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Abstract: 101 Oral: 101

MOLECULAR CYTOGENETIC STUDY OF MULTIPLE MYELOMA (MM)

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Background: One of the most important independent prognostic markers in patients with MM is specific chromosomal aberrations. The most frequent and prognostically most significant clonal aberrations in MM are rearrangements of IgH gene at 14q32 region (poor prognosis) and deletions of RB1 gene at 13q14 and/or loss of whole chromosome 13 (moderately adverse or medium prognosis). The translocation t(11;14)(q13;q32) is associated with longer overall survival and, in contrast to other IgH rearrangements, it is considered to be a favorable prognostic factor. Further structural and/or complex chromosomal aberrations are connected with worse prognosis. However, the detection of genetic aberrations involved in MM by conventional cytogenetic methods can be hampered by rather low proliferative index of plasma cells. Higher incidence of clonal chromosomal aberrations is found by interphase FISH (I-FISH) with specific DNA probes, nevertheless also classical FISH may be limited by the low extent of bone marrow involvement. The sensitivity and specifity of I-FISH analysis may significantly increase previous imunofluorescent labeling of malignant plasma cells. This method allows identification of chromosomal changes even in cases with low bone marrow infiltration. Aims: The aim of the study was to assess the frequency of the most significant chromosomal aberrations (abnormalities of IgH and RB1 genes) in labeled non-dividing plasma cells of patients with MM, to evaluate complex chromosomal aberrations by multicolor FISH (mFISH) and to establish correlation between molecular cytogenetics and other clinical and laboratory prognostic factors. Methods: I-FISH analyses were done on plasma cells labeled by the Amca Anti-Human kappa-chain, Amca Anti-Human lambda-chain and Amca Anti-goat IgG monoclonal antibodies (Vector Laboratories). For I-FISH directly marked locus specific DNA probes (Abbott-Vysis) were used. Detection of deletion/monosomy of chromosome 13 was performed by LSI 13q14 (RB1) and LSI 13q34 DNA probes. Aberrations of 14q32 region were proved by LSI IgH rearrangement probe and for detection of translocation t(11;14)(q13;q32) LSI IgH/CCND1 probe was used. Complex chromosomal rearrangements found by conventional cytogenetic methods were confirmed by mFISH with 24Xcyte probe kit (MetaSystems). Results: Altogether 76 newly diagnosed MM patients were examined by I-FISH. Mean age was 58,5 years (range 33-89). Deletion of RB-1 gene was found in 30% patients and monosomy 13 was identified in other 33% of them. Combination of both abberrations was proved in five cases. IgH translocation was detected in 34% of patients (deletions, partial trisomies and monosomies and numerical changes involving chromosome 14 were also found). Five out of 10 cases evaluated for t(11;14)(q13;q32) were positive. Twelve patients with complex karyotypes were examined by mFISH. Molecular cytogenetic results were correlated with laboratory and clinical data and will be presented in the poster. Conclusions: Detection of plasma cells by the imunofluorescent staining permits to increase the number of chromosomal abnormalities identified by IFISH. This method significantly contributes to the higher sensitivity and specifity of diagnostic procedures and is important for determination of prognosis and treat-

ment of MM patients. Supported by grant IGA NR/8183-4.

Abstract: 102 Oral: 102

THE EFFECTS OF CHEMOTHERAPY AND CLODRONATE TREATMENT ON BONE METABOLISM IN MULTIPLE MYELOMA PATIENTS

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Background: Pathological fractures are detected in almost 75% multiple myeloma (MM) patients due to osteoporosis and/or osteolisis. Osteoblasts activity markers are: C-terminal propertied of type I collagen (PICP), bone alkaline phosphates (BAP); Osteoblasts: C-terminal Telopeptide of type I collagen (ICTP). Aims: To assess the effects of clodronate treatment as a supportive care on bone metabolism in MM patients. Material and methods: The study group comprised 30 patients in the age 3583 years (median-59). Diagnosis was established on the base of common rules. Patients with renal failure were excluded of the study. The appearance of monoclonal protein IgG class was observed in the serum at 17 cases of the patients, IgA at 9, IgD at 1. At 2 patients was recognized light chain disease type kappa. At one case has been observed no secreting MM. Patients were in the stage of the clinical progression of the disease on base of Durie-Salmon scale: 7 patients at IA, 7-IIA, 2-IIB, 16-IIIA. In 26 (86.6%) patients bone change were detected in X-ray examination. Standard VAD (Vincristine, Adriamycin, Dexamethasone) chemotherapy treatment followed by high-dose therapy (melphalan) and auto-PBSCT were used in 17 patients. Thirteen patients were treated with conventional chemotherapy. All the patients were also treated as supportive care with clodronate-900 mg IV. every 4 weeks. Clodronate was given 3-8 times (average5) to each patients. Serum BAP, PICP, ICTP was elevated twicely-before and after the treatment (chemotherapy and clodronate). Control group was 20 healthy volunteers. Serum PICP and ICTP concentrations were analyzed by radioimmunoassays, BAP by ELISA method. All of the results have been statistically tested by using ANOVA test for the independent groups and Spearman correlation. For statistically significant results were $p<0.05$. Results: Median concentration of PICP before treatment was 115.3 µg/l (SD 71.67 µg/l), after treatment-207.32 µg/L (SD 131.72 µg/L). In control group median was 133.6 µg/L (SD 42.42 µg/L). Median concentration of BAP before the treatment was 37.8 µg/L (SD 19.82 µg/L), after treatment-38.65 µg/L (SD 18.4 µg/L). In control group median was 20.73 µg/L (SD 15.05 µg/L). Median concentration ICTP before the treatment was 3.76 µg/L (SD 10.06 µg/L), after the treatment-6.85 µg/L (SD 14.27 µg/L). In control group median was 4.72 µg/L (SD 2.43 µg/L). Concentration of PICP after the treatment were statistically significant elevated in comparison with those before the treatment ($p=0.001$), and in comparison with control group ($p=0.011$). Concentration of ICTP were statistically significant higher in comparison to control group ($p<0.05$). Concentration of BAP both before and after the treatment were statistically significant higher than in control group ($p=0.003$ and $p=0.018$ respectively). Concentration of PICP negatively correlation with clodronate dosage ($p=0.027$, $r= -0.43$). We obtained negative correlation between concentration of PICP and concentration of ICTP ($p=0,012$; $r= -0.98$). Positive correlation was observed between ICTP serum concentration and levels of phosphates ions ($p=0.032$; $r=0.96$), daily calcium secretion in urine ($p=0.043$; $r=0.95$). Results: Increasing PICP concentration can be the result of osteoblasts activate and stabilization activity of ICTP concentration may be the result of restraing osteoclasts activity followed by clodronate treatment.

Abstract: 103 Oral: 103

TREATMENT OF MULTIPLE MYELOMA WITH DOUBLE AUTOLOGOUS HAEMATOPOIETIC STEM CELL TRANSPLANTATION - A PROSPECTIVE SINGLE CENTER STUDY

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Treatment of advanced-stage multiple myeloma (MM) with high-dose therapy and autologous haematopoietic stem cell transplantation (AHSCT) improves response rates, disease-free survival (DSF), and overall survival (OS) in patients younger than 65 years when compared with con-

ventional chemotherapy. In addition transplant related mortality (TRM) is relatively low. Recent nonrandomised and randomised studies indicate survival benefit for MM patients treated with double AHSCT as compared to single AHSCT. We conducted a prospective trial of two successive high-dose therapies followed by peripheral blood stem cell rescue for patients with advanced stage MM. The aim of the study was to evaluate the feasibility, efficacy, and toxicity of the intensification with double high-dose therapy followed by autologous HSCT rescue. Patients who were pre-treated with melphalan or were treated with more than two chemotherapy regimens before AHSCT were excluded from the analysis. From December 1994 to December 2004 62 consecutive, previously untreated patients with MM stage II or III were included in the program of double HSCT. Median age of patients was 50 years (range 30-65 years), male/female ratio 32/30. After diagnosis was established they received 3 to 10 cycles of VAD therapy, few of them also other salvage regimen or thalidomide due to refractoriness to VAD protocol. After achieving partial response (less than 30% of plasma cells in the bone marrow aspirate) they proceed to mobilisation procedure with cyclophosphamide 4 g/m2 and G-CSF. Fifty four patients (87 %) actually received double transplant. Eight patients (13 %) received only single transplant due to progression of the disease, toxicity, no enough stem cells for second transplant, and low performance status or some refused second transplant. Conditioning regimen for majority of patients consisted of melphalan 200 mg/m2, 24 patients received melphalan 140 mg/m2 and fractionated TBI 800 cGy prior to second transplant. A complete or very good partial response was achieved in 55 patients (89%). With the median follow up of 38 months (range 10 to 139 months) 41 (66%) patients were alive in CR or VGPR, 12 (19%) were alive in relapse, and 8 (13%) patients died from relapse. Only one patient died from transplant related complication (2%). Survival was calculated from the time of diagnosis. Probability of EFS at 7 years after the diagnosis was 35%. Median relapse-free survival was 55 months. Probability of overall survival (OS) at seven years was 66% and the median survival was not reached. We conclude that double SCT is feasible in majority of patients, 87% of patients planned for double AHSCT actually received it. The toxicity is low even in older patient population, not higher than with single transplant. Double AHSCT achieve a favourable DFS and OS and is associated with low TRM rate. For all patients with MM in the stage II or III double AHSCT should be recommended and planned from the time of diagnosis.

Abstract: 104 Oral: 104

METABOLIZING ENZYME POLYMORPHISM STUDIES IN MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a B-cell neoplasm characterized by bone marrow infiltration with plasma cells resulting in pathological bone fractures and cytopenias. MM patients and their relatives are at high risk developing cancers other than multiple myeloma and there are literature data for the familial occurrence of the disease as well. This is why our scope was focused on searching predisposing factors rendering these subjects more susceptible for malignancies. Gluthatione S-trans-ferases (GSTs) are a large family of drug-metabolizing enzymes that participate primarily in detoxification mechanisms of genotoxic agents. The genes encoding GSTM1, GSTT1 and GSTP1 are polymorphic in human beings, and the variants of GSTM1 and GSTT1 with gene deletion do not express the enzyme. It is hypothesized that genetic polymorphism of GSTM1, GSTT1 and GSTP1 may influence the risk of the development of MM. AIMS The aim of our case - control study was to investigate the relation of GSTM1, GSTT1 and GSTP1 genetic polymorphism to the risk of MM. METHODS The study population comprised one hundred MM patients and one hundred hospitalized controls. Subtype distribution of MM patients were as follows: IgG: 63, IgA: 24, non-secretory: 3, biclonal: 1,lambda-light chain: 4, kappa-light chain: 5, IgD: 1. Controls were diagnosed for various unrelated diseases including hypertension, 2nd type diabetes, obliterative arteriosclerosis, metabolic syndromes, such as hyperuricacidaemia or hyperlipoproteinaemia. Exclusion criteria for controls were malignancies and immunpathological disorders. Mean age was 68 yrs both for the patients and their controls. The male/female ratio was 32/68 for the MM group and 45/55 for the controls group. GSTM1 and GSTT1 genotyping was carried out by multiplex PCR, GSTP1 Ile105Val genotyping by PCR-RFLP method RESULTS GSTM1 genotype frequencies were similar among cases and controls. There were 47.5 % positive genotypes, i.e. homozygous or heterozygous carrier of the gene, and 52.5 % null genotypes (homozygous

gene deletion) in the group of MM patients, and 51.5 % positive and 48% null genotypes among controls, respectively. There was no statistically significant difference between cases and controls for the GSTT1 polymorphism either, 73.3 % positive and 26.7 % null genotypes among cases, whereas 74.7 % positive and 25.3% null among controls. There was no significant difference in the genotype frequency of GSTP1 as well between the cases and the controls. The frequencies of the combined GST- genotypes were similar in cases and controls. CONCLUSIONS Our first results suggest that GSTM1, GSTT1 and GSTP1 genetic polymorphisms may not influence the risk for MM significantly in the Hungarian population. Literature data are controversial, both positive and negative findings have been reported. The available data suggest that the effect of GST genetic polymorphisms on the risk of development of MM may be weak if any and may vary in different geographical and ethnic populations that might necessitate further investigations.

Abstract: 105 Oral: 105

COMPLEMENT-MEDIATED CYTOTOXICITY OF RITUXIMAB TO A CD20 POSITIVE MYELOMA CELL LINE MC/ CAR

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Rituximab, chimera type anti-CD20 monoclonal antibody, is recognized to be effective against follicular B-cell lymphoma and used now clinically. It was examined whether it has efficacy to other type of B-cell malignancy. The anti-tumor action of Rituximab is thought to be through complement-dependent cytotoxicity (CDC) or antigen-dependent cell-mediated cytotoxicity (ADCC). We examined the CDC activity of Rituximab against a human CD20-positive myeloma cell line, and compared it with the CDC activity of a CD20-negative human myeloma cell line and a CD20-positive human cell line. Methods: The CDC activity of Rituximab against MC/ CAR (a CD20-positive myeloma cell line), U266 (a CD20negative cell line) and Pfeiffer (a CD20-positive lymphoma cell line) was examined by using Golay`s method. Namely, tumor cells at 1,500,000/mL were incubated with 20 microg/mL Rituximab for 10 minutes at the room temperature and thereafter they were incubated with 50% of normal human serum (complements) for 60 minutes at 37°. Apoptotic cells were detected by using Annexin V-FITC and propidium iodinde(PI) with flowcytometry or trypan blue staining. late apoptic cells were recognized as Annex-in V positive, PI positive cells while early apoptic cells were recognized as Annexin V positive, PI negative cells. Results: In MC/CAR cells, late apoptic cells were seen at 22%, early apoptic cells at 11.5%.and tripan-blue-posi-tive cells at22.6% after the incubation with rituximab and complement. When MC/CAR cells were incubated at 4° with 10 microg/mL of anti-CD59 antibody for 60 minutes before CDC incubation, early apoptic cells were 33.5%, that is, CDC was enhanced by anti-CD59 antibody. AntiCD55 antibody enhanced slightly CDC by Rituximab. Simultanous incubation with anti-CD59 antibody and anti-CD55 antibody enhanced the CDC by Rituximab as same as preincubation only with anti-CD59 antibody. The CDC activity by Rituximab was not seen in U266 cells. In Pfeiffer cells, late apoptic cells were 51.3%, early apoptic cells were 16%. However, Enhancement of Rituxi-mab-related CDC by anti-CD55 or anti-CD59 antibody was not clearly observed. Conclusion: MC/CAR, CD20-positive myeloma cells, showed Retuximab-related CDC activity and it was enhanced by the preincubation of anti-CD59 and antiCD55 antibody, but not by anti-CD46 antibody for 60 minutes.

Abstract: 106 Oral: 106

COMBINED ADMINISTRATION OF DARBEPOETIN AND ZOLEDRONIC ACID IN THE TREATMENT OF REFRACTORY MULTIPLE MYELOMA (MM)

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Background: Multiple myeloma (MM) is a neoplastic disease, that affects especially the elderly, even if in recent years it has also been observed in young patients. Painful osteolytic bone destruction is a frequent complication of MM. Bisphosphonate therapy has been shown to reduce complications of bone lesions in MM and in other malignancies. In details, it seems that zoledronate, a new generation aminobisphosphonate, also

exerts antitumor effects on myeloma cells; this drug has cytotoxic activity because it causes apoptosis and block of the proliferation. Another frequent issue in patients with MM is anemia that is due both to disease progression and chemotherapy. Aims: We have evaluated a combined administration of darbepoetin and zoledronic acid in the treatment of a subset of patients affected by refractory multiple myeloma in order to investigate the activity of the combination on the quality of life and survival. Methods: In our institution we are following 25 patients with stage II/III MM and 20 out of 25 are currently treated, independently from the adopted chemotherapy, with zoledronate because they had osteolytic bone lesions at the diagnosis. At 6 months from the beginning of the treatment (4mg i.v. every 28 days) all the 20 patients had a partial or complete regression of bone lesions. However, five out of 20 patients (4 F and 1 M, median age: 65 years, r.: 62-77 years), suspended chemotherapy after 12 cycles of Melphalan and Prednisone regimen for excessive toxicity even if they presented steady disease (SD) at clinical re-staging performed with cytological examination of bone marrow blood and of serum markers. On the basis of the anemia recorded in these patients, (median Hb: 8.2 g/dl, r. 7.8 - 9.2) they underwent a treatment with 500 micrograms s.c. darbepoetin every 21 days together with 4 mg i.v. zoledronate every 28 days. Results: After 6 weeks all the 5 patients had an increase of haemoglobin (median: +1.5g/dl, r.: +1.2 - 2). Interestingly, at a clinical re-staging performed after five months from the beginning of Zoledronate-Darbepoietin combined administration a partial remission was recorded in 4 out of 5 patients while the remaining was in SD. Conclusions: It is well known that myeloma cells compete with normal progenitors, and above all with erythroid precursors, for the same marrow microenvironment. The stimulation of erythroid precursors with growth factors in addition to the concomitant anti-proliferative effects exherted by zoledronate on myeloma cells likely induce the antitumour effects observed in these patients.

Abstract: 107 Oral: 107

IDENTIFICATION OF A NOVEL MUTATION IN A TURKISH FAMILY WITH PYRIMIDINE 5` NUCLEOTIDASE-1 DEFICIENCY

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Background: Pyrimidine 5` nucleotidase-I (P5N-1) deficiency is a rare autosomal recessive disorder characterized by hemophilic anemia, marked basophilic stippling and accumulation of high concentrations of pyrimidine nucleotides within the erythrocyte. P5N-1 gene has been identified and thirteen different pathogenic mutations have been described in the gene to date. We have previously reported three novel mutations in 4 unrelated Turkish families. Aims: The purpose of this study was to continue spending effort to define the mutational spectrum of P5N-1 gene by evaluating the molecular defect underlying the P5N-1 enzyme deficiency in a Turkish family. Methods: P5N-1 enzyme deficiency was detected by the method using red cell purine/pyrimidine ratio. Mutation analysis of P5N-1 gene was performed by PCR amplification of all coding sequences of the gene (exons 310) on genomic DNA and SSCP/HD analysis of PCR products. Aberrant bands detected in the analysis were evaluated for mutation by sequencing the related exon. The family members were screened for the mutation identified by developing specific PCR-RFLP method using restriction enzyme BseLI. Results: The 24 year old propositus is the first product of a consanguineous marriage. She was referred to the Hacettepe University Children`s Hospital for evaluation of the possible enzyme defect underlying chronic congenital hemolytic anemia. The propositus had 3 siblings. The patient and one sibling were found to have decreased purine-pyrimidine ratio. The affected sibling with mild chronic hemolytic anemia had retinitis pigmentosa that is another hereditary disorder unlinked to P5N-1 gene. Screening of all coding exons of the P5N-1 gene by SSCP analysis revealed aberrant bands in only exon 5 of both cases. Characterization of aberrant band by sequencing enabled the identification of a homozygous substitution of T220C (TGT>CGT) leading to the replacement of Cys with Arg at codon 74 (C74R). Screening of family members for this novel missense mutation by PCR-RFLP analysis revealed that two affected siblings were indeed homozygous, both parents, one of the health siblings and the son of the proband were heterozygous, other healthy sibling was normal for the mutation. Summary/Conclusions: This novel mutation was the forth distinct pathogenic sequence variation of P5N-1 gene in a total of 4 unrelated families, indicating that mutations related to this gene are quite heterogeneous in Turkish population. This study also shed some light into the diagnostic impor-

tance of mutation detection in P5N-1 gene especially in cases with atypical mild clinical phenotype. At the same time, it may be important to emphasize the variability of clinical manifestation of the disease in patients with the same mutation. gbalta@hacettepe.edu. tr This study was supported by Hacettepe University Research Fund (Project No: 02G116).

