence of relapse (REL) and non-relapse mortality (NRM) were taken into account as competing events.

RESULTS: In total, 22 patients (14 male, 8 female) of median age of 54 (range 30-72) were transplanted with active myeloid disease (de novo AML 5, sec AML 6, MDS 8, CMML 2, MF 1). Nineteen (19) patients had resistant disease while 3 MDS patients had not received prior chemotherapy. The median number of cycle treatments before transplantation was 2 (range 0-5). The median number of blasts on pre-treatment administration was 10% (range 1-45%). Pre-treatment was given on d-14 and included aracytin 1-4 g/mq bd administration, whereas 1 patient was given etoposide 100 mg/mq and in 1 patient the GCLAC regimen. The conditioning regimen was given from d-7 to d-3 (BCNU/Flu/Mel or TT n = 8, Busilphex/Flu/TT n = 11, Flu/Mel n = 3). The donors were siblings n = 7, 10/10-matched volunteers n = 10, 9/10-matched volunteers n = 4, and in 1 haploidentical donor. Engraftment was achieved in all patients with median time of WBC > 1.000/μl and platelets >20.000/μl at d+11 and d+13, respectively. Discontinuation of prophylactic cyclosporine was performed in all patients before d+120. The median follow-up was 322 days (range 14-3885). Of the 22 patients, 9 are alive and all are free of disease. Five patients relapsed (median day d+99, range 62-293) and all of them died (median day d+143, range 169-354). Eight patients died due to NRM (median day d+71, range 45-546). The estimated OS at 1 year was 33% and at 3 years 27%.

CONCLUSION: Performing allogeneic transplantation with the sequential administration of chemotherapy (pre-treatment) and administration of the conditioning regimen in the cytopenia phase is a therapeutic option for patients with active myeloid disease. Compared to published results, the present "sequential chemo-allo" strategy shows survival superiority over classical allo-HCT (Ferguson et al), but such a comparison is necessary in further retrospective and prospective studies with a larger number of patients.

P61. EARLY RELAPSE POST AUTOLOGOUS TRANSPLANTATION IS ASSOCIATED WITH VERY POOR SURVIVAL AND IDENTIFIES AN ULTRA HIGH-RISK GROUP OF MYELOMA PATIENTS: A 20-YEAR SINGLE-CENTER EXPERIENCE

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OBJECTIVE: Early relapses post high dose melphalan (HDM) and ASCT were associated with poor outcome in recent studies. The aim of this analysis was to describe the characteristics, management and outcomes of patients relapsing within 12 months from ASCT in a single center during a period of 20 years. **Methods:** The study analyzed the data of 297 consecutive patients who received HDM-ASCT as part of first line therapy from 1995 until June 2016 in our center. **Results:** The median age of all patients was 55 years. Induction included bortezomib-based in 47%, thalidomide-based in 13%, lenalidomide–based in 6%, and both bortezomib and an IMiD in 9% of patients. After ASCT, 35% received consolidation and 30% maintenance. Median follow up for all patients was 5 years; the median PFS post HDM was 36 months and 43 (13.5%) patients have progressed within <12 months post HDM. In univariate analysis, the baseline LDH ≥250 IU/L (37% vs 15%, p<0.001), eGFR <30 ml/min (22% vs 12%, p=0.055), hypercalcemia (26% vs 16%, p=0.069) and high-risk cytogenetics (39% vs 23%, p=0.084) were associated with early relapse. Response to induction therapy was not predictive of early relapse. The use of consolidation therapy post HDM was associated with lower rates of early relapses (4% vs 14% for maintenance and 26% for no post-HDM therapy, p<0.001). In multivariate analysis, LDH ≥250 IU/L (HR: 3.4, p=0.01) and hypercalcemia (HR: 3, p=0.006) at diagnosis increased, while the use of consolidation reduced the risk of early relapse (HR: 0.23, p=0.012). In multivariate analysis, the use of maintenance therapy (HR: 0.58, p=0.032) and sCR/CR post HDM (HR: 0.64 vs PR, p=0.06 and HR: 0.9 vs VGPR, p=0.17) were independently associated with PFS. In further analysis, PFS to 2nd line therapy was 14 months for all patients, but only 5 months for those with an early relapse vs 19 months for the other patients (P<0.001). Median PFS2 was 15.5 months for patients with a relapse <12 months post HDM vs 57% at 5 years for the other patients (p<0.001). The 5-years OS was 67%, but, median post-HDM OS was 18 months for those with an early relapse vs 71% at 5 years for those with post HDM PFS ≥12 months (p<0.001). In multivariate analysis PFS post ASCT <12 months (HR: 14, p<0.001) was the most important prognostic factor for poor OS; other prognostic factors for OS included post-HDM sCR/CR (HR: 0.48 vs PR, p=0.021 and HR: 0.54 vs VGPR, p=0.054), hypercalcemia at diagnosis (HR: 2.1, p=0.005) but not maintenance (p=0.1) or consolidation therapy (p=0.54) post ASCT.
CONCLUSION: Thirteen per cent of patients with newly diagnosed MM who receive HDM+ASCT at first remission relapse within 12 months after ASCT and they have very poor outcomes. These patients should be considered as an ultra high-risk group that urgently needs more effective therapies and innovative approaches through targeted clinical trials. The use of consolidation in patients at high risk for early relapse, such as those with high LDH and hypercalcemia at diagnosis, may reduce early relapses, and maintenance may prolong their PFS.

P62. SUCCESSFUL TREATMENT WITH GRANULOCYTE TRANSFUSION AND EARLY NEUTROPHIL ENGRAFTMENT IN ALLOGENEIC TRANSPLANT PATIENTS WITH FEBRILE NEUTROPENIA; DOES GRANULOCYTE TRANSFUSION EFFECT ON NEUTROPHIL ENGRAFTMENT?

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OBJECTIVE: Febrile Neutropenia is very severe and urgent early complication after bone marrow transplantation before engraftment. Infection delays engraftments in these periods. In this study we evaluated the effect and outcome of granulocyte transfusion on febrile neutropenia and neutrophil engraftment in patients receiving allogeneic transplantation. Between 2015-2017, sixteen patients receiving allogeneic bone marrow transplantation (BMT) were treated with granulocyte transfusion at the time of febrile neutropenia before engraftment. The reasons for the use of the granulocyte transfusion were prolonged febrile neutropenia episode.