Abstract: 108 Oral: 108

POSITIVELY CHARGED ALPHA-CHAINS CAN STIMULATE K-CL COTRANSPORT IN TRANSGENIC MOUSE RED CELLS

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Transgenic mice were generated with human alpha-chain anti-sickling mutations at contact sites for the HbS polymer. Some of these mice were found to have elevated K-Cl cotransport. Elevation of K-Cl cotransport in patients with homozygous HbS, HbC, or SC increases red cell MCHC and contributes to pathology. C57Bl mouse red cells (mRBC) and mRBC expressing only HbA have little volume-stimulated K-Cl cotransport. In contrast, we previously reported that mRBC expressing HbC have increased K-Cl cotransport and MCHC. We report here that positively charged alpha-chains also stimulate K-Cl cotransport in mRBC. Mice expressing alpha-chain mutants were generated: alpha49 (HbSavaria, alpha49S› R, +1 positive charge vs human alpha); alpha49-114, that expresses both alpha49 and alpha114 (HbChiapas alpha114P›R, +2); and alpha20-114 (that has no average charge difference from human alpha). Mice were bred with alpha-KO mice to produce mice expressing various levels of mutant alpha and mouse globins. We previously reported volume-stimulated K-Cl cotransport in C57Bl and HbAKO mice (that only express HbA) as 2.0±0.9 and 2.4±1.7 mmol/L cells x hr (FU) respectively. We found a similar value (1.7±1.4 FU, N=9) in mRBC expressing either 32% or 100% alpha20-114 (no charge difference from human alpha) that was not statistically different from C57 or HbAKO. mRBC expressing alpha49 at 44 or 100% had an average value of 10.8±2.4 FU, N=9; similarly, mRBC expressing 48% alpha114-49 averaged 8.5±1.3 FU, N=6; both of these differ from alpha20-114 with a p value >10-7. These results demonstrate that positively charged alpha-chains cause an increase in K-Cl cotransport while mutant alpha-chains without a charge difference do not. Density gradients detected increased red cell density relative to wild type mice (C57Bl) in mice expressing alpha-globins with a positive charge relative to human alpha. Advia measurements demonstrated that mRBC without added positive charge have normal MCHC and those with added positive charge have increased MCHC. These observations are consistent with the significantly increased intracellular potassium concentration found in alpha20-114 mRBC vs alpha49 and alpha114-49 mRBC that had 1250±89 vs 1110±64 and 1050±103 mmol/ Kg Hb with P>0.02 and 0.002, respectively. We conclude that a positive charge in excess of that found on human alpha plays a role in the acitvation of K-Cl cotransport and leads to increased MCHC in mRBC. In the presence of HbS, this effect results in mouse red cells with properties similar to human SC disease and prevents the birth of mice that are fully knocked out despite the presence of anti-sickling mutations. These observations could only have been made by creating a mouse model and imply that charge must also be considered when anti-sickling globins are proposed.

Abstract: 109 Oral: 109

COMPREHENSIVE ALPHA- AND BETA-THALASSEMIA GENOTYP-ING BY MEANS OF REVERSE-HYBRIDI-ZATION TESTSTRIPS

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Alpha- and beta-thalassemia (thal) are among the most common inherited diseases throughout Southeast Asia, India, the Middle East, parts of Africa and the Mediterranean area. Mutations in the beta-globin gene, or in one or both of the two alpha-globin genes, are leading to structural abnormalities (e.g. sickle cell anemia) or to haemoglobin imbalance due to the reduced synthesis or

complete absence of the respective globin chains. Unlike the prevalence of point mutations in beta-thal, the majority of alpha-thal alleles are derived from single or double gene deletions. We have developed reverse-hybridization assays (StripAssays) for the rapid and comprehensive genotyping of alpha- and beta-thalassemia. The tests are based on multiplex DNA amplification (including gap-PCR) and hybridization to teststrips presenting a parallel array of allele-specific oligonucleotide probes for each variant. The entire procedure from blood sampling to the identification of mutations requires less than 6 hours, and hybridization/detection may be carried out manually or essentially automated using existing instrumentation. The tests are simple and convenient, and require very small amounts of samples, which is of particular importance for prenatal diagnosis. Although the spectrum of alpha- and beta-thal mutations is known to be highly population-specific, the broad range of variants covered by the StripAssays should make them globally useful diagnostic tools.

Abstract: 110 Oral: 110

FREQUENCY OF ANTI-ERYTHROPOIETIN ANTIBODIES IN PATIENTS WITH END STAGE RENAL DISEASE TREATED WITH RECOMBINANT ERYTHROPOIETIN

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End Stage Renal Disease (ESRD) manifests hematologic, cardiovascular, inflammatory, and immunologic dysfunctions. Erythropoietin deficiency is present in ESRD leading to anemic states. ESRD patients receive regular/repeated administration of commercially available recombinant erythropoietin (r-E) (Epogen(r), Procrit(r)). Patients treated with r-E have been known to develop thrombotic complications during r-E therapy. Antibodies to erythropoietin are generated in some patients and lead to erythropoietic aplasia. The mechanisms of the anti-erythropoietin antibody induced aplasia and reported hypercoagulability state in patients treated with r-E are not fully understood. Anticoagulant drugs such as heparin are used to reduce the incidence of thrombotic complications during r-E therapy. To understand pathogenesis leading to hypercoagulable state and its potential link with r-E treatment, blood samples taken from a group of 60 patients with ESRD were drawn prior to initiation of antithrombotic therapy. These patients on periodic hemodialysis were administered r-E 6-8 weeks prior to blood drawn. Anti-erythropoietin antibody titer, anti-phospholipid antibody (APA) titer, CRP levels, fibrinopeptide-A (FPA), nitric oxide (NO) levels, asymmetric 1,3-dimethylarginine (ADMA) levels, and thrombin activatable fibrinolytic inhibitor (TAFI) levels were measured. Five of 60 patients (8.3%) showed higher titers of anti-erythropoietin antibodies. Three of 60 patients (5%) exhibited positive titers of anti-phospholipid antibodies. Marked elevation of the CRP, FPA, NO, ADMA, and TAFI levels were noted in comparison to match controls. Patients with positive levels of anti-erythropoietin antibody titer (3 to 5 folds) exhibited a simultaneous elevation of FPA, CRP and TAFI suggesting the potential role of anti-erythropoietin antibody in mediating these responses. These observations indicate that ESRD patients treated with r-E are at a higher risk of developing thrombotic complications. The upregulation of NO and ADMA in these patients is suggestive of ongoing inflammatory processes. Anticoagulants such as low molecular weight heparins may be useful in the management of these patients.

Abstract: 111 Oral: 111

EVALUATION OF RETICULATED AND ACTIVATED PLATELETS IN SICKLE CELL DISEASE

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Sickle cell disease (SCD) is considered a "hypercoagulable state". Several components of haemostasis are altered, and an increase in the adherence of sickle erythrocytes to vascular endothelium, mediated by adhesion receptor and other cellular elements of the blood, contribute to the development of vaso-occlusive crisis (VOC). Reticulated platelets (RP) are newly formed platelets that contain some rough endoplasmic reticulum and mRNA. RP measurement has had considerable clinical utility for monitoring thrombopoiesis and platelet turnover. The present study was performed to evaluate RP and their degree of activation in patients with SCD. Sixty-one adults with

SCD were studied: 22 in steady phase, 21 in hemolitic crisis (HC) and 18 in VOC. Platelet evaluation: platelet count, Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Platelet Larger Cell Ratio (PLCR) were determined by the Sysmex XE-2100 (Sysmex Inc., Kobe, Japan). RP were determined by flow cytometry in platelet-rich plasma. Platelets were identified by anti CD41-PeCy5 monoclonal antibody and RP were stained for RNA with thiazole orange (TO). Platelet activation was monitored using anti CD62P- PE. The action of interleukin on thrombopoiesis was verified by determination of serum IL-6 levels. Control group: thirty healthy subjects. Results: In relation to control group it was observed: the number of platelets was higher in SCD patients; MPV and % of P-LCR were lower in steady SCD patients. Those more numerous and small size platelets in steady phase probably are consequent to the lack of splenic sequestration due to functional asplenia. The absolute number of RP (TO+) and activated platelets (CDP62+) were significantly higher ($p < 0.005$) in the three phases of SCD when compared with control group, but not among patient subgroups. Using double fluorescence (anti-CD 62P-PE and TO) it was possible to determine the absolute number of activated RP. SCD patients presented higher number of activated RP than controls ($p < 0.0002$). Serum IL-6 levels were significantly higher in SCD patients (HC and VOC subgroups higher than steady phase) than in control group. It was not observed correlation between IL-6 levels and platelets parameters, suggesting that IL-6, although significantly increased, does not have a direct action on thrombopoiesis activity in SCD. It was shown a significant correlation between CD62P+ platelets and RP in percentage and absolute numbers, suggesting that those youngest platelets exhibit some activation degree. The increase of RP in SCD indicates an elevated activation-dependent turnover of platelets. Those activated platelets release thrombospondin and fibronectin, leading to further red cell adherence, potentially enhancing the microvascular occlusion. Patients with SCD present two factors, among others, that contribute to adhesion of sickle cells to endothelial cells: high number of reticulocytes, particularly active in adhesion mechanism, and young activated platelets. Our data confirm that platelet activation, potentially increased by activation from youngest platelets, is present in SCD and suggest that elevated number of activated RP participate in the cellular adhesion process and in the occurrence of vasocclusive episodes and of thrombosis. (Supported by FAPESP no. 02/13801-7)

Abstract: 112 Oral: 112

CLINICAL AND LABORATORY VALIDATION OF THE 2ND INTERNATIONAL STANDARD OF LOW MOLECULAR WEIGHT HEPARIN (IS 2003-01/608) IN THE ANTI-XA, ANTI-IIA HEPTEST AND PROTHROMBINASE INDUCED CLOTTING (PICT) TESTS

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Background: Because of the relative insensitivity of the global clot based assays such as the activated partial thromboplastin time (APTT), the low molecular weight heparins (LMWHs) are potency evaluated/optimized in the anti-Xa (AXa) and heptest. A new clot based assay namely, prothrombin induced clotting time (PiCT) is sensitive to the anticoagulant effects of LMWHs and related drugs. As the LMWHs are standardized using the anti-Xa methods, using the International Standards, this study was designed to cross validate the 2nd International Standard for LMWHs(NIBSC 01/608) in various assay methods. Methods: Commercially available LMWHs, Dalteparin (D), Enoxaparin (E), Tinzaparin (T) and the 1st International Standard (85/600) were crossed referenced against the 2nd International Standard (NIBSC 01/608) using an amidolytic AXa method. Each of these LMWHs were compared in the AXa, adjusted concentration range of 0-1.0 U/ml using the Heptest, AXa, AIIa and PiCT. In addition plasma samples from patients receiving a LMWH, E for therapeutic and interventional purposes were measured using various tests. Results: The AXa potency adjusted LMWHs (D,E, and T) and 1st International Standard provided superimposable concentration curves in the amidolytic AXa assays. However marked differences in the heptest and PiCT were noted. Major differences were noted in the AIIA levels, even between the two International standards. When patients samples (n=75) from a therapeutic trial (1.0 mg/kg BID/SC) were evaluated, assay based differences were further amplified. The amidolytic AXa assay consistently measured higher AXa levels. When the two standards were cross-referenced with one another in different assays, major differences were noted in the clot-based

assays. Even in the AXa assay at equivalent AXa levels, differences were obvious. Conclusions: These results suggest that both of the International Standards of LMWH are valid for only the cross standardization of the AXa activities of LMWHs. If any of the other methods were used, significantly different results were obtained with each of the individual LMWHs. Thus, the 2nd International Standard should only be used for amidolytic AXa assay for potency referencing purposes. Moreover, the stated potency of the 2nd Internation Standard may need to be readjusted against the 1st Standard to obtain valid results. These standards are of limited value in the clinical monitoring of LMWHs. It is therefore proposed that each of the LMWHs should be cross referenced by its own standard and the clinical monitoring of these drugs should only be carried out utilizing the specific drug used in a given patient. The PiCT test offers a global test which is capable of monitoring the effects of all components of heparins regardless of their affinity to serpines. Moreover, the effect of TFPI released on clotting processes is also measured. Thus, this test provides a physiologically relevant anticoagulant index.

Abstract: 113 Oral: 113

ANTI-ADAMTS-13 IGG ANTIBODY TITERS IN IDIOPATHIC TTP - EVALUATION OF A RAPID DETECTION METHOD (TECHNOZYM(r) ADAMTS-13 INH ELISA)

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Objectives: The current study evaluates the suitability of the TECHNOZYM(r) ADAMTS-13 INH ELISA for the management of patients presenting with symptoms of idiopathic thrombotic thrombocytopenic purpura (TTP). In up to 90% of these patients the underlying deficiency of the von Willebrand factor (vWF) cleaving protease ADAMTS13 is caused by autoantibodies. Therefore, early diagnosis of these antibodies and control of plasma exchange therapy is essential. Materials and Methods: The TECHNOZYM(r) ADAMTS-13 INH assay is based on a ELISA, determining the binding of circulating antibodies to immobilized recombinant ADAMTS-13. Results can be obtained within ~ 2 hours and thus timely enough to allow proper treatment in cases with antibody induced TTP. This assay has intra- and inter-assay variations of less than 7% and is linear over a wide range of dilutions of samples containing high anti ADAMTS-13 antibodies ($r > 0.99$). IgG antibody titers are obtained in arbitrary units (aU/ml). Results: TECHNOZYM(r) ADAMTS-13 INH ELISA specifically measures IgG antibodies directed against ADAMTS-13, because samples from patients with non-ADAMTS-13 autoantibodies such as Lupus patients or patients with increased IgG levels (Gammopathies) have titers not significantly different from healthy controls. In a pilot study, patients with acute and non-acute idiopathic TTP were tested. Both groups had significantly higher titers of anti-ADAMTS-13 IgG antibodies as com-pared to normal controls and the antibody titer in acute TTP was higher than in non-acute TTP. A second study to confirm these data is presently ongoing. Conclusion: The TECHNOZYM(r) ADAMTS-13 INH ELISA could be a valuable tool to confirm the diagnosis of idiopathic TTP as well as to control the course of antibody depletion or reoccurrence of antibodies against ADAMTS-13.

Abstract: 114 Oral: 114

ANTICARDIOLIPIN ANTIBODIES IN CHILDREN WITH HELICOBACTER PYLORI INFECTION

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Anticardiolipin antibodies (aCL) are antibodies directed against cardiolipin, which is a negative charged phospholipid in the organism. Anticardiolipin antibodies were associated with thrombosis and found to have an important role in the pathogenesis of thrombosis associated with autoimmune diseases like systemic lupus erythematosus and antiphospholipid syndrome. In addition, the presence of aCL has been reported in some infectious diseases, especially during the course of viral infections. Helicobacter pylori (H. pylori) has been reported as an etiological agent in chronic gastritis, gastric and duodenal ulcers, gastric malignancies, and iron deficiency anemia with an increasing prevalence in developing countries. Recently, it has been suggested that H. pylori infection may play a role in the pathogenesis of

some autoimmune diseases such as rheumatoid arthritis, chronic thyroiditis, immune thrombocytopenic purpura, and antiphospholipid syndrome. We investigated a possible relationship between H. pylori infection and aCL and initially aimed to determine the prevalence of anti-cardiolipin antibody positivity in children with H. pylori infection. This study was performed on 84 children (44 F/ 40 M) suffering from abdominal pain, anorexia, nausea and/or vomiting and diagnosed to have H. pylori infection on the basis of positive stool soluble H. pylori antigen test and C14 urea breath test. Serum levels of aCL IgG, IgA and IgM were measured before and 30 days after the treatment (clarithromycin + amoxicillin for 15 days and omeprazole 30 days). Pre-treatment aCL IgG, IgA, and aCL IgM levels were significantly higher than post-treat-ment and control values (Table 1). No complication was observed in patients with high anticardiolipin antibodies. Although aCLs were shown to constitute predisposition to thrombosis there was no clinical implication on children with H. pylori infection and high titers of aCLs. However, the presence of H. pylori infection should be considered in particular conditions with aCLs. The effects of H. pylori infection are not limited to gastrointestinal system and the onset of the systemic effects may extend to childhood. Thus, the eradication of H. pylori in children is exclusively important. In our particular experience, H. pylori was shown to cause aCL positivity in children and eradication of H. pylori provides the disappearance of these antibodies. Early and late clinical importance of these findings should be investigated in further large-scale studies.

Abstract: 115 Oral: 115

PREVENTION OF RECURRENT MISCARRIAGE SYNDROME WITH LOW MW HEPARIN AND DETECTION OF ACQUIRED AND HEREDITARY COAGULANT DEFECTS

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Thrombotic occlusion of placental vessels, both venous and arterial, affects fetal nutrition and viability leading to fetal miscarriage. The thrombotic defects associated with fetal wastage are thought to result from thrombosis of early placental vessels. Peak fetal wastage occurs mainly in the first trimester, but some cases also occur in the second and third t rimesters. The object of this paper is to present the results of the low MW heparin treatment of pregnant women with history of at least two fetal miscarriages, and the investigation in these patients of its association with anti-phospholipid syndrome or congenital deficiencies of blood coagulation proteins that could explain fetal wastage. MATERIALS AND METHODS: Sixty eight (68) pregnant women with diagnosis of recurrent miscarriage were studied. The patients were followed with daily fetal activity chart starting at week 28, biophysical profile and Doppler flow of the umbilical artery at week 32, 34, 36, and 38, and delivery at discretion of the obstetrician. The following tests were performed: hematology, prothrombin time, PTT, thrombin time, fibrinogen level, lupus anticoagulant determination, antinuclear antibodies, anti-DNA antibodies, VDRL, anticardiolipin antibody (lgG and IgM), antiphospholipid antibodies, determination of protein S and protein C antigenic and coagulant, plasminogen levels, antithrombin levels. The patients were treated with aspirin (100 mg daily), low weight heparin (40 mg subcutaneously or 5000 U per day), prenatal vitamins, iron, and folic acid. RESULTS: All patients had a viable fetus except one patient with associated HELLP syndrome, and two patients who received treatment only for two months and one month, respectively. The following abnormalities were found (19% of patients): antinuclear antibodies and anti-DNA antibodies (2 patients), only antinuclear antibodies (1 patient), antinuclear antibodies and antiphospholipid antibodies (2 patients), antinuclear antibodies and anticardiolipins (2 patients), protein C deficiency (3 patients), proteins C and S deficiency (1 patient). CONCLUSION. This study shows that the recurrent miscarriage syndrome may be associated with acquired or congenital hemostatic defects and the use of low molecular heparin and aspirin during pregnancy in women with history of recurrent mis-car-riages prevent fetal wastage probably due to prevention of thrombosis in placental vessels.