METHODS: Sixteen patients (9 AML, and 7 ALL) underwent allogeneic transplantation. Nine of them transplanted from match sibling donors, one from unrelated donor, and six from mismatch family donor (haploidentic transplantation). They had febrile neutropenia after transplantation, before engraftment. They were given antimicrobial therapy. Granulocyte was collected from unrelated and same blood groups donors. We started Granulocyte transfusion for three - four days. Mean infused granulocyte counts were 3 x 10^10 (1.2 - 4.8x10^10) /day, and about 15-20% of transfused granulocyte was monocyte. Before the granulocyte transfusion, on the 12th – 19th days of transplantation, their neutrophil counts were 0.02-0.09 x 10^3/dl.

RESULTS: Twenty-four hours after granulocyte transfusion, mean neutrophil counts were 0.7x10^3/dl (0.4-1.2 x 10^3/dl). Neutrophil counts were 2.2 x 10^3/dl, (1.7-2.6 x 10^3/dl), after 48 hour. After 72 hours, neutrophil counts were 3.2 x 10^3/dl. (2.0- 4.6 x 10^3/dl). After 4th days of granulocyte transfusion, neutrophil counts were normal level (> 0.5 x 10^3/dl.) in 12 patients, and less than normal level (< 0.5 x 10^3/dl) in 4 patients.

CONCLUSION: Granulocyte transfusions during the febrile neutropenia, helped to better-overcome febrile neutropenia periods in allogeneic transplant patients before engraftment. In addition, granulocytes transfusion also may help early neutrophil engraftments.The useful effect of granulocyte transfusion on neutrophil engraftment may be cause of cytokine (G-CSF) injection to donor before collection of granulocyte. Increased cytokine (G-CSF-GM-CSF-IL3) level of transfused neutrophil and monocyte can also effect the neutrophil engraftment.

P63. AUTOLOGOUS STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA AT TWO DIFFERENT TIME PERIODS, 1989-1999 VS. 2000-2012: COMPARISON OF PROCEDURES AND RESULTS; ONE CENTER EXPERIENCE

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OBJECTIVE: Autologous Stem Cell Transplantation (ASCT) is considered the standard of care for patients with multiple myeloma. The aim of this retrospective study is to compare the techniques and the results in our Center, at two different time periods (period 1: 1989-1999 vs. period 2: 2000-2012) and to evaluate the progress made in efficacy and safety.
METHODS: All data of interest retrieved from our Transplant Data Base during the two periods. We used the log-rank test for the Kaplan-Meier curves comparison and the Mann-Whitney test for age and CD34 cells number comparison.
RESULTS: Forty-eight patients (median age 49 years; 28M/20F) received an ASCT between 1989-1999 and 112 patients (median age 56 years; 59M/53F) received the auto-transplant between 2000-2012. Before ASCT, objective response (≥PR) was achieved in 46% and 87% of patients, respectively, reflecting the more effective anti-myeloma induction regimens used after 2000. Stem cells were harvested successfully in all patients. During period 1, five of patients were transplanted using bone marrow graft. Mobilization regimen included mainly the combination of cyclophosphamide, etoposide and G-CSF (34/48 patients, 71%) in period 1 and cyclophosphamide 2 g/m^2 + G-CSF (81/112 patients, 72%) in period 2. Furthermore, during period 2, three of the patients received plerixafor in addition to GCSF. Conditioning regimen included melphalan 140 mg/m^2 in combination with BiCNU, cyclophosphamide and etoposide or with busulphan in 45/48 (94%) patients between 1989-1999. On the other hand, melphalan 200 mg/m^2 was the main conditioning regimen after 2000 (107/112 patients, 96%). The median infused CD34(+) cells were 5.9x10^6/kg and 4.14x10^6/kg, in the two studied periods, respectively. Engraftment was achieved in all these patients but one in period 1. Oral mucositis and diarrhea was recorded in all patients but was more tolerable in those transplanted during period 2. The majority of the patients developed neutropenic fever, responding well to antibiotic treatment. Treatment related mortality (TRM) was 4.2% vs. 0.8% for period 1 and period 2, respectively. One patient died of septicemia due to graft failure in period 1. Median time to relapse was 22 and 31 months for the two studied periods, respectively (p=0.07), while the median overall survival was 65 and 72 months, respectively (p=0.125).
CONCLUSION: Safety and tolerability of ASCT in patients with MM had improved dramatically during the period 2000-2012. Yet, no statistically significant improvement in overall survival and time to disease progression was recorded.

P64. TRANSFUSION OF BLOOD PRODUCTS FOR PATIENTS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION; A SINGLE CENTER EXPERIENCE

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OBJECTIVE: High dose chemotherapy, followed by autologous stem cell transplantation (ASCT, is an effective treatment for patients with multiple myeloma (MM) and chemotherapy-sensitive relapsed non Hodgkin (NHL) and Hodgkin Lymphomas (HL). Due to the hematologic toxicity of the conditioning regimen, most of these patients need blood product transfusions. The aim of this retrospective study is to analyze the relevant data of our Transplant Center in the context of proper blood recourses management.

METHODS: We analyzed the transfusion needs during the first 100 days of 130 patients with MM, 59 with NHL and 36 with HL who had been transplanted between the years 2006-2015. The mobilization regimen was a combination of GCSF ±chemotherapy. The engraftment criteria were ANC ≥0.5x109/lt and PLT ≥20X109/lt without need of PLT transfusion. The threshold for RBC transfusion was Hb ≤8 g/dl, whereas for PLT transfusion was ≤10x109/lt without evidence of hemorrhage or fever and ≤20x109/lt if one of the above mentioned conditions was present. The SPSS 8.0 program was used for statistical analysis of the data.

RESULTS: The baseline characteristics of the patients are shown in Table 1, whereas their transfusion needs in Table 2. All transfusions of blood products have been administered up to day+30 after ASCT. Concerning patients with MM, 35.4% (46/130) did not need transfusion of RBC and 15.4% (20/130) transfusion of PLTs. For patients with HL the percentage was 19.4% (7/36) and 2.7% (1/36) respectively, whereas for patients with NHL the percentage was 15.25% (9/59) and 3.4% (2/59). The level of hemoglobin before ASCT was a prognostic factor in patients with MM concerning the need for RBC transfusion (p=0.04, data not shown). Levels of CD34+ cells did not seem to affect the transfusion needs in RBC and PLTs in all patients (all p values >0.05)

Table 1

	MM	NHL	HD	p values
Number (M/F)	77/53	45/14	21/15	
Age MT±SD Median (range)	 60.1±7.28 57 (37-72)	 49.98±11.75 52 (21-69)	 33.58±13.46 33 (15-57)	<0.05
Mobilization Regimen	86 Cy+G-CSF 10 G-CSF+Plerixafor 34 Other	15 Cy+G-CSF 13 ESHAP 31 Other	8 Cy+G-CSF 17 ESHAP 11 Other	
Conditioning Regimen	126 MELPHALAN	28 BEAM 8 BEAM reduced 4 BEAM-R	31 BEAM	
Infused CD34+ (x10^6/Kg)	5.07±2.60	8.63±8.76	9.31±6.42	NS
Days until **ANC** >0.5x10^9/l **PLT** ≥20x10^9/l	 11 (4-27) 10.4 (0-31)	 13.5 (3-26) 12.7 (6-41)	 13.5 (6-28) 11.3 (1-48)	NS

Table 2				
Transfusions	**MM**	**NHL**	**HD**	P values
RBC MT±SD Median (range)	 1.69±2.26 1 (0-16)	 3.05±3.18 2 (0-18)	 2,64±2,52 2 (0-13)	p<0.05
PLT MT±SD Median (range)	 2,50±5.53 2 (0-30)	 4.86±4.37 4 (0-29)	 3.47±3.41 2 (0-20)	p<0.05
FFP MT±SD Median (range)	 0.008±0.08 0 (0-1)	 0.068±0.36 0 (0-2)	 0	NS

CONCLUSION: Our results seem to agree with those of other published studies. A considerable percentage of patients undergoing ASCT do not need transfusion of any blood product. The differences between patients with MM and NHL/HL are due to the type of mobilization and conditioning regimens used, as well as to the stage of the disease at the time of transplantation (in lymphomas after the first relapse, in myelomas after the first remission). The different range of transfusion needs among patients emphasizes the importance of an individualized approach based on patients' characteristics.

P65. BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM: SINGLE CENTER EXPERIENCE ON A RARE HEMATOLOGICAL MALIGNANCY

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OBJECTIVE: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare, clinically aggressive and poor prognostic hematological malignancy. No consensus on treatment of these patients has been published yet. Although patients respond to intensive chemotherapy based induction regimens, relapse is common and overall survival (OS) ranges from 8 to 12 months. In this study, we aim to summarize the main clinical and biological characteristics, prognostic features and treatment of BPDCN patients diagnosed in our department. **Methods:** We retrospectively evaluated 8 BPDCN patients who had been diagnosed between July 2008 and July 2018 in Ankara University school of medicine. The diagnosis of BPDCN was based on clinical, morphological, and immunophenotypical features identified by the 2008 revision of the World Health Organization (WHO) classification of acute myeloid leukemia and related neoplasms. The medical records of Ankara University Faculty of Medicine were reviewed in terms of age, sex, clinical presentation, complete blood count, peripheral blood smear examination, bone marrow aspiration and biopsy, cytogenetic data, radiological imaging including computed tomography and positron emission tomography, and pathologic findings of the skin lesions. SPSS22 was used for statistical analysis and overall survival was assessed by of Kaplan–Meier method.

RESULTS: All of our patients (n=8) were male, median age was 61 (21-77). 5 of 8 patients (62.5%) had bone marrow infiltration, 4 of patients (50%) cutaneous lesions, 5 of them (62.5%) lymph node involvement and 1 patient (12.5%) central nervous system involvement at time of diagnosis. Complex karyotype was observed in 3 patients. CHOP regimen was given to 5 patients (62.5%), hyperCVAD regimen to 2 patients (25%) and FCM regimen to one patient as first line chemotherapy. Among the patients who responded to first line treatments, complete remission (CR) was achieved in 83.3% (n=5) and partial remission (PR) in 16.6%, (n=1). Progressive disease was observed in 25% of patients (n=2) after first line therapy. Two patients (25%)who received hyperCVAD regimen had allogeneic hematopoietic stem cell transplantation (AHSCT); one from HLA full match sibling donor, the other one from HLA full match unrelated donor in CR1. The median follow up time was 5.35 months (3-48.6 months). Estimated median overall survival was 6.5 +1.1 (%95 CI 4.3-8.7) months.

CONCLUSION: Diagnosis of BPDCN needs experienced hematopathologist. There is still no consensus on the treatment of these cases but chemotherapy regimens for agressive lymphoproliferative diseases followed by AHSCT in CR1 still remains to be the most appropiate choice.

P66. INVASIVE ZYGOMYCOSIS IN IMMUNOCOMPROMISED PATIENTS WITH HEMATOLOGICAL DISORDERS. ANALYSIS OF PREDISPOSING FACTORS IN A COHORT OF 20 PATIENTS