Abstract: 116 Oral: 116

INCORRECT USE OF THROMBOPROPHYLAXIS FOR VENOUS

THROMBOEMBOLISM: RESULTS OF A MULTICENTRIC, OBSERVATIONAL AND CROSS-SEC-TIONAL STUDY IN BRAZIL

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Inroduction: Although effective strategies for the prevention of venous thromboembolism (VTE) are widely available, a significant number of patients still develop VTE because appropriate thromboprophylaxis is not correctly prescribed. We conduced this study to estimate the risk profile for VTE and the employment of adequate thromboprophylaxis procedures in patients admitted to hospitals in the State of São Paulo, Brazil. Methods: Four hospitals were included in this study. Data on risk factors for VTE and prescription of pharmacological and non-pharmacological thromboprophylaxis were collected from 1454 randomly chosen patients (589 surgical and 865 clinical). Case report forms were filled according to medical and nursing records. Physicians were unaware of the survey. Three risk assessment models were used: American College of Chest Physicians (ACCP) Guidelines, Caprini score and the International Union of Angiololy Consensus Statement (IUAS). The ACCP score classifies VTE risk in surgical patients and the others classify VTE risk in surgical and clinical patients. Contingency tables were built presenting the joined distribution of the risk score and the prescription of any pharmacological and non-pharmacological thromboprophylaxis (yes or no). Results: According to the Caprini score, 57% of the patients were at the highest risk for VTE, and 29% of these patients were not prescribed any thromboprophylaxis. Considering ACCP and the IUAS, 37% and 29% of the patients under moderate, high or highest risk did not receive prescription of thromboprophylaxis, respectively. In contrast, 26.8% and 42.3% of the patients at low risk of VTE according to Caprini and IUAS, respectively, were receiving thromboprophylaxis with either unfractioned or low-molecular-weight heparins. Conclusion: Despite the existence of several guidelines, adequate thromboprophylaxis is not correctly prescribed: high risk patients are undertreated and low risk patients are overtreated. This situation determines a necessity to review its reasons to ensure that patients receive the care they need.

Abstract: 117 Oral: 117

ASPIRIN RESISTANCE FREQUENCY: A PROSPECTIVE STUDY OF 175 HEALTHY MALES

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Aspirin is the cornerstone of antiplatelet therapy in cardiovascular medicine today. However, aspirin has been shown to have variable antiplatelet activity in individual platelets. Previous studies have estimated that 5% and 45% of the population do not achieve an adequate antiplatelet effect from aspirin. This led us to prospectively evaluate the frequency of aspirin resistance among healthy males. A total of 175 participants who were > 19 years old and taking 100 mg of aspirin >7 days were investigated. Demographic information (age and cardiac risk factors; tobacca use, obesity, hyperlipidemia, family history of coronary arter disease) and laboratory data (blood count, erythrocyte sedimentation rate, fasting glucose and lipids) were collected. Aspirin resistance was detected by optical platelet aggregometry, a widely accepted method by using adenosine diphosphate(ADP) and arachidonic acid (AA). Aspirin resistance was defined as a mean aggregation of > 64% with 5 micromole ADP and a mean aggregation of >20% with 0.5 mg/ml. Aspirin semiresponders were defined as meeting one, but not both of the above criteria. 28% of the participants were aspirin-resistant, 62 % were aspirin-semiresponder and 10% were aspirin-sensitive.The aspirin-resistant individuals were older than the semiresponders ($p< 0.01$), had higher erytrocyte sedimentation rate than the semiresponders ($p< 0.01$) and sensitive individuals ($p<0.05$) and had higher platelet count than the semiresponders ($p< 0.05$).There were no significant differences between the groups when comparing tobacca use, obesity, family history of coronary arter disease as well as levels of hemoglobin, leucocyte, serum tryglyceride, HDL-choles-terol and glucose. Aspirin resistance is an important and real clinical diagnosis. Our study demonstrates it to be particularly important given the high frequency of aspirin resistance in our study population. The availability of safe, alternative long-term antiplatelet agents makes screening

for aspirin resistance, especially in cardiovascular and cerebral patients.

Abstract: 118 Oral: 118

ARE THE BLOOD PRODUCTS SAFE AS YOU EXPECT-ED-EXPERIENCES IN JAPAN CLEARLY INDICATE THE NEEDS FOR PATHOGEN INACTIVATION RATHER THAN LIMITED KNOWN PATHOGEN TESTING

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Background: The safety of the blood supply has been improved greatly since the implementation of donor screening and tests for known viruses such as HIV, HBV, HCV, and HTLV. Despite improved serological tests and the introduction of Nucleic Acid Test (NAT), there is still a risk of disease transmission through blood transfusions. The recent report of transfusion-transmitted HIV, HBV and HCV infection in Japan even after screening by NAT highlighted the vulnerability of today`s blood supply. The risk of transfusion-transmitted disease (TTD) is of particular concern because of the need for safety in the supportive care for patients that are immunocompromised. Aim: There were many suspected cases of TTD, which cannot be confirmed by blood products. In Japan, Japan Red Cross (JRC) archived 5 ml blood at each donation and stored frozen for 10 years. When any suspect case occurs, JRC can trace the blood product to the donor and confirm whether the disease is transmitted by blood transfusions. JRC followed these cases. To evaluate the improvement of test method, these cases were compared with serological test, mini-pool NAT and single NAT. Results: There were significant improvements regarding post transfusion hepatitis (PTH) since 1964. PTH was reduced from 51% to 16% by using only volunteer donors, to 14% with introduction of HBV serological test, and to 2.1% with introduction of HCV serological test. With an improved antibody assay, HCVAb2/HBcAb combination, PTH was lowered to 0.5%. Many cases of PTH were still reported. In 1999, JRC introduced NAT for HBV, HCV and HIV as well as HTLV I/II. Because of the 100-fold increase in test sensitivity, NAT was thought to be the best method to improve the safety of blood products. However, after introduction of NAT for HBV/HCV and HIV, there were over 100 cases/year for HBV and HCV and more than 10 cases of HIV through transfusion of NAT-negative blood. A recent case in Japan revealed that there are HBV carriers whose viral load is less than 40 copies, a level capable of inducing disease in transfusion recipients but can not be detected by even single donor NAT. This donor donated over 12 times and transmitted HBV to 8 transfusion recipients. Conclusions: With increased travel around the world and the lack of routine tests for emerging and migrating pathogens, more and more donors are being deferred, resulting in a significant impact on the donor population. We believe that it is time to change our paradigm from pathogen detection to pathogen inactivation. With pathogen inactivation one will not need to keep adding new tests whenever a new pathogen comes along that posts a risk to the blood supply. With pathogen inactivation the risk of bacteria contamination at the time of harvest can also be eliminated. Several pathogen inactivation technologies are under development for blood products. Intercept blood system is the only system that can inactivate pathogens in platelet, plasma and red cells.

Abstract: 119 Oral: 119

ALLOGENEIC LEUKOCYTES IN BLOOD TRANSFUSIONS INCREASE POSTOPERATIVE DEATH AFTER CARDIAC SURGERY DUE TO INFECTIONS

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BACKGROUND Leukocytes in allogeneic blood transfusions are believed to be the cause of immunomodulatory events. In two randomized controlled trials comparing standard, packed cells (PC) with leukocyte-depleted erythrocytes (LD), there was a dose-dependent and significant difference in favor of LD on the incidence of infections and mortality in patients undergoing cardiac surgery. AIMS To study the mechanism of increased mortality after cardiac surgery, we performed an extended analysis of data from these two randomised controlled trails. METHODS Patients in two unviversity hospitals in the Netherlands undergoing cardiac surgery were randomised to receive PC or LD, when transfusion was needed. The patients were followed for 60 days. The endpoints of this study were: onsewt of

infections, type of infections, cultured micro-organisms, causes of deaths and survival of patients with and without infections. RESULTS Of 1103 patients randomized patients (PC: 551 versus LD: 552), 1024 patients (92.8%) received one or more transfusions. Postoperative infections were significantly lower in the LD group (PC: 33.8 % versus LD: 23.7%, Odds ratio [OR]:1.64, 95% confidence interval [CI]:1.26-2.13, p<0.01). Although cultured micro-organisms and the type of the infections were similar in both randomization arms. In particular late onset infections were increased in the PC group. Dose-related with the number of transfusions, mortality was lower in the LD group (PC: 9.3% versus LD:5.4%, OR:1.78, 95% CI:1.11-2.83, p=0.02). Multiple-organ-dysfunction-syndrome (MODS) combined with infections was the most important cause for the difference in survival between PC and LD. CONCLUSIONS This study shows a dose-dependent deleterious effect of leukocytes in erythrocyte transfusions during cardiac surgery leading to more postoperative infections and a higher mortality rate due to infections associated with MODS. These results suggest that donor leukocytes have a longstanding effect and are relevant for the complications and outcome after cardiac surgery.

Abstract: 120 Oral: 120

RANTES AND TRANSFORMING GROWTH FACTOR B1 IN PLATELET CONCENTRATES AS PREDICTORS OF FEBRILE NON HEMOLYTIC TRANSFUSION REACTIONS IN CHILDREN

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Platelet-derived biologic response modifiers (BRMs) including transforming growth factor ß1 (TGF-ß1) and regulated upon activation, normal T-cells expressed and presumably secreted (RANTES) accumulate in platelets components during storage because of platelet activation and they may play a causative role in febrile non - hemolytic transfusion reactions (FNHTRs).OBJECTIVES:To study the levels of RANTES and TGF-ß1 accumulation in platelet (PLT) units and relation to in-vivo development of FNHTRs in transfused patients as well as their relation to the duration of platelet storage, use of leukofilters and type of platelet preparation.STUDY DESIGN: The study included 60 patients attending the Pediatric Hematology/Oncology Unit,Children`s Hospital, Ain Shams University, Cairo, Egypt.The patients were receiving platelet concentrates for hematological diseases:31 oncological patients and 29 non-oncological patients.Pre-transfusion investigations included blood grouping,complete blood count (CBC),as well as investigations to exclude sepsis and peripheral platelet destruction. Post transfusion follow up was done for 24 hours with calculation of platelet increment and corrected platelet count increment (CCI).Chemokine levels were assessed in transfused platelet units using the commercial enzyme-linked immunosorbant assay kits supplied from Diaclone Research,France.RESULTS:FNHTRs developed in 17 out of 60 transfused patients. Corrected platelet increment (CCI) was significantly higher in single donor compared to random donor PCs (P< 0.01),and CCI was significantly lower in patients who had FNHTRs compared to those who did not have reactions (P< 0.05). CCI was signicantly higher in non-oncological compared to oncological cases (P< 0.01).The levels of RANTES and TGF-ß1 in platelets concentrates (PCs) were significantly increased in patients who developed FNHTRs compared to patients who did not have FNHTRs (P< 0.05, for both). Leukofiltration has significantly reduced the levels of TGF- ß1 (P< 0.05) but the levels of RANTES did not reach a significant reduction.The levels of both chemokines increased with storage duration, however the levels of RANTES were significantly correlated to the duration of storage (r=0.881, P< 0.05),while TGF-ß1 was not correlated.There was significant correlation between both RANTES and TGF- ß1 in PCs and the PLT counts in PCs (r=0.250, P< 0.05 and r=0.324, P< 0.05,respectively).There was significant correlation between levels of both RANTES and TGF-ß1 in PCs and the corrected platelet increment (r=0.471, P< 0.05 and r=0.256, P< 0.05,respectively).There was no correlation between levels of RANTES and TGF-ß1 in PCs (r=0.035,P>0.05). CONCLUSION:RANTES and TGF-ß1 may play a key role in the pathogenesis of FNHTRs, leukofiltration might be useful for reducing their levels.In addition, single donor platelets should be encouraged for use.

Abstract: 121 Oral: 121

MEMBRANE PROTEIN ALTERATIONS IN CPDA-PRE-SERVED RED BLOOD CELLS

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Background: Several kinds of RBC anticoagulant-preservative solutions have been designed, in order to achieve increased cell viability for transfusion. Among them, the CPDA anticoagulant solution (composed of sodium citrate, citric acid, dextrose, monobasic sodium phosphate and adenine) has been employed worldwide. Red cells maintained in CPDA are viable for transfusion for up to 35 days after collection, because theretofore the RBC metabolism and the membrane keep satisfactory activity and integrity levels. Aims: The determination of the protein composition of RBC membrane in CPDA-pre-served cells in the course of transfusion period storage. The alterations that may be found would be utilized in subsequent studies for the optimizing of blood bank cells viability and transfusion effectiveness. Methods: RBC concentrates from five males eligible blood donors were prepared according to the standard operating procedures. Each RBC unit was followed up during the storage period of 35 days and shortly afterwards (samples prepared from days 2, 4, 8, 10, 17, 23, 28, 35 and 43 after collection day 0). The RBC ghosts of days 0-2 of these units, in addition to freshly prepared ghosts from 10 healthy subjects, were used as controls. Total ghosts and membrane skeletons were analyzed by SDS-PAGE densitometry and immunoblotted against a variety of erythroid-specific antibodies. Results: Disturbed electrophoretic ghost patterns were found in several RBC samples during CPDA preservation. Spectrin was present at the lowest normal levels during storage. The previously reported first storage days "cell shock", was found to be coupled with deficiencies in proteins band 3 and ankyrins, a variation which is subsequently regularized by day 17. The first days of storage the ghosts were also featured by a pathological increase of bound hemoglobin that followed a slight decline during the middling storage days, before it was picked up again on the way to the expiration day. The protein band 8 principally follows the fluctuations of membrane-bound hemoglobin during the storage period. By immunoblotting, several HbA-positive aberrant bands represented globin multimers and probable cross-linkings with other membrane proteins like spectrin and actin were revealed. The membrane skeletons were also characterized by hemoglobin binding above the normal range, especially the fourth day and the days 23 to 43, aberrant zones and tryptic peptides of spectrin. Conclusions: We conclude that the protein alterations of RBC membrane during the first days of banking in CPDA are associated with the spherocytosis-type physiological and morphological transformation that those cells undergo. Deficiencies of ankyrin or band 3 underlie the majority of hereditary spherocytosis cases. The augmentation of membrane and skeleton bound hemoglobin along with the other protein aberrations, are possibly indicative of membrane defects that signify the middling storage days in CPDA as more efficient for transfusion against the very first or final ones. This study was supported by the "Empirikion Foundation" and the Special Account for Research Grants of the University of Athens to I. S. Papassideri.

Abstract: 122 Oral: 122

INACTIVATION OF VIRUSES, BACTERIA, PROTOZOA, AND LEUKOCYTES IN PLATELETS AND PLASMA USING COMPOUNDS TARGETED TO NUCLEIC ACID

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Background: Collection of blood during the window period is likely the most important source of residual HIV infections. Recent findings add uncertainty to the true beginning of infectious viremia in HIV infection, which may precede ramp-up viremia by several weeks and may extend back close to the time of exposure, causing theoretic concern that infectious blood donations may escape detection by currently used screening algorithms. Neither the minipool nor the individual donation NAT has reliable sensitivity in the range of 1 to 10 copies/mL and thus would not consistently detect the low HIV concentrations associated with pre-ramp-up phase viremia. Pathogen inactivation methods seem to offer the best chance to counter the small theoretic risk of HIV transmission during the very early period of the infection. Methods: The INTERCEPT Blood System using amotosalen (150 micromoles/L) with activation by 3 J/sq cm long wavelength ultraviolet light (UVA, 320-400 nm) was developed to inactivate pathogens and nucleated cells in platelet

concentrates and plasma components. Integral disposable kits have been developed that allow the treatment of therapeutic doses of platelet and plasma components. Results: The amotosalen and UVA process inactivates high titers (4 to >6 logs) of enveloped (e.g., HIV-1/2, HBV, HCV, CMV, HTLV-I/II, WNV, SARS-HCoV) and non-enveloped (e.g., Human adenovirus 5, Parvo B19, Bluetongue) viruses, gram-positive bacteria (e.g., Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus pyogenes, vegetative Bacillus cereus, Propionibacterium acnes, Clostridium perfringens), gram-negative bacteria (e.g. Escherichia coli, Serratia marcescens, Klebsiella pneumoniae, Pseudomonas aeruginosa, Yersinia enterocolitica) and spirochetes (e.g., Treponema pallidum, Borrelia burdorferi), as well as protozoa (e.g., Trypanosoma cruzi, Plasmodium falciparum). Compared to inactivation of infectious pathogens, leukocytes are more sensitive to inactivation by this process. The formation of amotosalen-induced nucleic acid adducts results in inhibition of cellular proliferation and cytokine synthesis. Conclusions: While pathogen inactivation for plasma fractionation has been implemented for over 20 years, implementation for blood components is still not in practice today. The INTERCEPT Blood System inactivates contaminating pathogens and leukocytes in platelet and plasma components, thus potentially offers a one-step process that could increase the safety of platelet and plasma transfusions with respect to prevention of transfusion-transmit-ted diseases as well as reduction of transfusion reactions and transfusion-associated graft-versus-host disease.

Abstract: 123 Oral: 123

TRANSFUSION NEED INDICATORS IN MYELODYSPLASTIC PATIENTS: RETROSPECTIVE ANALYSIS ON 55 PATIENTS

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Background In myelodisplastic syndromes (MDS) anaemia level is imputable to blastic bone marrow infiltration and ineffective hemopoiesis. Hemoglobin concentration is frequently used in MDS prognostic assessment systems. Trasfusional need, depending from patient`s clinical conditions and hemoglobin level, is indirectly but quite accurately related to myelodysplastic pathology prognosis. Aims We try to identify transfusion need indicators in dysplastic hemopoiesis. With this purpose we used various laboratory parameters well-known as MDS prognostic factors or new parameters not formerly considered for this aim. Methods We reviewed 55 MDS patients followed at our day-hospital. Median follow-up was 10 months (range 3-12 months). Thirtythree patients were male and 22 female. Male to female ratio was 1,9. Median age was 74 years (range 36-84). Eighteen patients were affected by refractory anaemia, 7 by refractory anaemia with ring sideroblasts, 24 by refractory anaemia with blasts excess, 6 by chronic myelomonocytic leukemia with poor myeloproliferation and prevalent dysplastic trait. Thirtynine patients received blood transfusion and 13 platelets transfusion.We calculate mean monthly transfusion need for each patient. Complete blood count, reticulocyte count, immature reticulocyte fraction (IRF) were performed by ADVIA 120(r) (BAYER, Diagnostic Division, Tarrytow, NY) and ABBOTT CELL DYN 4000(r) (Abbott Diagnostics, Santa Clara, Ca), and reticulocyte fractions were performed by ADVIA 120(r) (BAYER, Diagnostic Division, Tarrytow, NY). Correlation between tested parameters and transfusion need was performed by Pearson`s r test and R2 test. Results We found that IRF (r-0.74), intermediate fluorescence reticulocyte fraction (MFR, r-0.81) and high percentage of bone marrow eosinophils (r-0.59) correlate with a higer effective hemopoiesis and with a lower erythrocyte transfusion need. High value of circulating erythroblasts correlate with high platelets (r+0.56) and red blood cells (r+0.68) transfusion need, but high medullary erythroblasts percentage correlate only with an high red cell transfusion need (r+0.68). Summary/conclusions It`s intresting to remark how, in our study, hig levels of circulating IRF and MRF correlate with high medullary eosinophils percentage and with low number of bone marrow erythroblasts. This may suggest that IRF and MRF correlate with a greater effective erythropoiesis. All mentioned parameters are normally present in common MDS follow-up tests and easy and not expensive to perform. Above mentioned indicators may be employed to: 1) correlate identified indicators with patients survival; 2) identify patients with greater transfusion need; 3) stratify patients in homogeneous groups which can be submitted to different therapy regimens (supportive, differentiative, cytotoxic); 4) monitor response to therapy. Evolution of this study is to verify validity of these indicators on a larger population of dysplastic patients.

Abstract: 124 Oral: 124

GENE EXPRESSION OF HEPCIDIN AND OTHER GENES INVOLVED IN IRON METABOLISM IN MICE WITH INHIBITED OR STIMULATED ERYTHROPOIESIS AND HEMOLYSIS

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Hepcidin, a peptide hormone produced in the liver, is a key regulator of iron kinetics in the organism. Control of hepcidin production and mechanism of its action were only partially elucidated. Transferrin saturation and cooperation of the both transferrin receptors, HFE and hemojuvelin in the hepatocyte were proposed to play a role in hepcidin gene expression. The iron exporter ferroportin was shown to be the target of hepcidin action, being internalized and degraded after hepcidin binding. We have compared expression of several genes involved in iron kinetics in the mouse liver using quantitative polymerase chain reaction. The expression of hepcidin, hemojuvelin, DMT1, ferroportin, ferritin, frataxin, transferrin receptors 1 and 2 was studied. A sublethal irradiation was used to suppress erythropoiesis and inhibit iron recycling. This was combined with hemolysis induced by phenylhydrazine to further increase the amount of iron that had to be relocated to iron stores. In opposite, iron was mobilized from the stores for hemoglobin synthesis after stimulation of the erythropoiesis by erythropoietin. Further disturbances of iron kinetics were induced by red blood cell transfusions. Expression of hepcidin showed expected responses to disturbed iron kinetics and in this respect hepcidin was used as a reference gene. Surprisingly, other genes involved in iron kinetics responded to the experimental manipulations very little or not at all. Obviously, the iron kinetics and the iron status of the hepatocytes are very differently transmitted to transcriptional or post-transcriptional control of mRNAs levels of the studied genes.

Abstract: 125 Oral: 125

RANDOMISED PROSPECTIVE 1-YEAR STUDY OF DAILY DEFERIPRONE PLUS TWICE WEEKLY DESFERRIOXAMINE COMPARED WITH SINGLE AGENT DEFERIPRONE IN THALASSAEMIA MAJOR

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The efficacy of combination treatment with deferiprone (L1) and desferrioxamine (DFO) at reducing liver iron concentration (LIC) and serum ferritin has been compared with the single agent treatment of deferiprone in a randomised prospective 1-year study. As control arm, patients treated with single agent desferrioxamine were included in the comparison, partly without randomisation. A total of 36 patients with thalassaemia major were included, i.e. 12 patients into one of the following 3 arms; L1 (LIPOMED AG, Switzerland) was given at a daily dose of 75 mg/kg either in combination with DFO (40-50 mg/ kg twice weekly) or as single agent and patients registered in the DFO control arm received 40-50 mg/kg s.c. DFO 5 days a week. All patients had been treated with DFO prior to the study. The baseline serum ferritin and LIC values of the patients ranged between 917-10 859 µg/L and 5.0-53.4 mg/g d.w., respectively, indicating that compliance to DFO was not uniform within the study cohort of patients. Serum ferritin (SF) was measured at 3-monthly intervals and LIC by biopsy prior to study start and after 1 year. The average urinary iron excretion (UIE) of all measurements during the study (W1, W12, W26, W38 and W54) was calculated. The patients` compliance and tolerance were also compared at 3-monthly intervals within the three regimens. In total, four patients (all treated in the combination arm) dropped out from the study: two patients had withdrawn from their informed consent, one died from arrhythmia induced heart failure just at the beginning of the study, and one developed agranulocytosis at week 26. Compliance with all chelation regimens was excellent in every patient during the study including the DFO arm. The mean UIE was more than two-fold higher with the simultaneous administration of both drugs compared with the single administration of L1 (Z -4.83, p=0.0001). The mean UIE was significantly higher in the L1 single agent arm compared to the DFO control arm (Z -3.56, p=0.0001). The majority of patients in all treatment arms showed a clear decrease in serum ferritin after one year (Table 1). LIC fell significantly by 43% in the DFO control group, by 32% in the combination arm and by 7%

in the L1 alone arm after one year of study treatment (Table 1). The decrease in LIC was achieved in higher percentage of patients in the combined arm compared with L1 single agent arm (87.0% v 41.7%). This study shows that the treatment arms including DFO were superior in reducing liver iron if compared to the L1 single agent arm. LIC can be efficiently reduced by the combination regimen of oral L1 at 75 mg/kg/day (in 3 divided doses) and 40-50 mg/kg DFO s.c. twice weekly (12-hour infusion during night).

Oral

DEFERIPRONE OR DEFEROXAMINE VERSUS COMBINATION THERAPY IN PATIENTS WITH THALASSEMIA MAJOR: A CASE STUDY IN TAIWAN

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BACKGROUND: Deferiprone (L1) has been suggested as an effective oral chelation therapy for regular transfusion thalassemia major patients. To assess its clinical efficacy, we compared L1 and Deferoxamine (DFO) alone, and L1 combined with DFO from multicenter experiences in Taiwan. The purpose of this study was to test the efficacy either of L1 or DFO alone, or combination use, and also monitor the safety of L1. MATERIALS AND METHODS: From April 1999 to December 2004, 114 thalassemic patients from 5 treatment centers were enrolled in this program. L1 at the standard dose of 75mg/kg was given to 57 patients. The mean administered dose of L1 was 72.5mg/kg body weight divided into three doses per day. DFO at the standard dose 30-50mg/kg/day at least 5 days/week was given to 26 patients, and the mean DFO dose was 46.5mg/kg/day more than 5 days/week. Combined therapy of daily L1 with subcutaneous DFO was given 2 to 6 days each week to the other 31 patients. The mean administered dose of DFO was 45.7mg/kg body weight 2 to 6 days per week, and the mean L1 dose was 71.5mg/kg/day divided into 3 doses per day. Clinical and laboratory examinations were performed regularly throughout the study. The therapeutic efficacy and potential side effects on cardiac and/or hepatic systems of these patients were assessed by left ventricular ejection fraction, T2-weighted magnetic resonance imaging (T2-MRI), biochemical parameters and liver biopsies. RESULTS: Most patients tolerated oral L1 well. No significant liver function impairment, neutropenia or arthropathy ever occurred. Only one patient was found to have transient leukopenia (he had a coincidental virus infection at that time) 3 patients had temporary GI upset but no one required discontinuation of L1. The serum ferritin levels reduced significantly in 3 of 5 groups ($P<0.01$ each). The combined therapy group witnessed a more significant ferritin decrease than the L1 alone or DFO alone groups and hepatic function improved or stabilized notably. Besides a decrease of iron, no marked pathohistological changes were observed in the liver biopsies. GPT had a decreasing trend in 3 subgroups of L1 & combination therapy group, however there was no stastic significance. Cardiac studies showed a marked recovery of signal intensity in the heart T2-MRI and increased LVEF, indicating a significant reduction of iron load in the heart in L1 use and in over 40 -month combination therapy groups. CONCLUSIONS: We collected data from these 114 patients with BACKGROUND: Deferiprone (L1) has been suggested as an effective oral chelation therapy for regular transfusion thalassemia major patients. To assess its clinical efficacy, we compared L1 and Deferoxamine (DFO) alone, and L1 combined with DFO from multicenter experiences in Taiwan. The purpose of this study was to test the efficacy either of L1 or DFO alone, or combination use, and also monitor the safety of L1. MATERIALS AND METHODS: From April 1999 to December 2004, 114 thalassemic patients from 5 treatment centers were enrolled in this program. L1 at the standard dose of 75mg/kg was given to 57 patients. The mean administered dose of L1 was 72.5mg/kg body weight divided into three doses per day. DFO at the standard dose 30-50mg/kg/day at least 5 days/week was given to 26 patients, and the mean DFO dose was 46.5mg/kg/day more than 5 days/week. Combined therapy of daily L1 with subcutaneous DFO was given 2 to 6 days each week to the other 31 patients. The mean administered dose of DFO was 45.7mg/kg body weight 2 to 6 days per week, and the me

Abstract: 127 Oral: 127

PRENATAL DIAGNOSIS BEFORE TERMINATING AN E BETA-THALASSEMIA FETUS: FETAL DNA ANALYSIS TO RULE OUT BETA -28(A->G)/BETA E-THALASSEMIA

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Background: Thalassemias represent a serious public health concern and in some countries to introduce abortion in the severe forms, including the E beta form, is justified. And, though, E beta-thalassemia is a thalassemia intermedia, its` symptoms can range from a mild to severe disease. Specifically, the interaction of the beta+ and beta E alleles result in mild manifestations, however, the evidence is limited and other non-genetic factors may also affect the clinical expression. Aims: To identify the factors contributing to the severity of E beta-thalassemia, from a mild to severe form, with the intention to avoid unnecessary abortion in a mild thalassemia. Methods: Genotype and phenotype of E beta-thalassemia in pediatric patients at the Department of Pediatrics, Faculty of Medicine, Khon Kaen University and at Khon Kaen Regional Hospital between March 2003 and 2005 were studied. The clinical severity was classified as severe or non-severe E beta-thalassemia according to Ho`s criteria. Results: A total of 136 E beta-thalassemia patients were included (67 males, 69 females; mean age +/- SD 11.7 +/- 4.5). Seven (5%) of them (4 males, 3 females; median age 13, range 9-15) had beta-28 (A->G)/beta E-thalassemia. The median of their steady hemoglobins was 9.9 g/dL (range, 9-11). The median age at diagnosis was 6 years (range, 4-11), mostly diagnosed accidentally after having some illness. All of the children had normal growth and development; thalassemic face was not noted as well as no or mild hepatosplenomegaly was observed. They had mild thalassemia without any effect from alpha-thalassemia co-inheritance or G gamma XmnI(+) polymorphism. The prevalence of beta -28(A->G)/beta E-thalassemia in Thailand is around 5%, this figure seems under-estimated. Furthermore, beta -28(A->G)/beta E-thalassemia has very mild or no anemic symptoms that most test-positive individuals suffer no ill-effects. Conclusions: Our study confirms that beta -28(A->G)/beta E is a mild form E beta-thalassemia and fetal DNA analysis is needed definitely and morally to rule out this condition before decision of an abortion.

Abstract: 128 Oral: 128

CLINICO-HEMATOLOGICAL PROFILE OF PNH: INDIAN EXPERIENCE

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Background- Paroxysmal nocturnal hemoglobinuria (PNH) is a rare disorder with an estimated annual incidence of 1-10 cases per million persons. Important differences in clinical presentation and complications have been observed between Caucasian and Asian patients but Indian data is still sparse. Aims- To study the clinical and haematological profile of Indian PNH patients. Methods- The case sheets of all patients of PNH seen in the hematology department of All India Institute of Medical Sciences, New-Delhi, India, from January 1995 to January 2005 were analysed. Diagnosis was based on positive Ham`s and Sucrose lysis tests and absence of CD-55 and CD-59 by gel card or flow cytometry. Results- A total of 54 cases of PNH were diagnosed over the 10 year period with median age 27 years (range 12-56) and male: female ratio 2.8:1. Presentation was with (a) anemia- 95% (b) jaundice- 39% (c) cola-coloured urine- 37%. Acute renal failure developed in 2 patients due to severe haemolysis. Thrombotic episodes were seen in 3 patients: deep vein thrombosis in leg veins-1; cerebral venous sinus thrombosis-1 and arterial stroke-1. At presentation the hematological profile was as follows-median hemoglobin -6.0gm/dl (range 2.1 to 15.4), median total leucocyte count - 4100 /cu mm (range 1700-9700), median platelet count - 97000/cu mm(range 10000-364000). The reticulocyte count varied from 0 to 18% with a mean of 8.6%. The records for plasma haemoglobin were available in 16 patients and it was raised in 15 cases (93%), range- 3mg/ dl to 20 mg/dl. Urine hemosiderin was tested in 19 and was positive in 16 (84.2%). Bone marrow examination was done in 29 cases and the findings were: hypocellularity-14, erythroid hyperplasia without dyspoiesis-8, dys-erythropoiesis-5 and dyspoiesis in three cell

lines-2. The follow up period was for a median of 12 months (range 1-120). There was one reported death due to septicemia with no transformation to acute leukemia or myeloproliferative disorder. Specific treatment was given to 46, of which 44 were evaluable. Response criteria were-Com-plete Response (CR): Sustained levels of all of the following- Hb >12gm/dl without transfusions, ANC > 2000/cu mm, Platelets > 120000/cu mm. Partial Response (PR): Sustained improvement in any of the following- ANC >/= 500/cu mm above baseline, platelets >/= 30000/cu mm above baseline, transfusion independent Ten received prednisolone, of which 6 showed no response(NR), 2 achieved a CR while another 2 achieved a PR (overall response rate of 40%). Eight received both danazol and prednisolone of which 3 achieved PR (overall response 37.5%). Twelve cases were given danazol of which 9 showed a response (PR-8, CR-1, overall response-75%). All the 14 cases with hypocellular marrow received stanazolol, cyclosporine or a combination of both. Five patients were given stanazolol alone and all 5 showed a PR(100%). Of 6 who received both cyclosporine and stanazolol, 4 (66.66%) showed a PR while 2 had NR. Only cyclosporine was given to 2 cases and none of them responded. In a lone case receiving both prednisolone and cyclosporine there was NR. Conclusion- In Indian PNH patients, few characteristics stand out- a younger age at presentation, a clear cut male preponderance and the rarity of thromboses (5.6%). In the pure PNH group danazol appears to be a good treatment option.

Abstract: 129 Oral: 129

DEFERIPRONE IN THE TREATMENT OF IRON OVERLOAD IN PATIENTS WITH HB H DISEASE

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Background: Patients with Hb H disease, due to loss of function of three out of the four alpha globin genes, present with a thalassaemia intermedia phenotype. They are not transfusion-dependent, but iron overload, is a major cause of morbidity and mortality. Aims: To study the efficacy and toxicity of deferiprone in the treatment iron overload in patients with Hb H disease. Methods :17 adult Hb H disease patients with serum ferritin >2200 pmol/l were treated with deferiprone at 50-75 mg/kg/ day until serum ferritin falls below 881 pmol/l on two consecutive visits (4 wks apart) or after 18 months` therapy (whichever occurred sooner). Serum ferritin level, magnetic resonance imaging (MRI) of the abdomen and echocardiography were serially performed for all patients as well as a group of aged and Hb H genotype matched control (with ferritin <2000 pmol/l). Results: Drug dosage was increased from 50 to 75 mg/kg/day in all but 2 patients between wk 16-48. The drug was well-toler-ated in all but one patient. No evidence of neutropenia or agranulocytosis was seen. Gastrointestinal upset and arthralgia subsided after the initial few weeks. Serum ferritin level fell in all subjects, 8/16 had levels <881 pmol/l before 18 months. Signal intensity ratio (SIR) of the liver versus paraspinous muscle with the T2-weighted gradient echo sequences improved in 12/16 patients (pre-treat-ment 0.172 ± 0.076, post-treatment 2-6 months, 0.522 ± 0.484). Two patients had normal post-treatment SIR of 1.81 and 1.07 respectively. On echocardiography, the prolonged isovolumic relaxation time (IVRT) in the treatment group did not change significantly after 1 year, but deteriorated upon stopping treatment. Conclusions: The lowering of serum ferritin by deferiprone was accompanied by concomitant decrease in liver iron in most subjects. A much longer treatment period may be necessary to demonstrate unequivocal improvement in myocardial function using the less sensitive ultrasonographic technique.

Abstract: 130 Oral: 130

GRAFT-VERSUS-ADULT T-CELL LEUKEMIA (ATL) EFFECT FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION

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A. Background Adult T-cell leukemia (ATL) is a T-cell malignancy with poor prognosis. Recent advance in allogeneic stem cell transplantation (allo-SCT) has produced favorable results in the

treatment of ATL. A graft-versus-ATL (Gv-ATL) effect might underlie such clinical outcome. B. Aims In order to assess the Gv-ATL effect following allo-SCT, we analyzed 21 ATL patients who underwent allo-SCT retrospectively. C. Patients and Methods A total of 21 patients with ATL who underwent allo-SCT at Imamura Bun-in Hospital from June 1998 to March 2005 were estimated clinically. These patients include 18 patients with acute type ATL, 2 with lymphoma type, and 1 with chronic type. Response to induction chemotherapy was evaluated as complete remission (CR), partial remission (PR), stable disease (SD) or progressive disease (PD). The overall survival (OS) period from the transplantation was analyzed by the Kaplan-Meier method. D. Results The median age at the time of allo-SCT was 49 years (range; 37-62). Before allo-SCT, all patients received standard-dose chemotherapy, which resulted in CR in 7 patients, PR in 1, SD in 5 and PD in 8. Bone marrow transplantation, peripheral blood stem cell transplantation, or cord blood transplantation were conducted on 5, 13, and 3 patients, respectively. The myeloablative regimen was employed for 10 patients and ablation with reduced intensity was applied for 11 patients for the pre-transplantation conditioning. The primary engraftment was rejected or failed in 4 patients. The second allo-SCT was carried out in 3 patients. The median survival period after transplantation was 8.4+ months (range; 1.4-83.7+) and the median observation period of 7 surviving patients was 28.0+ months (range; 4.3+-83.7+). The 3-year survival rate of the 21 patients was 33.2% ± 10.9%. After the transplantation, 16 patients survived more than 100 days and ATL relapsed in 10 of these 16 patients. ATL lesions relapsed on the skin in 9 patients, in the peripheral blood in 4 patients, in the lymph nodes in 3 patients, and in the central nervous system in 1 patient. Of the 9 patients who had relapse of skin lesions, 5 had the lesions exclusively on the skin. Of these 5 patients, 4 experienced the second CR after the reduction or cessation of immunosuppressive agents. E. Summary/Conclu-sions Our treatment outcome was comparable to that of the previous reports. Induction chemotherapy resulted in SD or PD in 13 patients, who underwent allo-SCT in such condition. CR was obtained in 5 of these 13 patients, 2 of whom were alive in CR for 44.8+ and 28.0+ months. Relapse of ATL was observed in 10 patients and the skin was most frequently affected; 9 of 10 patients had relapse on their skin. CR was achieved again in 4 patients out of 5 who had relapse exclusively on the skin after the reduction or cessation of immunosuppressive agents. This strongly suggest that the Gv-ATL effect is important in the clinical effectiveness of allo-SCT for ATL.