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OBJECTIVE: Invasive zygomycosis is a severe, potentially fatal invasive fungal infection, mainly presented as rhinocerebral mucormycocis. Novel antifungal treatments may not be sufficient and surgical intervention and debridement might be needed to cure this infection. We describe 20 patients, investigating for possible predisposing factors and analyzing the significance of each of them.
METHODS: All cases of invasive zygomycosis, diagnosed among patients with various hematological disorders in the University Hospital of Patras, were retrieved, the clinical manifestations, and laboratory features were retrospectively analyzed, and the preexistence of known predisposing factors was investigated. The course and evolution of these patients was then compared, in relation to the presence of these factors.
RESULTS: Patients were 12 males and 8 females, with a median age of 60 years (range 21-85). Seventeen had typical rhinocerebral mucormycocis, one had pulmonary, one vertebral and one invasive skin disease. Eighteen patients had an underlying hematological disorder, neoplastic in 16 (ALL in 4, of whom 1 allotransplanted, with grade-1 acute GVHD, AML 2, MDS 4, multiple myeloma 4, non-Hodgkin's Lymphoma 2) and non-neoplastic in 4 (Anemia various 2, Common Variable Immunodeficiency 1, β-Thalassemia major 1). Twelve patients were severely neutropenic (absolute neutrophil count <0.2/μl), 13 severely lymphopenic (absolute lymphocyte count <0.5/μl, and 13 were diabetics, of whom 4 poorly controlled. Thirteen patients had previously received various immunosuppressive treatments (methotrexate 5, lenalidomide 4, cyclosporine-A 3, cyclophosphamide 2, ATG/Campath 2, fludarabin 1, other 4). No patient was HIV positive or received parenteral nutrition. Three patients had previous colonization with Candida species, 14 were receiving broad-spectrum antibiotics, and 9 were receiving antifungal prophylaxis or preemptive treatment with imidazole derivatives (7) or echinocandins (2), (breakthrough zygomycosis). At diagnosis of zygomycosis 16/19 patients (84%) exhibited severe iron overload, defined by transferrin saturation >50% and/or by serum ferritin >1000 ng/ml, attributed to previous transfusions in 11. Median serum ferritin was 2450 ng/ml (range 346-8014 ng/ml) and median transferrin saturation 69.2% (range 33.2-91.4%), rendering iron overload the dominant feature for this patients. Two patients were treated with Amphotericin-B deoxycholate and the remaining 18 with Ambisome 5-10 mg/kg/d for a median of 15 days (range 5-57). The course was fatal in 15 cases and curative in 5, but in one patient zygomycosis relapsed 6 weeks later, and he finally succumbed. The 4 patients, who survived, underwent repeated surgical debridement, and 2 of them also received iron-chelation treatment. These patients finally died for reasons related to their underlying disease, without recurrence of zygomycosis. Survivors had lower mean transferrin saturation and serum ferritin levels, compared to patients who died (59.1±20.1% versus 71.6±11.6% and 1800±864 versus 3369±2054 ng/ml, respectively), yet these differences were not statistically significant (p=0.186 and p=0.197, respectively).
CONCLUSION: Although already recognized, iron overload, either attributed to previous red-blood cell transfusions or not, appears to be a dominant risk factor for the development of invasive zygomycosis, in patients, either with an underlying hematological malignancy or not. Iron status should be examined in all cases, and if possibly, iron overload should be reversed with the appropriate iron-chelating treatment.

P67. HEMATOLOGIC MALIGNANCIES ASSOCIATED WITH PRIMARY AND ACQUIRED IMMUNODEFICIENCY SYNDROMES

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OBJECTIVE: The prevalence of hematologic malignancy in primary immunodeficiency cases as well as acquired immunodeficiency is increasing. The objective of this study was to evaluate hematological malignancy frequency in primary and acquired immunodeficiency patients.
METHODS: Thirty patients with primary and acquired immunodeficiency that who admitted to Hacettepe University Hospital between the years of 1992 and 2018 were evaluated.
RESULTS: In this study, the most common type of immunodeficiency is common variable immunodeficiency (CVID) (60%), followed by predominantly acquired immune deficiency syndrome (AIDS) (26.7%), lipopolysaccharide-responsive and beige-like anchor (LRBA) deficiency (6.7%), congenital neutropenia (3.3%) and chronic granulomatosis disease (3.3%). Hematologic diseases were developed in 40% of the patients. B-cell Non-Hodgkin`s lymphoma was observed in 7 (23.3%) of the patients, two patients had T cell lymphoma (16.7%), one patient had acute lymphoblastic leukemia (3.3%), one patient had acute myeloid leukemia (3.3%) and one patient had aplastic anemia (3.3%). The 5-year overall survival for patients with no malignancy and patients with hematologic malignancy were 83% and 70%, respectively (p=0.06)
CONCLUSION: Hematological malignancies are frequently observed in patients with primary and secondary immunodeficiency. Patients with a diagnosis of immunodeficiency are usually referred to the hematology outpatient clinic with B symptoms and pancytopenia. Several types of PID and AIDS, lead to an increased risk of a hematopoietic malignancy and that it is clinically important to be aware of this.

P68. TRANSFUSION MANAGEMENT OF RED BLOOD CELLS (RBC) AND PLATELETS (PLT) IN TRANSPLANTED AND NOT-TRANSPLANTED HEMATOLOGICAL PATIENTS