Abstract: 131 Oral: 131

INCREASING POST-TRANSPLANT CYTOMEGALOVIRAL COMPLICATIONS IN PATIENTS WITH B-CELL NON-HODGKIN`S LYMPHOMA RECEIVING RITUXIMAB THERAPY AND ALLOGENEIC STEM CELLS TRANSPLANTATION

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Background: Several reports had confirmed the efficacy of rituximab combined with stem cell transplantation (SCT) in the treatment of non-Hodgkin`s lymphoma. However, severe viral infections after rituximab therapy had been reported in some cases recently. Only limited reference of post-transplant cytomegaloviral (CMV) complications after rituximab and SCT was reported. Aims: To evaluate the occurrence of CMV complications in B-cell NHL patients received either autologous or allogeneic SCT with or without rituximab in a single institute. Methods: Forty-two patients with relapsed indolent or high-risk aggressive B-cell NHL treated by SCT were retrospectively studied. Pre-transplant and post transplant CMV infectious conditions, conditioning regimens, transplant types, post-transplant complications were analyzed. Among these 42 cases, 15 received rituximab therapy and 11 received allogeneic transplantation. Results: Seven of 42 patients suffered from CMV infection and 4 of them developed CMV pneumonitis. Six of 11 allogeneic SCT patients developed CMV infection and 1 of 31 autologous SCT patients developed CMV infection. Six of 15 Rituximab-treated patients had CMV infections and only 1 of 27 non-Rituximab-treated patient developed CMV infection. The risks to have CMV complications after SCT were significant in patients receiving allogeneic SCT ($p < 0.001$) and Rituximab therapy ($p<0.001$). The most important risk factor of increasing CMV complication after allogeneic SCT was graft-versus-host disease (GVHD) ($p = 0.06$). Conclusions: The patients with B-cell NHL receiving Rituximab therapy or allogeneic SCT had

higher risk to develop CMV complications after SCT. Prophylactic use of anti-CMV agents should be routinely considered in these patients.

Abstract: 132 Oral: 132

THE IMPACT OF METHYLENE-TETRAHYDROFOLATE REDUCTASE C677T GENE POLYMORPHISM ON ENGRAFTMENT AFTER ALLOGENEIC HEMATOPOETIC CELL TRANSPLANTATION IN PATIENTS RECEIVING METHOTREXATE IN GRAFT VERSUS HOST DISEASE PROPHYLAXIS

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Methotrexate (MTX) is an antifolate chemotherapeutic drug and is used to prevent graft versus host disease (GVHD) in allogeneic hemapoietic cell transplantation (AHCT). The effectiveness of MTX is largely attributable to its role of MTHR and its gene polymorphism is a common (10-12% homozygote and 40% heterozygote) variation in the population. It was shown by Ulrich et al that C677T polymorphism leads to variations in toxicities. Depending on to this finding, we investigated whether methylenetetrahyrofolate reductase (MTHFR) C677T gene polymorphism has any affect on engraftment kinetics of patients undergoing ASCT to whom MTX is given for GVHD prophylaxis. We retrospectively analyzed our cohort of 82 allogeneic stem cell recipients whose MTHFR gene polymorphism of C677T region was analyzed by RT-PCR for the pretransplant evaluation of hereditary thrombophilia. The patient`s median age was 31 (range, 14-50) years, with a M/F: 50/32 and diagnosis; 35 AML, 26 CML, 12 ALL and 9 other. Nearly all of the patients were given standard conditioning regimen consisting of busulphan and cyclophosphamide or total body irradiation and cyclophosphamide. All of the patients received cyclosporine A and short term (MTX) for GVHD prophylaxis. Stem cell source was bone marrow (BM) in 23 and peripheral blood (PB) in 59 of the patients. MTHFR gene polymorphism was detected in 32 (39%) of all patients, whose 90% were heterozygote (MTHFR HeZ). When we compared the engraftment kinetics, granulocyte engraftment was found to be late in MTHFR HeZ group (neutrophil 1000 median 19 vs 17days; p=0.01) but not different for neutrophil 500 and platelet engraftment. In order to eliminate the effect of stem cell source on engraftment kinetics we have done the same analysis for BM and PB group separately. We have observed that MTHFR gene polymorphism had a prominent effect on BM recipients, as both neutrophil 500 and 1000 and also platelet engraftments were affected (granulocyte 500 median 21 vs 15 p=0.005; granulocyte 1000 median 22.5 vs 17 p=0.0001 and plt 20 median 27 vs 21 p=0.03) significantly. On the contrary, there was no difference in the PB group. When we compare the side effects of MTX such as nausea and vomiting, diarrhea, mucositis; There was no difference in acute GVHD incidence. Our knowledge on epigenetic data will help us on tailoring the chemotherapy regimen for conditioning and GvHD prophlaxis in transplant recipients. Our data on a limited patient size suggests that the presence of MTHFR HeZ may have an impact on allo HCT recipient engraftment kinetics while using MTX for GVHD prophylaxis and BM as stem cell source.

Abstract: 133 Oral: 133

SUCCESSFUL AUTOLOGOUS TRANSPLANTATION WITH NON-CRYOPRESERVED UNMANIPULATED PERIPHERAL BLOOD STEM CELLS: EXPERIENCE AT A SINGLE INSTITUTE IN THAILAND

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BACKGROUND High dose chemotherapy with autologous stem cell rescue is extensively used in the treatment of both hematologic malignancies and solid cancers. The procedure has been expensive for developing countries. With logistic limitation of cryopreservation, simple and feasible procedures are needed. AIMS To evaluate hematological reconstitution and clinical outcome in the patients receiving autologous transplantation with non-cryopreserved unmanipulated peripheral blood stem cells. METHODS Between January 1996 and May 2005, we performed 30 autologous stem cell transplantations in 24 patients at Songklanagarind Hospital, Thailand. There were 17 males and 13 females. The median age of the patients was 38 years, with a range of 7-53. There

were 11 patients with multiple myeloma, 7 with non-Hodgkin`s lymphoma, 5 with acute leukemia (4 ANLL, 1 ALL), 3 with chronic myelogenous leukemia, and 4 with solid tumors. Mobilized peripheral blood stem cells were collected by the CS3000+ cell separator, and were stored in unmanipulated and non-cryopreserved setting at 4 degrees C in a conventional blood bank refrigerator. RESULTS The median number of leukapheresis was 3 (range 2-5). The median total numbers of mononuclear cells and CD34+ cells collected were 3.94x10^8/kg (range 1.73-19.35) and 6.92x10^6/kg, respectively. The apheresis products were kept with the median maximal duration of 6 days (range 4-7) and the median cell viability of 92% (range 82-98). All patients engrafted. The median recovery time to neutrophil count > 0.5x10^9/L was 14 days (range 831) and the median time to platelet count > 20x10^9/L was 16 days (range 9-35). Febrile neutropenia developed in 80% of the patients at the median time of day 5 and lasted for the median duration of 8.5 days (range 4-17). One patient died of intracerebral hemorrhage causing the transplant-related mortality of 3.3%. CONCLUSIONS Unmanipulated stem cell storage at 4 degrees C for up to 7 days appears to be a simple and feasible source of adequate viable stem cells with the acceptable capacity for hematological reconstitution. It is a safe method and also results in satisfactory outcome and a substantial decrease of the cost of procedures.

Abstract: 134 Oral: 134

SUCCESSFUL AUTOLOGOUS HAEMATOPOIETIC STEM CELL TRANSPLANTS FOR SEVERE MULTIPLE SCLEROSIS WITH FLUDARABINE AND CYCLOPHOSPHAMIDE CONDITIONING

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Introduction: Severe multiple sclerosis (MS) refractory to standard therapy results in progressive disability. Its pathogenesis is generally accepted to have an autoimmune basis and thus immunomodulation with high-dose immunosuppressive therapy and autologous haematopoietic stem cell transplant (AHSCT) has been employed in severe cases. To date, there is no published data on patients in Asia treated in this manner. Aim: To study the feasibility and tolerability of AHSCT in the treatment of severe MS in a Singapore centre Methods: Three patients with secondary progressive MS underwent AHSCT between 2002 and 2003. They were mobilized with cyclophosphamide and subcutaneous granulocyte colony stimulating factor and peripheral blood stem cells harvested via apheresis to obtain a yield of 12 to 18 x 10^6/kg CD34. They were conditioned with a unique regimen of fludarabine 30 mg/m^2 d-5 to d-3 and cyclophosphamide 50 mg/kg d-5 to d-2 (FC) and CD34selected or enriched PBSC was reinfused on day 0. The expanded disability status scale (EDSS) scores were monitored prior to and serially after AHSCT. Results: Neutrophil engraftment occurred in all 3 patients by day+9 and platelet engraftment by day +13. Mobilization or conditioning chemotherapy was complicated by sepsis in all 3 patients which responded to antibiotics. All 3 patients are surviving at median follow up of 19.3 months (17.1 to 30.2 months). The patients had EDSS ranging from 3.5 to 9.5 at baseline. There was definite improvement in limb power and the EDSS improved by between 1.0 and 2.5 in the 3 patients from baseline to 18 months post transplant. Conclusion: AHSCT with the FC conditioning regimen was tolerated in these 3 patients and is a feasible modality of treatment of severe MS in Asian patients. It warrants further study in a larger group of patients.

Abstract: 135 Oral: 135

EARLY DETERMINATION OF DOMINATING UNIT IN DOUBLE UNRELATED CORD BLOOD TRANSPLANTATION FOR PEDIATRIC HEMATOLOGIC DISEASE

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Unrelated cord blood transplantation (UCBT) is a promising alternative modality of allogeneic stem cell transplantation. However, limited cell dose compromises the outcome. To enhance engraftment, UCBT was performed with 2 partially HLA-matched units and the early engraftment kinetic was analyzed. Six patients (4 AML, 1 ABL, 1 SAA)

were given transplants of 2 umbilical cord blood units with various conditioning according to the disease status. Serial analysis of chimerism from peripheral blood was examined with STR method. The median age and body weight of patients (M:4, F:2) were 13 years (6-17) and 52.5 kg (23-62.9), respectively. The median number of the infused nuclear cells by the sum of 2 units before freezing was 4.36x10E7/kg (2.75-6.51). The median number of days required for ANC of more than 500/uL and 1,000/uL were 19.5 days (14-32 days) and 23.5 days (16-34 days), respectively. No serious complications occurred during UCBT including veno-occlusive disease, and there was no transplantation related mortality. Grade II acute GVHD occurred in 4 patient but solved after treatment. Except one patient who had severe systemic CMV disease, all other 5 patients are still alive without disease recurrence. The early engraftment kinetics reveals that dominancy of one of two units determined from the day of engraftment (ANC > 500/uL). The median value of percentage of predominant unit at day 21 was 92.5% (60-100). UCBT with 2 units was safe, effective and promising alternative option for pediatric transplantation with good engraftment potential. The determination of dominancy was very early event after UCBT and the analysis of influencing factor is warranted.

Abstract: 136 Oral: 136

INVOLVEMENT OF PROTEIN KINASE C-E IN SIGNAL TRANS-DUCTION OF THROMBOPOIETIN AND G-CSF IN ENHANCEMENT OF INTERLEUKIN-3-DEPEND-ENT PROLIFERATION OF PRIMITIVE HEMATOPOIETIC PROGENITORS

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Background We studied the effect of thrombopoietin (TPO) on interleukin-3 (IL-3)-dependent bone marrow cell colony formation of mice to clarify the role of protein kinase C (PKC) in the signal transduction of TPO for the proliferation of primitive hematopoietic progenitors. TPO might hasten the appearance of colonies by shortening the dormant period (G0) of primitive progenitors. Immunocytochemical studies on PKC isoforms in progenitor cells stimulated with TPO have revealed that the expression pattern of PKC-e is changed, but not that of PKC-a, -b, -g, -d, or -z. Selective PKC inhibitors, such as GF 109203X and PKC-e-specific translocation inhibitor peptide, abrogated the enhancing effect of TPO on IL-3-dependent colony formation and the changes in the intracellular expression pattern of PKC-e (JPET 297:868, 2001). Aims As granulocyte-colony stimulating factor (G-CSF) has also been reported to shorten the G0 of primitive progenitors, we address the role of PKC-e among the signaling pathways of TPO and G-CSF-mediated proliferation of hematopoietic progenitors to obtain a more accurate picture of the action mechanism of TPO and G-CSF. Methods Cell Preparation. A single cell suspension was prepared from the Male BDF1 mice. They had been intravenously injected with 5-fluorouracil (5-FU) 2 days before examination (5-FU marrow cells) to enrich their noncycling hematopoietic primitive progenitors. Factors and Agents. In the presence or absence of PKC-e translocation inhibitor peptide (Calbiochem, USA), purified recombinant human TPO or G-CSF (Kirin Brewery, Japan) was pre-incubated with 5-FU marrow cells. Clonal Cell Culture. After washing, methylcellulose cell culture was performed in 35-mm suspension culture dishes containing 5 X10 4 5-FU marrow cells, 30% fetal bovine serum, 1% bovine serum albumin, and 200 U/ml recombinant IL-3. Serial Observation of Colony Formation from Progenitors. The location and proliferation of emerging blast cell colonies were recorded. Cell doubling time, and the average number of days required for the colonies to reach 100 cells, were estimated by connecting the endpoints of their growth curves. Immunocytochemical studies on PKC isoforms. After pre-incubation with TPO or G-CSF in the presence or absence of PKC-e translocation inhibitor peptide, lineage marker negative 5-FU marrow cells were stained with anti-PKC-e antibody (Life technologies, USA) followed by staining with FITC-conju-gated second antibody. The smears were observed under an laser scanning confocal imaging system (BioLad,USA) and photographed. Results/ Summary/ Conclusions TPO and G-CSF stimulated proliferation of the primitive progenitors by shortening their G0 phase. TPO has a direct effect on primitive progenitors and enhances IL-3-dependent colony formation, at least partly through the activation of PKC-e. Studies on PKC-e in signaling of GCSF are underway.

Abstract: 137 Oral: 137

INTERLEUKIN-1BETA SUPPRESSES HYPOXIC INDUCIBILITY OF THE ERYTHROPOIETIN ENHANCER BY INHIBITING HEPATOCYTE NUCLEAR FACTOR - 4ALPHA

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The suppression of hypoxia induced erythropoietin (EPO) expression by inflammatory cytokines like interleukin - 1 (IL-1) is considered a major step in the development of the anemia of chronic disease (ACD). However, the precise mechanism of this suppression is unclear. The enhancer of the EPO gene mediating the transcriptional response to hypoxia can bind the transcription factors hypoxia inducible factor (HIF), hepatocyte nuclear factor -4alpha (HNF-4alpha) and chicken ovalbumin upstream promoter transcription factor (COUP-TF). Furthermore, CBP/P300 and SRC-1 are required as transcriptional co-activators. Earlier studies from this laboratory have shown that the DNA binding activity of HIF-1 is induced by IL-1beta. As HIF-1 is known to stimulate EPO transcription, this result did not provide an explanation for the suppressive effect of inflammatory cytokines on EPO production. The aim of the present study was to investigate whether IL-1beta inhibits the activity of the EPO enhancer despite HIF activation. In particular, the effect of IL-1beta on the level and DNA binding capacity of HNF-4alpha was studied in EPO producing HepG2 and in HNF-4alpha negative non-EPO producing U2OS cells. In HepG2 cells IL-1beta inhibited HNF-4alpha mRNA expression. Moreover, we demonstrated that stimulation with IL-1beta induces ubiquitination and proteasome dependent degradation of HNF-4alpha protein. Thus, treatment with IL-1beta appears to lower HNF-4alpha levels both by transcriptional and posttranscriptional mechanisms. Studies by EMSA revealed that treatment of the cells with IL-1beta suppressed DNA binding activity of HNF-4alpha at the corresponding binding site of the EPO enhancer. Furthermore, reporter gene studies were carried out with U2OS cells transiently transfected with a reporter plasmid containing all response elements of the EPO enhancer, including the HIF binding site. As U2OS cells do not express HNF-4alpha intrinsically we co-transfected a HNF-4alpha expression plasmid. IL-1beta completely blocked the hypoxic induction of luciferase activity in HNF-4alpha gene transfected U2OS cells. Since HNF-4alpha is generally considered a constitutive factor for EPO expression, the data presented herein are the first to suggest a modulation of EPO transcription through an altered activity of HNF-4alpha. Transfection studies utilizing a reporter plasmid in which the HIF binding site was deleted showed that the suppressive effect of IL-1beta on EPO enhancer activity requires the HIF binding site. This finding supports the concept that both the response element for HNF-4alpha and the HIF binding site are necessary for the assembly of a transcriptionally active complex of HNF-4alpha, HIF and the transcriptional co-activators CBP/P300 and SRC-1. We conclude that IL-1beta, at least partly, reduces hypoxia induced EPO expression by downregulation of HNF-4alpha.

Abstract: 138 Oral: 138

DECOY RECEPTOR 3 EXPRESSION IN HEMATOLOGIC MALIGNANT DISEASES

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Decoy receptor 3(DcR3), a soluble decoy receptor, belongs to the tumor necrosis factor receptor superfamily. Decoy receptor 3 can bind Fas ligand, LIGHT, and TL1A. Activated cytotoxic T cells and NK cells destroy tumor cells by the interaction of Fas ligand and Fas. After binding of Fas ligand, Fas receptor will induce apoptosis by caspasedependent pathway. DcR3 is expressed in several types of malignant tumors, and is postulated to endow tumor cells to escape immune surveillance. We investigated the DcR3 level of bone marrow blood serum in patients with AML, ALL, CML, MM, and MDS, and also evaluated the clinical relevance of DcR3 in AML patients. We examined DcR3 levels of bone marrow blood serums with ELISA in 23 healthy bone marrow donors, 95 AML patients, 21 ALL patients, 16 CML patients, 19 MM patients, and 18 MDS patients. We examined the DcR3 mRNA expression by RT-PCR and compared with £]-actin expression. The average DcR3 levels (mean"bS.D.) were

0.42"b0.35ng/ ml in healthy bone marrow donors, 5.97"b18.12 ng/ml in AML patients, 21.95"b82.54ng/ml in ALL patients, 0.83"b1.48 in CML patients, 0.19"b0.31ng/ml in MM patients, 1.40"b2.06ng/ml in MDS patients. We investigated the DcR3 mRNA expression by RT-PCR and compared with£]-actin expression. The DcR3 mRNA expression was correlated with the DcR3 protein level. There is no significant correlation between DcR3 levels and clinical manifestations in AML patients. The DcR3 levels have no significant association with overall survival. We examined the DcR3 levels before and after induction chemotherapy in 7 AML patients who had clinical response. The DcR3 levels significantly decreased after induction chemotherapy. This is the first study to investigate the DcR3 expression in acute leukemia, CML, MM, and MDS. A significant portion of AML leukemic cells express the DcR3. The serum DcR3 level of bone marrow blood could be a good parameter for treatment response in AML.