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OBJECTIVE: Hematological patients require often and prolonged hospitalisations during their course of treatment, in part due to increased and frequent transfusion needs.The objective of the study was to assess the factors affecting transfusion demands in a Hematology Department (bone marrow transplant unit- BMTU, post-transplant unit-PTU, hematology clinic).
METHODS: Patients hospitalized between 1/1/2015 and 31/12/2015 were analyzed. Data regarding the underlying disease, the disease status, type of transplant, duration of marrow aplasia and donor-patient blood group mismatch were obtained from the medical records. The analysis was restricted to the transfusion of packed RBCs and units. Differences between groups were assessed using non-parametric statistics (Kruskall-Wallis and Mann-Whitney U-test).
RESULTS: There were 523 admissions of 256 different patients. Complete data for analysis could be obtained for 487 admissions of 237 patients (92.6% of patients, 93.1% of admissions), corresponding to 10,673 days of hospitalization. Total number of blood products transfused was 2284 packed RBC units, 13883 PLT units (apheresis platelets counted as 5 units). Values are reported as median (range), unless otherwise specified. In the BMTU, the type of transplant was correlated with transfusion needs: number of RBC units transfused per admission was 2 (1-5) for autologous transplanted (AUTO) patients, 4 (1-28) for allo-transplanted (ALLO) (no difference between sibling and matched unrelated donors), and 7 (1-14) for haplo-identical transplantations (HAPLO), p=0.001. Platelet units requirements were respectively 15 (5-45) for AUTO, 20 (5-205) for ALLO and 50 (30-130) for HAPLO, p<0.001. The length of stay was 18 (13-23) days in AUTO, 22 (16-44) in ALLO, 30 (29-40) days in HAPLO transplantation, p<0.001, while the duration of aplasia in days was 9 (4-19) in AUTO, 13 (5-32) in ALLO and 25 (20-38) in HAPLO, p<0.001. Greater transfusion needs were correlated with longer duration of aplasia and hospitalization. In the PTU there was no statistically significant difference in transfused RBC or PLT units with regard to transplant type. Disease status (response versus active disease) was only correlated with RBC units transfused in PTU [2 (1-29) vs 6 (1-56) units respectively, p=0.006]. Donor – patient blood group mismatch was correlated with increased transfusion demands in BMTU for RBCs [4 (1-28) vs 2 (1-5), p<0.001] and PLTs [25 vs 15, p<0.001]. In hematology clinic, transfusion needs in RBC and PLTs were correlated with the underlying disease. The higher needs in RBCs and PLTs were observed in patients with AML, whereas patients with lymphoma had the lowest needs in RBC transfusions. Disease status was not correlated with transfusion needs. The duration of aplasia was correlated with the number of RBC units (Pearson's r = 0.66, p<0.001, r 2 =0.435) and of PLTs transfused (Pearson's r = 0.78, p<0.001, r 2 =0.61).
CONCLUSION: The main determinants of transfusion requirements are the duration of aplasia, the type of transplant and the disease, with myeloid malignancies requiring more transfusions. The establishment of haplo-identical transplantations has increased the transfusion needs due to longer period of aplasia.

P69. DENOSUMAB INCREASES BONE MINERAL DENSITY AND REDUCES BONE RESORPTION AND OSTEOCLAST STIMULATORS IN PATIENTS WITH THALASSEMIA MAJOR AND OSTEOPOROSIS: FINAL RESULTS OF A RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE BLIND, PHASE 2B CLINICAL TRIAL

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OBJECTIVE: Osteoporosis is a common complication of thalassemia major (TM). The pathophysiology of bone loss in TM is multifactorial and includes an imbalance in osteoclast and osteoblast function. We have previously shown that circulating receptor activator of nuclear factor kappaB ligand (RANKL), the most potent osteoclast activator, is elevated in TM patients and is associated with low bone mineral density (BMD). Denosumab (DMB) is a fully human monoclonal antibody against RANKL that has been licensed for the treatment of different types of osteoporosis. However, there are no prospective data for the effects of DMB on TM-induced osteoporosis.
METHODS: This is a single-site, randomized, placebo-controlled, double blind phase 2b clinical trial to evaluate the effects of DMB in TM osteoporosis. The primary objective of this study was to assess the changes on lumbar spine (L1-L4) BMD in TM patients with osteoporosis who received DMB versus placebo at 12 months. Secondary endpoints included the evaluation of the effects of DMB versus placebo on femoral neck (FN) and wrist (WR) BMD at 12 months, the safety profile of DMB as well as its effects on bone turnover markers, on osteoclast and osteoblast regulators. Main inclusion criteria included TM patients >30 years of age with BMD T-score between -2.5 and - 4.0 in at least one of the examined sites (L1-L4, FN, WR). Main exclusion criteria included: impaired renal function (eGFR of ≤30 mL/min); elevated ALT and/or AST >2 fold the upper limit of normal (UNL), or elevated direct bilirubin >1.5xUNL; heart failure (NYHA above 2); administration of bisphosphonates within one year of study enrollment; presence of any other disorder that affects bone metabolism. Patients were assigned into two treatment groups: in group A, 60 mg DMB was administered sc, every 6 months for 12 months for a total of 2 doses (day 0 and day 180); in group B, placebo was administered sc, at the same time. All patients received calcium and vitamin D supplementation. Measurement of BMD with dual energy X-ray absorptiometry at three body sites (L1-L4, FN, WR) was performed during the screening period and at the end of the study. The following biochemical markers were evaluated on the day 0 and then every 3 months up to 12 months (every patient had 5 measurements): i) osteoclast regulators: sRANKL and osteoprotegerin (OPG); ii) osteoblast inhibitors dickkopf-1 (Dkk-1) and sclerostin (SOST); iii) bone resorption markers: C-telopeptide of collagen type-I (CTX) and tartrate-resistant acid phosphatase isoform-5b (TRACP-5b); and iv) bone formation markers: bone-specific alkaline phosphatase (bALP) and osteocalcin.

RESULTS: Sixty-three patients participated in the study (group A, n=31; group B, n=32) and they were well-balanced regarding their baseline BMD of all evaluated sites. Patients of group A (DMB arm) achieved an increase in both L1-L4 BMD ($p<0.001$) and FN BMD ($p=0.022$), while there were no changes in WR BMD. Patients of group B (placebo arm) achieved a slight increase in their L1-L4 BMD and a significant decrease in their WR BMD ($p=0.008$). The percentage increase of L1-L4 BMD was higher in DMB arm than in placebo arm (6.02±5.30% vs. 3.11±5.46%, respectively; $p=0.03$), while the advantage of DMB regarding WR BMD was much higher compared to placebo (-0.22±5.40% vs. -4.15±8.58%, respectively; $p=0.02$). No grade 3 or 4 toxicity was observed in this study. Patients who received DMB showed a dramatic reduction of sRANKL, sRANKL/OPG ratio, CTX, TRACP-5b, bALP between baseline and 12th month ($p<0.01$ for all comparisons) without changes in Dkk-1, SOST and OC. On the contrary, placebo patients showed an increase in sRANKL, OPG, Dkk-1, CTX, TRACP-5b, bALP during the study period ($p<0.01$ for all comparisons) along with a slight increase of SOST and OC (p=NS).
CONCLUSION: DMB, given twice per year in TM patients, increases the BMD of the L1-L4 more efficiently than placebo after 12 months, with excellent safety profile. Furthermore, DMB increased the FN BMD, which was not increased in the placebo arm, along with a positive effect on WR BMD compared to placebo. DMB also reduced markers of bone resorption and osteoclast activation without affecting Dkk-1, which was increased in placebo arm patients in whom there was a significant increase in osteoclast activators and both bone resorption markers. These data support the use of DMB for the management of TM-induced osteoporosis.