Abstract: 139 Oral: 139

A PHOTODYNAMIC PATHWAY TO APOPTOSIS INDUCED BY HYPERICIN IN HUMAN MYELOMA ARH77 CELL LINE: POSSIBLE RELEVANCE TO PHOTODYNAMIC THERAPY

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Hypericin, a photosensitizing plant pigment from Hypericum perforatum, is a protein tyrosine kinase inhibitor that has been exploited in models for anti-tumor and anti-viral activity. In this study, we investigated the effects of hypericin on the human myeloma cell line ARH77 as a model to determine whether hypericin-induced cell death is available. The cells were incubated with hypericin at concentrations ranging from 0.001 to 10 microg/ml in RPMI at 37°C in 5% CO2 atmosphere for 4 h. Then, the cells were irradiated at 532 nm (fluence=24 J/ cm2) using a dye laser pumped by an argon laser (Orion). After 72 h exposure, the IC50 of hypericin was 0.005 microg/ml as determined by the MTT assay. Hypericin exerted phototoxic effect on ARH-77 cells, while it did not produce toxic effect in the absence of irradiation. After 72 h exposure to 0.005 microg/ml photoactive hypericin, apoptosis was assessed by morphological changes, DNA fragmentation and flow cytometric analysis using Annexin V and propidium iodide staining. Most of the cells were accumulated in the late stage of apoptosis and these cells were brightly stained and fragmented nuclei and cytoplasmic blebbing were observed. A decrease in the number of apoptotic cells was detected when protein kinase C was inhibited by addition of staurosporine to photoactive hypericin induced ARH-77 cells. From these results, we demonstrated that exposing myeloma cell line ARH-77 to photoactive hypericin inhibits cell growth in a dose dependent manner, induces apoptotic cell death by protein kinase C activation, and provides a rationale for potential applications in vivo.

Abstract: 140 Oral: 140

TELOMERE LENGTH, TELOMERASE ACTIVITY AND EXPRESSION PROFILES OF ASSOCIATED GENES IN MYELODYSPLASTIC SYNDROMES: TELOMERE LENGTH AND THE HTERT GENE EXPRESSION ARE THE MOST HELPFUL FOR DISEASE PROGNOSIS

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Background: Knowledge of telomere-telmerase complex brings important sign into molecular pathology of myelodysplastic syndromes (MDS). Data on telomere erosion, activity of ribonucleoprotein telomerase, and expression profiles of associated genes: the hTERT encoding catalytic sub-unit of telomerase, the tankyrase (TNKS) and the TEP1 encoding telomere associated protein, may be valuable from the viewpoint of disease prognosis and prospective molecular target therapy. Aims: To ascertain variation of telomere-telomerase complex components in MDS patients with the aim to evaluate their association with genome instability and disease progression. Methods: The study was done on mononuclear bone

marrow or peripheral blood samples from 74 (telomere length), 50 (telomerase activity) and 22 (expression profiles) MDS patients divided according to both, FAB and WHO criteria. Average TRF (Terminal Repeat Fragment) from all chromosome ends was measured in DNA performing TeloTAGGG Telomere Length Assay. Protein extracts were tested for telomerase activity by TRAP Assay (TeloTAGGG Telomerase PCR ELISA kit). Expressions of hTERT, TNKS and TEP1 RNA were assayed by quantitative real-time PCR with specific Taq-Man probes. Mononuclear BM cells of age matched donors served as controls. Data on telomere length were discussed together with karyotype findings (G-binding, FISH, mFISH, I-FISH), risk score established according to the International Prognostic Scoring System (IPSS) and results of the DFS analysis. Results: Significantly reduced telomeres were detected in 66% of MDS patients: in all patients with positive telomerase activity and also in some patients with its negative level. Patients with RA/RARS and RCMD with reduced telomeres showed significant decrease of median survival (P=0.04) and estimated 3 years survival (P=0.01), compared with those with normal telomere length. Positive levels of telomerase activity/expression of hTERT gene were obtained in 40/50% patients with early forms of MDS and in 50/75% patients with advanced forms of MDS. Nevertheless, telomerase activity shows low variability in the course of MDS with significant increasing in patients with conversion to AML In sequential samples, taken at the time of diagnosis and during the course of MDS, different expressions of the hTERT gene were observed. In patients with RAEB and RAEB-t notable increase of the hTERT expression was found in contrast to no significant changes of telomerase activity. Thus, positive telomerase activity represents a later event than telomere erosion and increased expression of the hTERT gene. Disease progression does not seem to be associated neither with the TNKS, nor the TEP1 genes, showing only small expression differences depending on clinical outcome. Summary/conclusion: Detection of TRF and the hTERT expression in early MDS may have prognostic value for stratification and treatment of MDS patients according to their individual risk. The grants NC7606-3 and NK7713-3

Abstract: 141 Oral: 141

JAK2, AND PTPN11 MUTATIONS IN THE PATIENTS WITH MYELODYSPLASTIC SYNDROME

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In the developmental program of normal hematopoiesis, cytokines play an important role. Cytokines bind to the receptors and then activate cytoplasmic Janus kinase (JAK) and signal transducer and activator of transcription (STAT) to induce intracellular signaling. Recently, a somatic point mutation of JAK2 (V617F), which results in constitutive activation of the tyrosine kinase and factor-independent growth of hematopoietic cells, has been found in patients with myeloproliferative disorder. The PTPN11 gene encodes SHP2, a non receptor protein-tyrosine phosphatase that also participates in signaling events downstream of receptors for growth factors, cytokines, hormones, and extracellular matrixes in the control of cell growth, differentiation, migration, and death. Dominant mutations in PTPN11, which result in gain of function of SHP2, have been demonstrated in juvenile myelomonocytic leukemia. The studies concerning JAK2 and PTPN11 mutations in myelodysplastic syndrome (MDS) have been scarce. In this study, we analyzed the genetic mutations of JAK2 and PTPN11 genes in 134 patients with primary MDS according to FAB criteria, including 25 RA, 10 RARS, 37 RAEB, 28 RAEB-T, 27 CMMoL and 7 acute myeloid leukemia (AML) transformed from MDS. Additional 10 patients with juvenile myelomonocytic leukemia (JMML) were also studied. Forty-two patients were serially studied during the course. Four (3%) MDS patients had JAK2 V617F mutation. In two of them (1 RAEBT, 1 CMMoL), the mutation was detected at diagnosis, but in one RA patient, it was not shown until the disease was transformed to AML and in the remaining one patient, the mutation was not demonstrated until 153 months after the diagnosis of CMMoL. Three of the four patients with JAK2 V617F mutation had normal karyotype, compared with 71 (59%) of the 121 patients without the mutation who had cytogenetic data did so. The patients with JAK2 V617F mutation had higher platelet count (P=0.014) than others. None of the JMML patients had JAK2 mutation. Four (3%) MDS patients (2 RAEB, 1 RAEBT, and 1 CMMoL) had PTPN11 mutation compared with 6 (60%) of the JMML patients did so. Two patients had mutations at exon 3 and the remaing two, exon 13. The mutations were de-

tected at diagnosis in all 4 patients. In two patients who achieved a CR after hematopoietic stem cell transplantation, the mutations disappeared after the procedure. In one of the JMML patients who had PTPN11 mutations, the mutation was not detected at diagnosis, but appeared 3 month later. Among the 4 MDS patients with PTPN11 mutations, one had cytogenetic abnormality -7 and the other three patients had normal karyotype. The 4 MDS patients with PTPN11 mutations was significantly younger ($p<0.001$) than other patients without the mutation. The mutations of JAK2 and PTPN11 were mutually excluded. In conclusion, JAK2 and PTPN11 genetic changes can be detected in a few MDS patients, usually in those with high-risk subtypes, RAEB, RAEB-T or CMMoL, or low-risk subtype in transformation. The mutation might occur either at diagnosis or during serial follow-up.

Abstract: 143 Oral: 143

BFM-TR ALL 2000: FIRST TURKISH MULTICENTRIC STUDY IN THE TREATMENT OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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With the aim of achieving standardization and repeating the successful results reported by the BFM Group on a nationwide basis, the first Turkish multicentric observa-tion study BFM-TR ALL 2000 has been opened to patient accrual in January 2000. A modified ALL-BFM 95 protocol has been administered by reducing the methotrexate dose to 1g/m2, infused over 36h, in order to limit toxicity. Intermediate and high risk patients received 1200 cGy cranial irradiation. Until January 2005, 1020 patients have been enrolled from 20 centers. Half of the patients were treated in 12 university hospitals and the rest in state and foundation hospitals. The centers participating showed a wide geographic distribution and reported 2 to 113 patients. The median age at diagnosis was 5 years, range 0,2-17 years, and the male to female ratio was 1,2. The median WBC count was 14.000/µL, range 500/µL-1.350.000/µL and 25% had counts over 50.000/µL. Initial CNS involvement was present in 3,8% and testicular involvement in 0,4% of the patients. Immunophenotyping showed T-ALL in 20%. According to ALL-BFM 95 criteria, 27,7% of the patients were standard, 54,4% intermediate and 17,9% high risk. Remission was achieved in 96% of the patients and 87% had prednisone good response. Totally, 16% of the patients died, 1,7% during induction and 0,4% in first remission and the rest after relapse. Relapse was observed in 8,5% of the patients, the bone marrow being involved in 7,7%, the CNS in 1,9%, the testis in 0,5%. The probability of EFS at 4 years was 72 ± 2% in the whole group, and 89 ± 3%, 73 ± 3% and 44 ± 5 % in the standard, intermediate and high risk groups, respectively. In conclusion, the preliminary results of the first Turkish multicentric clinical study for pediatric ALL showed the feasibility of applying an intensive BFM-based chemotherapy protocol in a high number of centers in a standardized form. The primary cause of failure was the high rate of deaths in remission, the prevention of which should be the aim of continuing and future studies. info@tr-bfm.org Principal investigators and institutions of the Turkish BFM Group: G. Aydoğan, Z. Salcıoglu (Bakırköy Hosp., İstanbul), A. Tanyeli, B. Antmen (Çukurova Univ., Adana), C.Timur, A. Yörük (Göztepe Hosp., İstanbul), A. Erbay, E. Kazancı (Behçet Uz Hosp., İzmir), H. Oniz, B. Atabay (Tepecik Hosp., İzmir), T. Dagoglu, M. Karakukçu (Erciyes Univ., Kayseri), U. Ezer, (LOSEV, Ankara), I. Yıldız (İstanbul Univ., İstanbul), A. Yeşilipek, V. Hazar (Akdeniz Univ., Antalya), M. Söker (Dicle Univ., Diyarbakır), S. Vural (Sisli Etfal Hosp., İstanbul), F. Pekün (Okmeydanı Hosp., İstanbul), O. Bor (Osmangazi Univ., Eskisehir), N. Sarper (Kocaeli Univ., Kocaeli), N. Çetingül (Ege Univ., İzmir), E. Guler (Gaziantep Univ., Gaziantep), A. Polat (Pamukkale Univ., Denizli), B. Biner (Trakya Univ., Edirne), I Ayan (İstanbul Univ., İstanbul), K. Çağlar (Dr. B. Nalbantoğlu Hosp., Lefkosa), L. Yüksel-Soycan, B. Goksan (Halıc Univ., İstanbul)

Abstract: 144 Oral: 144

PROGNOSTIC FACTORS AND CLINICAL OUTCOME OF 124 ADOLESCENTS AND YOUNG ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Aims. Prognostic value of basic clinical and biological features and clinical outcome of adolescents and young adults with ALL on therapy about pediatric protocols ALL-BFM-90m and

ALL-MB-91 were evaluated. Metods. From 1991 to 2003, 124 adolescents and young adults diagnosed with ALL were treated with one of the two pediatric protocols: ALL-BFM-90m (n=78) and ALL-MB-91 (n=46). The comparative analysis of clinical outcome were executed on three age groups: 10 - 14 years-old (m-41; f29; median 12.1 years) - 70/56.5% and 15 - 19 years-old (m-19; f-11; median 16.8 years) - 30/24.2% and 20 - 29 years-old (m - 17; f - 7; median 21.6 years) - 24/19.3%. The importance of initial prognostic factors was investigated by Cox Regression model. Results. The complete remission (CR) were achieved in 109/124/88.6 % of the patients (88.6% -10 - 14 years and 86.7% -15 - 19 years and 87.5% - 20 - 29 years; p=0.963). 6y-OS were 65.3% (64.3% - 10 - 14 years and 66.7% -15 - 19 years and 66.7% - 20 - 29 years; p=0.405). 6y-EFS were 61.3% (62.9% -10 - 14 years and 63.3% -15 - 19 years and 54.2% - 20 - 29 years; p=0.564). From 109 patients achieved a CR 24/124/19.4% was developed relapses. Statistical difference in frequency of relapses between age groups it was not revealed: 10 - 14 years - 14/20.0% and 15 - 19 years - 4/13.3% and 20 - 29 years - 6/25.0% p=0.547). In the single-step test of Cox regress it has been revealed that for adolescents and young adults on therapy under protocols ALL-BFM-90m and ALL-MB-91 the initial hyperleukocytosis . 50000/μl (p=0.002); BFM-RF>0.8; CNS involvement (p=0.003); risk group (p<0.001); the slow response on steroids on 8 day . 1000 blasts/μl in blood (p=0.008); blasts in bone marrow >25% on 15 day (p=0.001) and >5% on 33/36 day (p<0.001) have adverse prognostic value. The age (p=0.366); sex (0.472); T or B immunophenotype (p=0.267) and enlargement of a mediastinum (p=0.099) was found out have no independent prognostic value. The blasts in bone marrow >25% on 15 day (p=0.002); BFM-RF>0.8 (p=0.010) and initial CNS involvement (p=0.001) were confirmed the high prognostic importance in the multialternative Cox analysis of the basic prognostic features which have been identified in the single-step test. Conclusions. Pediatric protocols of ALL-BFM-90m and ALL-MB-91 are highly effective for treatment of adolescents and young adults with ALL providing 6y-EFS at a level of 61.3%. The basic prognostic factors which were revealed earlier in study ALL-BFM-90m and ALL-MB-91 at children keep the value concerning adolescents and young adults with ALL treated on the same reports. The initial hyperleukocytosis . 50000/μl; BFM-RF>0.8; CNS involvement and all variants of the early response to therapy have the greatest prognostic value.

Abstract: 145 Oral: 145

EXPRESSION LEVEL OF NOTCH1, NOTCH2, NOTCH3 AND DELTA LIKE 4 IN T CELL ACUTE LYMPHOPLASTIC LEUKEMIA AND IN NORMAL HEMATOPOESIS

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Background: Notch family of proteins are single-pass transmembrane cell surface receptors which were highly conserved through evolution. Four Notch homologs, Notch 1-4, have been identified in mammals. These signalling pathway is triggered by interaction with Notch ligands which are known as DSL (Delta, Serrate, Lag-2). Also this pathway have been shown to participate in key cellular functions, such as differrentiation, proliferation and apoptosis in many organ and hematopoiesis. T cell acute lymphoblastic leukemia is one of the agressive cancer which is commanly associated with acquired chromosomal translocations and other genetic abnormalities. Recent studies have been shown Notch receptors and their triggering ligands of this pathway could be affective in T-ALL tumoro-genesis either by mutation or with overexpression of genes in mouse models. Aim: In this study we aimed to research expression level of Notch 1, 2, 3 and DII4 genes in T-ALL samples and in normal controls to determine their roles in tumorogenesis. Materials and Methods: To test this hypothesis we investigated Notch1-3, Dll4 genes expression in bone marrow sample of 27 patient with de novo pediatric T-ALL (The age range was 3 to17; mean=8.65, median=8) and 7 control bone marrow samples from healthy volenteers and additionally CD4 and CD8 positive samples using quantitative real-time PCR technique. The levels of housekeeping gene, beta 2 microglobulin and abl were used as an internal control for normalisation of RNA quantity and quality differences in all samples. Results: Notch1, Notch 2 and Notch 3 have significantly different distributions for patients and control-CD4, CD8 positive cell lineage. Descriptive statistic provided that the expression level of all three genes were elevated in patients. We compare means of expression in both patient and CD4CD8 positive samples group by independent two sample t-test. According to statistic result Notch1 (p<0.001, 2-tailed), Notch2 (p=0.006, 2-tailed), Notch3 (p=0.003, 2-tailed) were affected but we couldn`t get simillar result for DII4 (p=0.097, 2-tailed). Also we investigate the posible affects on WBC count,

extramedullary involvement, response on Day 8, Outcome, and relapse. None of the four expression variables seem to have significant effect on those clinical features of patients. Amongs those analysis only DII4 expression has a nearly effect on Outcome and when the expression DII4 increases by 0.001 units, the odds of being alive decreases about 40%. Discussion: These result was showing that Notch1, 2, 3 expressed differently in T-ALL from control grup. DII4 expression wasn`t related with the tumorogenesis or oncogenesis of T-ALL but any increase in the expression of DII4 would decreases the survival of the patients. We got simillar result with the expression of Notch 2 but its affet was not strong as much as DII4 and the correlation of these two gene couldn`t reach to the significance(p=0.1367).

Abstract: 146 Oral: 146

UNEXPECTED PROTECTION FROM INFECTION BY TWO BOOSTER HEPATITIS B VIRUS VACCINATION IN CHILDRENS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background and Aim: The vaccination programmes in patients with malign disease is the current approach but with undefined schedule for today. Impaired immunity to infectious agents contributes to poor outcome or important sequel in cured patients. Both the high incidence of hepatitis B virus (HBV) and lack of immunization in underdeveloping countries increase the risk of HBV infection in children with acute lymphoblastic leukemia (ALL). Protective power of the vaccination against HBV infection has not been examined in seronegative patients with ALL. The aim of the study was to evaluate the incidence of HBV infection in vaccinated patients with or without seroconversion and compared this group with unvaccinated patients. Material-Methods and Results: The study group included 111 male and 85 female ALL patients with a mean age of 6.23±4.10 years and divided into three groups. Group 1 (n=82) included patients who were vaccinated during maintanence chemotherapy, Group 2 (n=87) included unvaccinated patients, Group 3 (n=27) included patients who were vaccinated prior the diagnosis of ALL. Patients in group 1 were vaccinated during maintenance chemotherapy period. Serological tests for HBV and liver function test were done in all patients before vaccination. The patients who had normal test results were vaccinated and included in the study. Immunization schedules consisted of five doses which were administered at 0, 1, 6, 12, 15 or 0, 1, 2, 6, 12 months for hepatitis B vaccination. Seroconversion was obtained 35.4% of patients (29/82) in group 1. Altough the follow-up period was significantly longer in group 1 than group 2 (57,7 months and 46,1 months respectively, p=0.008), the incidence of HBV infection rate was significantly higher in group 2 (25/87, 28.7%) than group 1 patients (4/82, %4.8). The HBV infection rate was also higher in group 2 patients than vaccinated but seronegative patients in group 1 (4/53, 7.5%) (p=0.001). No patients in group 3 (n=27) had HBV infection. Conclusion: The cellular immunity, and cytotoxic T cell function may have a potential role in the protection against viral infection in the vaccination of immunocompromised patients. In our study, these strike results suggest that protective role of HBV vaccination was independent from seroconversion. This finding should be evaluated and investigated with further studies.