P70. DENOSUMAB EFFECTS ON SERUM LEVELS OF THE BONE MORPHOGENETIC PROTEINS ANTAGONIST NOGGIN IN PATIENTS WITH TRANSFUSION-DEPENDENT THALASSEMIA AND OSTEOPOROSIS

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OBJECTIVE: Osteoporosis is a common complication of beta-thalassemia major (TM). Denosumab (DMB) is a fully human monoclonal antibody that binds RANKL, decreases osteoclast formation and function and is used for the treatment of osteoporosis. Noggin (NOG), a secreted homodimeric glycoprotein, is an antagonist of bone morphogenetic proteins (BMPs) that predominantly binds BMP-4 and BMP-2 and antagonizes their bioactivities by preventing their binding to the respective receptors. Thus, NOG seems to have a profound impact on osteogenesis and may be influenced by anti-resorptive therapy. The aim of the study was to evaluate NOG in patients with TM and osteoporosis under DMB therapy.
METHODS: NOG was measured in 63 TM patients with osteoporosis who participated in a randomized, placebo-control, phase 2b study. In that study, patients received either 60 mg DMB, sc, every 6 months for 12 months for a total of 2 doses (n=31) or placebo (group B, n=32). Measurement of bone mineral density (BMD) of three body sites (L1-L4, Femoral Neck, Wrist) was performed using DXA before treatment and after 12 months, along with a series of bone remodeling indices: sRANKL and OPG, Dkk-1 and SOST, CTX and TRACP-5b, bALP and osteocalcin. NOG was measured, for the first time in TM patients, on days 0 and 180, using a recently developed high sensitivity fluorescent immunoassay based on plasmonic microtiter plates which increase the signal of fluorescent dyes several hundred-fold. Briefly the assay protocol includes: adsorptive coating of capture antibody in 50mM phosphate buffer (PBS)/150mM NaCl pH 7.4, over-night at 4°C followed by washing with PBS containing 0,1% Triton x100. Blocking of unspecific binding was achieved with a proprietary solution of FIANOSTICS containing synthetic polymers and mercapto-compounds. After another washing step, 20ul duplicates of standards/samples (serum) together with 25ul of anti-human NOG antibody labeled with AlexaFluor680 were incubated over night at RT temperature in the dark. Measurements were done using a standard fluorescence micro-plate reader. Samples reading above 100 pmol/l NOG were diluted with assay buffer and re-run to check for linearity of the signal.
RESULTS: The effects of DMB on BMD are presented in the main abstract on this conference (see P68). Both groups showed an increase in noggin serum levels after 12 months compared to baseline. In the denosumab group the values (mean±SD) were 44.2±112.4 and 19.4±49.1, $p<0.001$, respectively, whereas in the placebo group the corresponding values were 120.3±478.0 and 12.2±22.1, $p<0.0001$, respectively. It becomes evident that the increase was higher in the placebo group. Importantly, we noted a strong correlation between serum noggin levels and wrist BMD. Noggin levels were negatively associated with BMD values ($r=-0.641$, $p=0.002$). However, this finding was not reproduced among patients in the placebo group.
CONCLUSION: This is the first study where NOG is measured in TM. NOG elevation was lower in DMB than in placebo patients. Only in DMB group NOG changes correlated with WR-BMD. Since our assay detects free, bioactive NOG, higher NOG levels reflect more BMP inhibition, which in turn led to less bone formation of placebo group. Mechanistically these results suggest that denosumab possibly regulates NOG and leads to increased BMD in TM patients with osteoporosis via another mechanism of action.

P71. THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP): ONE CENTER EXPERIENCE

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OBJECTIVE: TTP is a rare disease with an incidence of 4.5 new cases per million of people per year. It may be either Idiopathic or secondary to other medical conditions. The purpose of this study is to present our Center's experience with the therapeutic approach and the overall survival of patients with newly diagnosed or relapsed TTP.
METHODS: We have studied retrospectively the data of all our patients with TTP from 1989 up to 2015. Disease diagnosis was made according to international criteria (microangiopathic hemolytic anemia, thrombocytopenia, fever, neurologic symptoms and renal impairment). The therapeutic approach for all patients was immediate plasma exchange and high dose corticosteroids (except for three patients, who did not receive corticosteroids). Platelet number ≥150.000/mm3 for two consecutive days was defined as treatment response, whereas, a new episode after 30 days of remission was defined as disease relapse. Whenever possible we evaluated ADAMTS13 levels.
RESULTS: Twelve patients (4 men/8 women), were diagnosed with TTP. Median age at the time of diagnosis was 53 years (range 34-72). All patients but 4, who did not have neurologic symptoms, met all the above diagnosis criteria. Four patients whose ADAMTS 13 levels had been evaluated had ADAMTS 13 deficiency. Plasmapheresis was performed once daily right after the diagnosis was made. Upon treatment, response patients were kept at the same schedule for at least ten days, followed by gradual tappering. The median number of plasmapheresis sessions was 13 (range 2-36). In 6 patients who did not respond or had disease progression after five days, two plasmaphereses per day were initiated. Three patients received rituximab after ten days of plasmapheresis due to partial response. Six patients relapsed; two of them had received rituximab. Two out of six relapsed patients had more than one relapses. The median time to first relapse was 1.7 months (range 0.8-13). One patient relapsed after 71.6 months. Three patients died of the disease during the first week of treatment mostly due to delayed diagnosis. Three more patients died later of other causes.
Conclusion: Due to the fact that the disease is rare, our sample was small. Nevertheless, we can conclude that early diagnosis and especially immediate initiation of plasmapheresis are critical for the survival of patients with TTP. Rituximab infusion in patients who do not respond well to plasmapheresis is important for the disease control and hematological recovery.