Abstract: 147 Oral: 147

EVALUATION OF NEPHROTOXICITY IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH BFM 95 PROTOCOL

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Background: Survivors of childhood leukemia often have treatment sequelae in various tissues and organs. High dose methotrexate (HDMTX) and cyclophosphamide in acute lymphoblastic leukemia (ALL) treatment protocols as well as certain antimicrobial agents have renal toxicity. The aims of present study were to evaluate the incidence and time course nephrotoxicity associated with BFM 95 protocol in children with ALL. Methods: Forty two children with standard or median risk ALL were studied. They were classified into three groups according to the time of

examination: group 1 was examined between 1 to 6 months, group 2 between 13 to 42 months and group 3 between 43 to 80 months after the completion of consolidation therapy with four doses of 5 gr/m2 MTX. Results: The levels of blood creatinine and urinary excretion of microalbumin, ß2 microglobulin, N-acetyl glucoseaminidase and fractioned phosphorus were significantly higher and tubular phosphorus reabsorption was significantly lower in group 1 than in age matched healthy controls. Increased proteinuria, microalbuminuria and ß2 microglobulinuria persisted in group 2 whereas there was only increased ß2 microglobulinuria in group 3. Urine osmolality was higher in group 1 and group 2 whereas, the mean values of BUN, electrolytes, Ca, P, Mg and creatinine clearance in each group were not different from those of controls. Conclusions: BFM 95 protocol is not associated with a notable glomerular toxicity in children with ALL. Acute tubular damage detected after the completion of consolidation therapy gradually resolves over the next two years in most cases, although mild subclinical tubular abnormality as indicated by increased ß2 microglobulin excretion may persist longer.

Abstract: 148 Oral: 148

LONG-TERM OUTCOMES OF 231 PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA: PROGNOSTIC FACTORS TO BLEEDING, THROMBOSIS, MYELOFIBROSIS AND LEUKEMIA

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Background: Essential thrombocythemia is a clonal, myeloproliferative disease associated with thrombohemorrhagic complications and myeloid transformations such as myelofibrosis (MF) and acute myeloid leukemia (AML). Methods: A multi-center study of essential thrombocythemia (ET) was conducted to define the long-term outcome and prognostic factors for bleeding, thrombosis and myeloid transformations in 231 consecutive patients with ET. Literature on leukemogenic risk of hydroxyurea (HU) was reviewed. Results: Median age was 65 years. Thrombotic rates at and after diagnosis were comparable to Caucasian patients but bleeding rates at and after diagnosis were much less frequent. Projected 10-year thrombosis-free (TFS), bleeding-free (BFS) and overall survival (OS) were 66%, 83% and 80% respectively. In particular, there was no death in young patients under 60 years of age with a maximum follow-up of 15 years, and splenomegaly at diagnosis appeared to protect from thrombosis. In multivariate analysis, advanced age predicted inferior TFS and OS; and male gender predicted inferior BFS. Half of the deaths were ET-related. Seven patients evolved to MF, of whom two subsequently developed AML. Probability of MF transformation is 9.7% at 10 years. Five patients developed AML/MDS (median latency: 10 years), of which three (1.3%) received hydroxyurea only. Prior myelofibrosis (p=0.008) and use of melphalan (p=0.002) were risk factors for AML evolution. Conclusion: ET is a relatively benign disease of the elderly. Chinese patients have lower risk of bleeding, and prior MF is a major risk factor to AML. Leukemic transformation with hydroxyurea alone is rare, and warrants further prospective studies.

Abstract: 149 Oral: 149

THE BCR/ABL TRANSCRIPT WITH TWO DIFFERENT PCR TECHNICS IN PATIENTS WITH CML

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BCR-ABL gene was proved to play the principal role in CML pathogenesis. It is a hallmark of CML used in diagnostics and monitoring the response to therapy. The most sensitive method of detecting BCR-ABL aberration is RT-PCR which is able to find a single leukemic cell between 10(6) normal leukocytes. Monitoring of BCR-ABL transcript level by quantitative RT-PCR (Q-RT-PCR) has high prognostic value. The RT-PCR negativity, low level, or decreasing BCR-ABL transcript number denotes good response to treatment and good prognosis. Q-RT-PCR can detect changes in disease status several weeks or even months earlier than other methods. However, with the recent introduction of STI571 (Imatinib) for treatment of CML, and other BCR-ABL targeted drugs in the research pipeline, there is a vital need for a sensitive and reliable monitoring test. The aim of this

study is to compare the Q-RT-PCR results with nested-RT-PCR in patients with CML who demonstrated negative BCR/ABL levels with Q-RT-PCR. We re-investigated 18 CML patients` [9 males and 9 females, median age 44 years] RNA samples who developed BCR-ABL negativity with Q-RT-PCR. After total RNA extraction, copy DNA was prepared and these C-DNA samples were used in both real-time RT-PCR (LightCycler-Roche) and nested-RT-PCR. P-190 transcript was also searched in patients who demonstrated negative p210 transcript. We found negative all 18 samples with LightCycler again but, we found 9 of them p210 positive (9/18, 50%), 6 of them p190 positive (6/18, 33,3%) and only 3 samples were negative (3/18, 16,7%) with nested-RT PCR. Our findings indicated that the real-time PCR assay was less sensitive than the nested RT-PCR. Real-time RT-PCR has great advantages for estimating transcript levels in a variety of situations. It has also many advantages such as relative rapid assay times, low contamination risk, and reliability and ease of performing replicate analyses. The increased sensitivity of the nest-ed-RT-PCR was readily apparent in patients with minimal residual disease, which were negative in the majority of patients by the real-time. As a result we can perform real-time PCR during the follow-up period of patients but we should perform nested-RT PCR when we find negative results with real-time PCR.

Abstract: 150 Oral: 150

UTILITY OF OBSERVATION OF ENDOGENOUS COLONY FORMATION IN DIAGNOSIS OF POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA

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Polycythemia vera (PV) and essential thrombocythemia (ET) are malignant disorders of hemaopoietic stem cells which are characterized by clonal hyperproliferation. The aims of our study were to evaulate the usefulnes of endogenous colony formation of erythroid (EEC) and megakaryocyte (EMC). The bone marrow mononuclear cells of 17 patients with polycythemia (7 PV, 10 secondary eythrocytosis) and 27 patients with thrombocythemia (6 ET, 21 secondary thrombocytosis) were cultured by cytokine free methyl cellulose media (Methocult TM H4230) for endogenous colony evaluation. The sensitivity of EEC was 80 % in diagnosing PV, and the specificity of EEC was 0.57 %. The sensitivity of EMV was 73 % in diagnosing ET while the specificity of EMC was 0.67 %. In total, the sensitivity was found as 81%, and specificity 62%. Thus, we can concluded that the observation of endogenous colony formation is a sensitive and specific parameter reflecting the abnormal hemaopoietic clone burden induced by these myeloproliferative disorders PV and ET.

Abstract: 151 Oral: 151

CLINICAL AND LABORATORY DATA OF THE PATIENTS WITH PRIMARY HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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Background: Primary hemophagocytic lymphohistiocytosis (primary HLH) is a life threatining disorder that usually presents during infancy or early childhood. Aim: Evaluate clinical and laboratory data of the patients with FHL. Methods: As of June 2005, multicenter data from 11 different centers of HLH Study group in Turkey; were collected and analyzed by SPSS 10. Results: Study group contained 29 female, 30 male total 59 patients, with a mean age at diagnosis 1.3 (1.7±1.9). In the history 81% had fever (. 7 days) and 98% had splenomegaly (. 3 cm below costal margin) on admission. Anemia, thrombocytopenia, neutropenia were detected in 92%, 90%, 53% of patients respectively. Hypertriglyceridemia 66% (mean triglyceride level 474±283 mg/dl), hyperferritinemia 66 % (mean ferritin 3352±8346 mg/dl) and hypofibrinogenemia 37 % (mean fibrinogen level 172±174 g/l) were suggestive laboratory values for HLH. Diagnosis based on, observation of hemophagocytosis in bone marrow aspirations in 54 (92%) of patients and in other biopsies materials in 11 (19%) of patients, perforin mutations was detected in only in 5 (8%). In the history of patients, parental consanguinity was present in 38 (64%) of patients, affected sibling history was in 24 (41%) of patients. Twenty four (41%) of patients had both affected sibling and consanguinity history. On admission, other findings were lymphadenopathy 42%, skin eruptions 27%, cerebromeningeal symptom 31%, bleeding 17% of patients. Other laboratory results in patients and

mean values (±SD) were as follows: elevated LDH 68% (mean level 1387 IU (±1050)), ALT level 71% (247 IU (±372)) and AST level was 61% (165 IU (±194)). Fifteen patients received supportive treatment and only 3 is alive. Three of 8 patients receiving HLH 94 treatment protocol was survived, on the otherhand, survivors in familal HLH 2004 treatment protocol group were 6 from 10. Bone marrow transplantation has been done to two patients and both are alive. Totally 59 patients with primary HLH, 36 (61%) deceased, 10 (17 %) is in remission and 13 (22%) patients are still receiving treatment at the time. Study Group: Selin AYTAC1, Turkan PATIROGLU2, Akif OZDEMIR2, Tiraje CELKAN3, Lebriz YUKSEL3, Mehmet ERTEM4, Canan UCAR5, Ümran CALISKAN5, Elif KAZANC6, Canan VERGIN6, Özcan BOR7, Tunç FISGIN8, Feride DURU8, Emel OZYUREK9, Namik OZBEK9, Cengiz CANBOLAT10, Erol ERDURAN11 and Aytemiz GURGEY1. 1 Hacettepe University Ped. Hematology Dep. 2 Erciyes University Ped.Hematol-ogy Dep. 3 İstanbul University Faculty of Cerrahpaşa Medicine Ped.Hematology Dep. 4 Ankara University Ped. Hematology Dep. 5 Selçuk University Ped.Hematology Dep. 6 Behçet Uz Children`s Hospital 7 OsmanGazi University Ped.Hematology Dep. 8 Ondokuz Mayıs University Ped.Hematol Dep. 9 Başkent University Ped.Hematology Dep. 10 Marmara University Ped.Hematology Dep. 11 Karadeniz Technical University, Faculty of Medicine, Ped. Hematology Dep.

Abstract: 152 Oral: 152

THE RELATIONSHIP BETWEEN ZAP-70 EXPRESSION AND THE TIME FROM DIAGNOSIS TO INITIAL THERAPY IN PATIENTS WITH CLL

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The clinical course of chronic lymphocytic leukemia (CLL) is heterogeneous. Many patients have an indolent, asymptomatic disease, that never needs treatment for many years. Some patients have an aggressive clinical outcome and require therapy within a relatively short time after diagnosis. Therapy is currently recommended only for patients with disease that is progressive or symptomatic. Currently, the presence or absence of somatic mutations in the variable region of immunoglobulin heavy chain gene (IgVH gene) is the best predictor of clinical outcome. Patients whose CLL cells have unmutated IgVH genes have a significantly worse prognosis than those with unmutated IgVH genes. However, IgVH mutation analysis is based on DNA sequencing, which is time-consuming, expensive and not widely available for routine clinical use. Recently ZAP-70, a member of the protein tyrosine kinase family, which is a key signaling molecule for T lymphocytes and natural killer cells, was shown to serve as a surrogate marker for the IgVH mutation status in CLL patients. There was a significant association between the presence of unmutated IgVH genes and ZAP-70 positivity. ZAP-70 protein expression can be detected by flow cytometry and could routinely be assessed at any time of the disease. We measured the proportion of cells for ZAP-70 expression in the cell population, defined by gating on forward-side scatter and subsequently on (CD5+/ CD19+) lymphocytes. A CLL population was considered ZAP-70 `positive` when at least 20% of the gated cells expressed it. Then we investigated the association between the ZAP-70 positivity and the time from diagnosis to initial therapy. Our patients were treated when symptomatic or progressive disease developed, according to NCIWG criteria. We studied 48 patients with CLL; 16 (33%) were women and 32 (67%) were men. Median age was 64 years (range, 44-86 years). The median follow-up of the study group was 26 months, with a range of 1 to 104 months. The leukemia cells were ZAP-70(+) in 25 patients (52%) and ZAP-70(-) in 23 patients (48%). 22 of 25 ZAP-70(+) patients (88%) had received or were on chemotherapy at the time of analysis, whereas only 5 of 23 (21%) ZAP-70(-) patients had been treated for CLL. The median time from diagnosis to initial therapy was 5 months in the group of patients who were ZAP-70 (+) and 59 months in the group of patients who were ZAP-70 (-).The difference was very significant ($P < 0.0001$) (Fig. 1). Our study shows that CLL cases with ZAP-70 positivity are characterized by an unfavorable clinical course and a significantly high therapy need as compared to ZAP-70 negative patients. We conclude that ZAP-70 is a useful clinical test and increased expression of ZAP-70 by CLL cells is a strong predictor of the need for treatment in CLL. Larger studies with longer follow up are needed to confirm the role of ZAP-70 expression in CLL patients. Figure 1.

Abstract: 153 Oral: 153

EFFECT OF HLA-G EXPRESSION ON TREG AND CD19+CD25+/- CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Introduction: In CLL, there is a progressive accumulation of the leukemic lymphocytes, without an increase in their rate of proliferation. HLA-G molecule owns a restricted tissue distribution and it is physiologically expressed on thymus and extravillous cytotrophoblasts. Recent studies indicate HLA-G an ectopic up-regula-tion in tumor cells which may favour their escape from anti-tumor immune responses The aim of the study is to find out if there is an association between HLA-G serum levels, expression on Treg and B cell surface and progression and effects on treatment of the CLL patients. Patients and Methods: The aim of this study was to investigate whether there is an association between soluble and membrane bound HLA-G levels on Treg cells and B cell and prognosis of CLL patients. For this purpose 20 patients whom diagnosed CLL and 13 healthy blood donors were included in the study. In order to investigate the expression of HLA-G on the surface of PMNL cells, we have used CD4, CD25, CD19, CD45, and HLA-G monoclonal antibodies and assessments were made by using flow cytometry. HLA-G serum levels from all the serum samples and cell culture supernatants were determined by using ELISA. Results: Comparing the results we have found differentiation between patients sHLA-G serum and plasma levels (47.9 ± 69.5 µ/ml and 120.3 ± 145.2 µ/ml) and healthy controls sHLA-G serum and plasma levels (7.2 ± 8.1 µ/ml and 61.7 ± 57.2 µ/ml). On the other hand we found a correlation of CD4+CD25+HLA-G+ cells between in control group, patients group with and without treatment (Table 1). Discussion: More than 95% of patients have a B cell phenotype (B-CLL). B-CLL has a variable clinical course. Some patients have an excellent prognosis whereas in others survival is short despite early treatment. Expression of HLA-G has been suggested to contribute to immune evasion of tumour cells. In an effort to gain inside in to the role of HLA-G in B-CLL we evaluated its expression in Treg cells and CD19+CD25-/+ cells. Comparing the results of HLA-G expression on Treg cells in patients we found that HLA-G expression on Treg cells were higher than the control group. On the other hand we found similar expression levels of HLA-G in treated patients with control group. We can said that Treg cells suppress the effector mechanisms and produced immune response to Th2 in CLL patients. Treg cells may be a usefull parameter in outcome of treatment in CLL patients. When we evaluated the CD19+HLA-G+CD25- cells were increased in B-CLL patients than both control group and patients with treatment group. As a result we suggest that follow up HLA-G expression on Treg cells and CD19+ HLA-G+CD25- cells may be a predictive factor for treatment and prognosis in CLL.

Abstract: 154 Oral: 154

ORIGINAL LEUKOCYTE CULTURE METHODS FOR THE DIAGNOSIS OF IMMUNOPHENOTYPICALLY DIFFICULTY DIAGNOSED HEMOBLASTOSES AND CHLAMYDIA TRACHOMATIS INFECTION

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In spite of inculcation modern technologies and diagnostic methods in oncohematology and infective diseases, doctors do not meet with the difficulty diagnosed or unclear cases. Original bone marrow (BM), blood leukocyte and cerebrospinal (CS) liquid culture methods (Georgia Patent 2136) had been worked out. These methods help to maintain physiological environment for cells to reveal their proliferative and functional activity. Due to the high proliferative activity of malignant cells of patients with hemoblastoses, significant increase of blast cells is observed in the cases of acute leukemia (AL). When percent of blast cells in blood or BM of patients with AL falls at the range of 20-40 % and immunophenotyping can`t reveal the malignant clone, culture method helps to precise the diagnose. Blast cells in vitro maintain their morphological and cytochemical peculiarities that help to determine form and variant of

AL. Proliferating in vitro blast cells can be examined also by immunophenotype assay. At the stage of clinico-hematological remission increase of blast cells in BM cultures or their appearance in blood or CS cultures of patients with AL points to the existence of minimal residual disease. In cases of malignant lymphoma in vitro investigations reveal the presence and proliferation of malignant cells - lymphocytes with misshaped, abnormal nucleus, or with 2 or 3 nuclei and figures of pathological mitosis. Amount of malignant cells in vitro reflects the degree of malignancy of lymphoma and represents prognosing factor for patients with malignant lymphoma and helps physician to determine therapy strategy. BM and blood leukocyte cultures of patients with Chlamydia (C) trachomatis infection reveals morphological inclusions of this bacteria in monocytes, macrophages, neutrophillic leukocytes, lymphocytes and erythrokaryocytes as well. According to cultural data C trachomatis infection was diagnosed to 150 patients investigated by us for different reasons. Among them in 85 cases this infection was revealed for the first time. Leukocyte culture method offered by us can successfully be used for the examination of antibiotics in vitro. Electron microscope study revealed colonies of C trachomatis at different stage of developmental cycle in macrophages and neutrophiollic leukocytes. Investigation of these patients in dynamic revealed though significant decrease but existence of chlamydial inclusions after treatment pointing to the persistence of infection in organism. Results of our investigations shows that cultural studies are useful to detect proliferating clone and minimal residual disease in the patients with hemoblastoses, to reveal concealed and latent forms of C trachomatis infection and to evaluate the efficacy of treatment. Isolation of C trachomatis in patients` leukocyte culture enables to examine and estimate new antichlamydial drugs in vitro.