P72. THROMBOTIC THROMBOCYTOPENIC PURPURA IN TREATMENT WITH SUNITINIB FOR RENAL CELL CARCINOMA: CASE REPORT

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BACKGROUND: Sunitinib malate is an oral multitargeting tyrosine kinase inhibitor approved for the first line treatment of metastatic renal cell carcinoma. Sunitinib administration is associated with several adverse events including fatigue, diarrhea, skin toxicity, hypothyroidism, and cytopenia. Herein, we present a case of thrombotic thrombocytopenic purpura within sunitinib therapy.
CASE REPORT: A 50-year-old male with metastatic renal cell carcinoma presented with bruise around the right eye two years after starting sunitinib 50 mg daily. He was found to have schistocytes in peripheral blood smear, in addition to significant thrombocytopenia and anemia. Serum creatinine and LDH level of the patient was 1.7 mg / dl and 563 U/L, respectively. Current clinical and laboratory findings were thought to be consistent with thrombotic thrombocytopenic purpura caused by sunitinib. Sunitinib was stopped and plasmapheresis started. On day 3rd of plasmapheresis, LDH levels were decreased and platelet counts reached normal levels. She recovered completely after plasmapheresis.
DISCUSSION: To our knowledge, this is the fifth case report of thrombotic thrombocytopenic purpura secondary to sunitinib. Oncologists should be aware of this rare but potentially fatal adverse event. We recommend monitoring the number of platelets routinely and frequently after starting sunitinib.

P73. THROMBOTIC MICROANGIOPATHY-LIKE HEMOLYSIS IN VITAMINE B12 DEFICIENCY-RELATED MEGALOBLASTIC MACROCYTIC ANEMIA

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BACKGROUND: Vitamin B12 deficiency may present with hemolytic anemia, thrombocytopenia, and schistocytosis, mimicking a microangiopathic hemolytic anemia. This is known as pseudothrombotic microangiopathy. A case of pseudothrombotic microangiopathy due to severe vitamin B12 deficiency is presented.
CASE REPORT: An 87-year-old male patient presented with fatigue for one year. The patient with a hemoglobin level of 6.8 g / dl was admitted to the internal medicine clinic for further examination and treatment. At the first examination of the patient, the temperature was 37 C, blood pressure was 125/67 mm/Hg, pulse rate was 82/ min, respiratory rate was 18/min. No pathological finding was found on physicial examination. The laboratory findings of the patient were as follows; WBC: 5100/mm3, Hgb: 6.8 gr/dl, PLT: 71.000/mm3, MCV: 115.4 fl, Fe: 19 ug/dl, ALT: 25 U/L, AST: 84 U/L, ALP: 60 U/L, LDH: 1953 U/L, INR: 1.02, APTT: 18.7 second, Vitamin B12: 88 pg/dl, folic acid: 3.56 ng/ml, Total bilirubin: 2.37 mg/dl, Direct bilirubin: 0.32 mg/dl, HBsAg (-), AntiHBs (-), AntiHAV IgM (-), AntiHAV IgG (-), AntiHCV (-), AntiHIV (-), Anti-CMV IgM (-), AntiToxo IgM (-), AntiRubella IgM (-),, EBV VCA IgM (-), ANA (-), AMA (-),and Anti-SM (-). İndirect and direct coombs was negative and positive, respectively. The patient`s periferic blood smear was compatible with macrocytic anemia, hypersegmented neutrophils and schistocytes (Figure 1). The patient considered a clinical picture similar to a microangiopathic hemolytic anemia due to severe B12 deficiency. Patient without symptoms of anemia did not receive erythrocyte suspension. The patient underwent intramuscular B12 replacement. After B12 replacement, the patient`s hemoglobin level rose to 8.7 gr / dl. Peripheral blood smear after vit B12 replacement did not show schistocyte. The patient was discharged with the recommendation of the outpatient clinic follow-up.
DISCUSSION: Pseudothrombotic microangiopathy is a clinical picture due to severe B12 deficiency and is characterized by anemia, thrombocytopenia and schistocytosis. The number of platelets in the pseudothrombotic microangiopathy is about 70,000, which is higher than the platelet count in TTP. Assessment of lactate dehydrogenase, reticulocyte count, bilirubin and platelet counts is most useful when a pseudothrombotic microangiopathy is distinguished from a true microangiopathic hemolytic anemia. Assessment of lactate dehydrogenase, reticulocyte count, bilirubin and platelet counts is most useful if a pseudothrombotic microangiopathy is distinguished from a true microangiopathic hemolytic anemia.

P74. A CASE OF FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN AN ADULT MIDDLE- AGE FEMALE PATIENT

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BACKGROUND: Hemophagocytic lymphohistiocytosis (HLH) is a complex syndrome, characterized by hyperactivation of the immune system and hyper-inflammation. Characteristic clinical features include fever, hepatosplenomegalia, pancytopenia and neurological abnormalities. HLH is divided into two types: (1) primary or familial HLH that is inherited in an autosomal recessive manner and (2) secondary HLH. Here we present a rare case of familial HLH (FLH).

CASE REPORT: A 48 -year-old lady was admitted to the 1st Medical Propedeutic Department of Internal Medicine, in December 2017 due to fever, hepatosplenomegalia and pancytopenia. In particular, blood tests revealed decreased hemoglobin levels of 9.1 (normal range, 12–18 g/dL), thrombocytopenia of 18-20K/μL (normal range, 150–450 K/μL), leukopenia of 2.29 (normal range, 3.8–10,5× K/μL) with 36.25% neutrophils and 59.4% lymphocytes, and 14% atypical lymphocytes. Previous medical history of stroke was reported. Whole body FDG PET/CT imaging revealed hepatosplenomegalia and increased bone marrow uptake. After additional testing with chest/abdomen CT, transthoracic cardiac ECHO, bone marrow aspiration and trephine, she was found to fulfil the diagnostic criteria for HLH. She was immediately started on therapy according to the HLH-94 treatment protocol. This protocol included an initial intensive therapy with immunosuppressive and cytotoxic agents (etoposide in combination with dexamethasone) for 8 weeks in order to induce remission of the disease activity. Maintenance therapy consisted of pulses of dexamethasone in combination with etoposide and cyclosporine for 9 weeks. The patient showed marked improvement. The PRF1 gene coding sequence showed a null homozygous variant c.1247-1G>Cin the STXBP2 gene that is classified as pathogenic for FLH and subsequently she became a potential candidate for an allogeneic stem cell transplantation.

DISCUSSION: As in our case, when there is a strong indication of HLH- even at an advanced age-, genetic testing of the probability of the inherited type of the syndrome should be performed from the early beginning, since the most beneficial treatment option is the bone marrow transplantation as early as possible.

P75. TWO CASES OF MALARIA IN A GENERAL HOSPITAL DIAGNOSED BY LIGHT MICROSCOPY

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BACKGROUND: Malaria is a mosquito-borne infectious disease caused by the parasitic protozoans belonging to the Plasmodium type. Malaria is endemic throughout most of the tropics. Although the incidence of malaria has decreased globally, in 2016, there were 216 million cases of malaria worldwide resulting in an estimated 445,000 deaths mainly children under 5 years of age in sub-Saharan Africa. Therefore, prompt and accurate diagnosis of malaria is critical for implementation of appropriate treatment to reduce associated morbidity and mortality. Forms of parasite diagnosis include light microscopy, rapid diagnostic tests, and molecular techniques detecting parasite genetic material.

CASE REPORTS: We present two cases of malaria diagnosed by light microscopy and further confirmed by rapid diagnostic test and PCR. Both male patients had a past medical history of malaria 4 and 6 months ago respectively and reported to have received relevant treatment. Patient #1 came to the hospital due to fever, whereas patient #2 presented with fever, headache and photophobia over the last 3 days. Both patients had an unremarkable full blood count (FBC). Patient #1 had the following parameters WBC $7.77x10^9$/L (neutrophils 87.3%, lymphocytes 5.8%, monocytes 6%), Hb 13.8 g/dl, Ht 39.8%, PLT $204x10^9$/L. Biochemistry tests revealed mild transaminasemia. A thick blood smear and a Giemsa stained smear of peripheral blood were prepared, and the ring form of the parasites was found in both. The one-step malaria rapid test was also performed and was positive for Plasmodium falciparum. The patient was successfully treated with mefloquine. Patient #2 had the following FBC WBC: $8.36x10^9$/L (neutrophils 76.5%, lymphocytes 10.8%, monocytes 12.4%), Hb 16.3 g/dl, Ht 46.1%, PLT $153x10^9$/L. Biochemistry tests revealed mild transaminasemia and an increased total bilirubin. On physical examination, he was found to have splenomegaly A thick blood smear and a Giemsa stained smear of peripheral blood were prepared, and the ring form of the parasites was found in both. The one-step malaria rapid test was also performed and was positive for Plasmodium non-falciparum. A multiplex PCR was positive for Plasmodium vivax. The patient was successfully treated with primaquine.

DISCUSSION: Malaria should be suspected in the setting of fever and relevant epidemiologic exposure. Parasite-based confirmation should be pursued whenever malaria is suspected clinically. Detection of parasites on Giemsa-stained blood smears by light microscopy remains the standard tool for diagnosis of malaria. It is labor intensive and requires substantial training and expertise. Hematologists and microbiologists should be appropriately trained and alerted since malaria still exists and carries significant morbidity and mortality.

P76. ACTIVITY OF THESSALONIKI CORD BLOOD BANK (TSCB) GREECE

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OBJECTIVE: Public TSCB established in 2009 and accepts cord blood units (CBUs) for allogeneic transplantation. Housed in the G.Papanicolaou Hospital, Department of Hematology-BMT Unit, National Health System. Umbilical cord blood (UCB) represents a stem cell source for transplantation. UCB offers advantages over bone marrow or mobilized peripheral blood including ready availability, ethnic diversity in the world-wide UCB banks, reduced incidence of graft versus host disease, less strict HLA matching. In parallel there are some disadvantages in UCBT such engraftment delay. In principle, transplant graft content on CD34+ progenitors and especially CFUs correlate with the speed of engraftment. In patients undergoing allogeneic stem cell transplantation have been detected small membrane vesicles, cell-derived microparticles (MPs), which are considered as markers of cell activation and apoptosis. Aim of this study was to analyze certain parameters of UCBs as well as MPs of CD34+ cells in cord blood units and to investigate their potential role to the cord blood quality.

METHODS: Volume reduction and RBC depletion is performed by the automated system SEPAX (Biosafe). Controlled-rate freezer is used after 10% DMSO addition and transplants are stored in liquid nitrogen tanks. Labeling system follows NETCORD-FACT standards. Evaluation of total nucleated cell number (TNC) pre- and post-processing and blood group typing, nucleated RBC, post-thawing CD34+cell number and viability analysis is performed routinely. Molecular intermediate resolution HLA typing (A,B,C and DRB1) analyzed in EFI accredited laboratory. Assessment of the colony forming units (CFU) was performed using complete methylcellulose medium (Stem Cell Technologies). Cells plated in duplicate for 14 days at 370C and 5% CO2. Granulocyte-macrophage (CFU-GM), erythroid (BFUE) and multipotential (CFU-GEMM) colonies were scored by microscopic examination. The MPs were isolated after centrifugation of the plasma and their number was determined after incubation with Annexin V and CD34 by flow cytometry.

RESULTS: TSCB is a member of Bone Marrow Donors Worldwide, the Hellenic Transplant Organization and the Netcord Foundation (associate) having stored more that 5600 units CBUs. The post-processing number of CD34+cells was 3.3x10^6/unit and their viability 91,08%. Univariate analysis using Spearman`s correlation of 29 CBUs showed that the pre-processing CD34+ number is significantly positive correlated to the post-processing (p=0.00, rho=0,918) and post-thawing CD34+number (p=0,002, Spearman`s rho=0,665 respectively). The recovery of colony-forming units (CFUs) recovery pre-processing vs.post-thawing was 94,5%. CD34+number is significant correlated with the CFUs in each step of processing. The mean TNC number was 77,92x107/unit with recovery was 82,37%. The mean number of annexin V and CD34 positive microparticles (AnnV+/CD34+MPs) increased significantly during post-processing compared to the number of pre-processing AnnV+/CD34+MPs.

CONCLUSION: The number of CD34+cells and AnnV+/CD34+MPs can be valuable parameters to evaluate the CBU quality.