Abstract: 155 Oral: 155

THE ROLE OF MBL, IL4, ACE, CCR5 AND IL-1RN GENE POLYMORPHISMS IN FEBRILE NEUTROPENIA IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Aim: The aim of this study was to investigate the mannose binding lectin (MBL) gene codon 54, 57, interleukin 4 (IL4) gene (-590), angiotensin converting enzyme (ACE) gene insertion (I)/deletion (D), C-C chemokine receptor 5 (CCR5) gene (32 bp deletion) and IL1-R antagonist (IL1-RN) (VNTR in intron 2) gene polymorphisms (GP) in different hematological malignancies and to evaluate their roles in febrile neutropenia (FN) resulting from chemotherapy (CT). Methods: The study included 11 acute lymphoblastic leukemia (ALL) patients and 33 acute myelogenous leukemia (AML) patients hospitalized between the period of 1 July 2004 and 1 June 2005. Fifty subjects formed the control group. Median age of the AL patients and controls were 42 years (16-69) and 38 (16-52) years, respectively. Polymorphisms for the genes ACE, CCR5 and IL1-RN were detected by PCR. MBL and IL4 polymorphisms were typed by PCR-RFLP. Genotypes and allelic frequencies for these genes were compared in patient and control groups. The relationships between the genotypes and the body distribution of infections, pathogens, the duration of neutropenia and febrile episodes in acute leukemia (AL) patients were evaluated. Results: No significant differences in either the genotype distribution or the allelic frequencies of IL4, CCR5, ACE, IL-1RN GPs were observed between patients and healthy controls. MBL AB/BB genotype (55%) and B allele frequency (33%), which are important in susceptibility to infections, were found to be significantly higher in the AL patients compared to control groups (p=0.000). The median duration of neutropenia (neutrophil count<500/ µl) was 15 days (6-56 days) and the median duration of febrile periods was 5 days (1-30 days). There were no correlations between the presence of MBL and CCR5 polymorphisms and infectious pathogens, the duration of neutropenic episodes and febrile episodes, the presence of fungal pneumonia and mortality due to FN. Overall bacteremia and gram-positive bacteremia were more common in MBL AB/BB group compared to AA group (p=0.000 and 0.01 respectively). Median duration of febrile episodes were significantly shorter in ACE II group compared to ID/DD group and IL4 TT group compared to CT/CC group (p=0.008 and 0.041 respectively). Mortality within 28 days due to FN was not observed in IL1-RN 2 allele group compared to 3/4/5 allele groups (p=0.049). Conclusion: The frequencies of AB/BB alleles resulting from polymorphisms in the exon 1 of the MBL gene were significantly higher in patients with AL group and the patients who carried this polymor-

phism were more likely to have sepsis in FN episodes. Empiric antibiotic treatment can be considered in FN patients who carry variant MBL alleles. These results suggest that further research in larger patient populations is needed to elucidate the role of MBL, ACE, IL4 and IL-1RN in FN.

Abstract: 156 Oral: 156

TOTALLY IMPLANTABLE CENTRAL VENOUS ACCESS DEVICES IN CHILDREN WITH HEMATO-ONCOLOGIC MALIGNANCIES: EVALUATION OF COMPLICATIONS AND COMPARISON OF INCIDENCE OF FEBRILE EPISODES WITH SIMILAR PATIENTS WITHOUT CENTRAL VENOUS ACCESS DEVICES

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Background: Central venous access device (CVAD) is a requirement for pediatric hematology-oncology patients. The incidence of complications of CVADs must be related to underlying disease, intensity of the chemotherapy and frequency of CVAD manipulations. The purpose of this retrospective study is to evaluate complications of totally implantable CVAD (ports) in patients with hemato-onco-logic malignancies. Taking into consideration the low rate of microbiologic documentation we also compared the incidence of febrile episodes with similar patients without CVAD. Procedure: In our center ports could be inserted to only some of the patients either due to unavailability and/or refusal of the patients or parents. Records of the patients hospitalized between January 2002-May 2005 were evaluated. Patients with ports were matched with patients without any CVAD but having the same malignancy, the same anti-neoplastic chemotherapy and almost similar age. The corresponding phases of the chemotherapy were compared. Results: Low profile ports (Deltec) with silicone catheters were inserted to 31 patients with a median age of 4.3 years (range 9 months-14 years). There were 19 ALL, 3 AML, 1NHL, 1 Wilms, 2 RMS, 1 retinoblastoma, 2 hepatoblastoma, 1 osteosarcoma. Catheters could be inserted in the median 24 days (1-197 days) of the chemotherapy without any antibiotic prophilaxis. The total number of catheter days was 5268, with a median catheter life of 174 days (range 9-493 days). Eight catheters (26%) were removed due to following complications: 1 leakage, 1 dislodgement, 1 obstruction, 1 tunnel infection, 1 persistant CVAD associated bacteriemia, 3 persistent febrile neutropenia. Another obstruction could be managed with streptokinase and one port pocket required revision. There was 14 infective episodes with a rate of 2.6/ 1000 catheter days (no clinic CVAD infection, 5 proven CVAD infection, 3 CVAD associated bacteriemia, 4 CVAD unrelated sepsis and 2 tunnel/pocket infection). All isolates were gram positive bacteria (55% staphylococcus species), no fungus could be isolated. Total number of mechanical complications was 6 and overall rate of complications were 3.7/1000 catheter days. Only 25 of the patients with ports could be matched with patients without CVAD. Patient with ataxia telengiectasia+NHL, osteosarcoma, infant ALL, high risk ALL and patients with hepatoblastoma can not be matched due to underlying disease or difference in chemotherapy regimens. During the evaluated phases of chemotherapy, total number of febril episodes were 54 and 41 in the CVAD and non-CVAD group, respectively (p:0.11). Duration of neutropenia per febril attack was 9.6 and 7.4 days (p:0.047) and duration of fever per febrile attack was 5.6 and 4.4 days in the CVAD and non-CVAD group, respectively (p:0.56). Conclusion: Although majority of our patients had hematologic malignancy, neutropenic periods and required frequent manipulation, the rate of the complications were acceptable and similar to developed countries. Larger studies comparing the infection rate in hemato-oncology patients with and without CVAD`s are necessary. Nevertheless CVAD`s are inevitable for delivery of anti-neoplastic chemotherapy in the majority of the children.

Abstract: 157 Oral: 157

SERUM GALACTOMANNAN ANTIGEN IN INVASIVE ASPERGILLOSIS: OPTIMAL CUT-OFF VALUE

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Background: Invasive aspergillosis (IA) is a serious problem for immunocompromised patients, especially for haematologic malignancies. Since clinical symptoms are often similar to those of other infectious diseases, the diagnosis of this infection is difficult. An early diagnosis of IA may improve the therapeutic outcomes but only few tolls are available for this purpose. Aimes: Although the recent advent of an improved commercial serum enzyme-linked immunosorbent assay (ELISA) for the detection of circulating galactomannan (GM), a major constituent of Aspergillus cell walls, has contributed to the diagnosis of invasive aspergillosis, the optimal `cut-off` value of the test is a matter of debate. Methods: This study evaluated the specificity and sensitivity of the detection of GM for the diagnosis and prediction of IA in neutropenic haematologic patients. In a series of 165 consecutive high-risk treatment episodes that were stratified according to the probability of IA based on recently accepted case definition sets, the potential of serial screening for circulating GM in predicting IA was validated. In a total of 165 febrile neutropenic episodes, 1385 serum samples were studied. Results: Among the episodes studied, proven, probable, possible and non IA constituted four, eleven, sixtyfive and eightyfive of the total respectively. As the optimal threshold for positivity remains a matter of debate, we calculated the value of different thresholds. Different cut-off values that were used for the estimation of sensitivity and specificity were 1.5, 1.0, 0.8, 0.7 and 0.5. By using 0.8 cut-off value, the best sensitivity (66%) and specificity (88%) was achieved. When 0.8 cut-off value was determined on two sequential sera, 10 of 15 `proven and probable IA` episodes were positive. If only proven episodes were considered 100% sensitivity with the same spesificity was obtained. According to ROC analyses the optimal cut-off value was 0.85. When `single` 1.0 cut-off value was used, GM became positive before empirical antifungal therapy in 5 of 11 episodes but when the cutoff value was lowered to 0.8, GM became positive in 7 episodes. Conclusions: Without considerable change in specificity, better sensitivities can be obtained by reducing the cut-off value to 0.8 instead of 1.0 and in high risk patients more frequently screening of the test should be required for predicting IA earlier.

Abstract: 158 Oral: 158

THE USE OF CASPOFUNGIN IN THE TREATMENT AND PROPHYLAXIS OF INVASIVE FUNGAL INFECTION IN PATIENTS WITH ACUTE LEUKAEMIA AND IN STEM CELL TRANSPLANT RECIPIENTS

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BACKGROUND: Invasive Fungal Infection (IFI) is a major cause of morbidity and mortality in the setting of both chemotherapy-induced myelosuppression and stem cell transplantation (SCT). Caspofungin is a novel antifungal agent of the echinocandin class of compounds. In a randomised trial of empirical antifungal therapy in patients with persistent fever and neutropenia, caspofungin was as effective as liposomal amphotericin B and associated with less nephrotoxicity (Walsh TJ, NEJM 2004). AIMS: To assess caspofungin in the treatment and prophylaxis of IFI in patients with acute leukaemia and in stem cell transplant recipients. METHODS: From March 2003 to May 2005, caspofungin was used for the treatment of proven or probable IFI in patients with acute leukaemia and in stem cell transplant recipients. Proven IFI required histopathological or microbiological documentation of fungal species from biopsied tissues. Probable IFI was defined as the presence of characteristic clinical signs, symptoms and radiological evidence consistent with invasive aspergillosis, which included the CT halo sign, or progression from multiple nodules to cavitation or air-crescent formation. The presence of small, peripheral abscesses in the liver and/or spleen suggested a diagnosis of chronic disseminated candidiasis. Caspofungin was commenced empirically after 72 hours of antibiotic-resistant neutropenic fever with negative blood cultures or earlier at the discretion of the physician. A modified regimen (70mg daily on alternate days) was simultaneously used as antifungal prophy-laxis in patients undergoing induction or consolidation chemotherapy for acute leukaemia or in the setting of stem cell transplantation. RESULTS: A diagnosis of proven or probable IFI was made in 24 patients in this cohort over the 26 month period. Sixteen patients were allograft recipients, 2 were autograft recipients and the remaining 6 had acute leukaemia. The median age was 31 years (range 18-62 years). Eighteen were male and 6 female. There was only 1 death due to IFI. Importantly, given the inclusion of patients receiving ciclosporin and tacrolimus as prophylaxis against Graft-versus-Host Disease, there was no need for dose reduction due to hepatic or other toxicity. Hepatic biochemical data are provided.

Seventeen patients received anti-fungal prophylaxis with modified-dose caspofungin. Supportive pharmacokinetic data including trough drug levels are provided. Four of these patients developed IFI and proceeded to treatment doses of caspofungin. All survived. SUMMARY: Caspofungin is an effective agent in the treatment of IFI. Its role in prophylaxis requires further study.

Abstract: 159 Oral: 159

LONG-TERM OUTCOME AFTER THE PROPHYLACTIC LAMIVUDINE USE ON CHEMOTHERAPY INDUCED HEPATITIS B VIRUS (HBV) REACTIVATION IN PATIENTS WITH NON-HODGKIN`S LYMPHOMA: THE EFFECTS OF PRE-CHEMOTHERAPY HBV VIRAL LOAD

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Hepatitis B surface antigen (HBsAg)-positive malignant lymphoma patients are frequently complicated by hepatitis B virus (HBV) reactivation after cytotoxic chemotherapy. Pre-chemotherapy viral load may be a risk factor for HBV reactivation during cytotoxic chemotherapy. Hepatitis B e antigen (HBeAg)-positivity which is correlated with elevated HBV-DNA levels is associated with increased viral load. The aims of this study were to investigate the long-term treatment outcome of lamivudine in preventing HBV reactivation and its associated morbidity according to the pre-chemotherapy HBeAg status. Twenty-four adult NHL patients with HBsAg-positive undergoing various intensive chemotherapies (first-line or salvage) were analyzed. All patients received lamivudine 100 mg daily before the initiation of chemotherapy. The median duration of lamivudine therapy was 11.5 months (range: 1-54). The median number of chemotherapy cycles the patients received during lamivudine prophylaxis was 6 (range: 1-16). The initial steroid containing chemotherapy regimens used in 18 patients (75%), and anti-CD20 monoclonal antibody containing chemotherapy regimens used in 6 patients (25%). Four patients received autologous peripheral blood stem cell transplantation without the resultant HBV reactivation. Among 14 patients with pre-chemotherapy HBeAg-positive, hepatitis associated with HBV reactivation developed in 1 patient and there were no patients who had developed hepatitis due to HBV reactivation in 10 patients with HBeAg-negative. One patient developed HBV reactivation during the follow-up visit after lamivudine withdrawal, and 4 patients developed YMDD (tyrosine-methionine-aspartate-aspartate) mutation during lamivudine therapy. There were no statistical differences between patients with HBeAg-positive and those with HBeAg-negative in HBV reactivation rate during chemotherapy and overall survival. Our results demonstrate that lamivudine should be considered preemptively before the initiation of chemotherapy for all HBsAg-positive NHL patients regardless of pre-chemotherapy HBeAg status (HBV viral load) undergoing various intensive chemotherapies to prevent chemotherapy related HBV reactivation. And compared with the general population, similar rate of hepatic problems (HBV reactivation after lamivudine withdrawal and developed YMDD mutant) on the long-term evaluation were observed in the NHL patients.

Abstract: 160 Oral: 160

USE OF THROMBIN GENERATION TEST. F1+2 AND TAT AS MARKERS OF PROTHROMBINASE ACTIVITY IN HAEMOPHILIACS

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It has been seen that a significant number of haemophiliacs show a co-existence of mutations related with an increased risk of thrombosis (Foka et al 2003). It has been also suggested that these thrombophilic genetic defects may actually protect haemophiliacs from recurrent bleeding episodes or may even lead to thrombosis in several cases (Makris et al 2003). In order to study their coagulation activity F1+2 and TAT were selected as markers of the generation of prothrombinase. We simultaneously applied the Hicks & Pitney test to compare our results, and we used the Thrombin Generation test as a confirmatory standard test. Materials and Methods: 65 subjects were studied (58 haemophiliacs 42±15 year old and 7 normal subjects 45±10 year old). We divide our subjects in four groups: 1- 27 haemophiliacs without mutations (HN), 2- 15 with FVLeiden

and/or FIIG20210A mutations (HHet), 3- 16 homozygotes in MTHFR (HTT) and 4- 7 normal subjects (NS). The levels of F1+2 and TAT were tested with ELISA. The Hicks & Pitney test was applied in 10 consecutive incubations in 37oC. Initially we added Cephaline and CaCl2 in equal and sufficient quantity of diluted PPP (1/10). 0,1ml from this mixture is added to a new tube containing 0,1ml substrate normal plasma and 0,1ml CaCl2. Simultaneously, 0,1ml from each incubation is frozen for measurement of F1+2 and TAT. Concerning the thrombin generation (TG) test or Endogenous Thrombin Potential (ETP) we used the Thrombogram-ThrombinoscopeTm assay. TG was studied according to the assay described by Hemker et al (2000). Our results presented graphically with the 5 pictures. In these graphs it can be seen that haemophiliacs with no mutations show a gradual prothrombinase generation. On the contrary, haemophiliacs with mutations show a rapid generation that is also expressed by the concomitant increase in the levels of F1+2 and TAT mainly in the group of carriers of mutation FVL or/and FIIG20210A. A similar response was observed in the group of normal subjects. This can be explained as an adjustment of the coagulation mechanism of haemophiliacs with coexistence of thrombophilic mutations, possibly capable of preventing the high frequency and severity of bleeding episodes. These results were more revelatory using the TG test. The comparison between the different groups using the t-test distribution curve was as follow: a- Comparison between normal subjects (NS) and haemophiliacs with FV-Leiden mutations t-test, $p<0.1$ (ns), in contrary the comparison between the NS curve and all the rest groups was statistically different ($p<0,05$). Conclusion: The above results are in line with the observation that haemophilic patients bleed only after trauma, surgical intervention etc. It seems probably that during the million years of human life on earth one or more compensatory mechanisms have developed. These appear to play a significant role in the rate of bleeding episodes in haemophiliacs and may also be able to explain the increasing frequency of thrombotic events in these patients.

Abstract: 161 Oral: 161

INHIBITOR INCIDENCE AND PREVALANCE RATES IN TURKISH PATIENTS WITH HEMOPHILIA-A 10 YEARS OF EXPERIENCE IN EGE HEMOPHILIA CENTER, İZMİR, TURKEY

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The development of factor VIII inhibitors is the most serious, life-threatening and expensive complication of hemophilia therapy. Every hemophilia patient should be screened for inhibitor formation. The epidemiology of inhibitors generally evaluated with prevalance and incidence studies. "Prevalance" is defined as the percentage of patients with hemophilia who have inhibitors at any given time and they are cross-sectional or retrospective studies. "Incidence" is defined as the occurrence of inhibitors over a particular period of time and they are prospective cohort studies. The aim of this screening study was to determined the incidence and prevalance of inhibitor development ratios in Turkish patients with hemo-philia-A who followed up in Ege Region of Turkey. Since 1996, inhibitor tests have performed regularly in 384 hemophilia-A patients. All cases were severe(FVIII<2%). All patients had have one test at least in a year. In most of patients, blood drawing was performed in our hospital during routine controls and hemophilia society meetings. Other plasma samples collected from other hospitals from Ege Region were transferred to our hospital. Mean age was 17.5 years (range:1-45 yr). In 2005, ten years of duration was completed (mean; 5 yr). All patients used blood products (FVIII concentrates and FFP) were included the study. Inhibitor screening tests and titrating the inhibitors were performed with Bethesda method. The cut-off level for inhibitor positivity was defined as >0.6 BU/ml. The Nijmegen modification was used for confirmation of lower values. FVIII activities were determined by an one stage assay with STA-Compact Analyser (Diagnostica Stago, France). Totally 77 patients (20%) was determined inhibitor positive. Of these cases 27 were LR inhibitor (7%) and 50 were HR inhibitor (13%). Mean age was 14 (range; 1-45 yr). However, 31 inhibitor were transient (LR=15 / HR=16 pts). Resolution time for transient inhibitors was 7.8±2.9 months for LR and 16.3±5.7 months for HR patients. Permanent inhibitor rate was 12% (46 pts; LR=3% / 34 pts; HR=9%). Inhibitor titrages were 2.0±1.5 BU/ml (0.8-4.8) for LR and 76.2±11.8 BU/ ml (5-800) for HR cases. Incidence rate was determined with prospective observation and testing in previously inhibitor negative patients. Totally 322 severe HA cases was inhibitor negative at the beginning of study. Previously inhibitor positive patients (n=62) were excluded. After 5 years of duration only 13 patients became inhibitor positive. Nine patients were LR (2.7%) and 4 patients

were HR (1.3%). Incidence rate was determined as 4%. Reported rates for inhibitors in literature were heterogenous, ranging from 6-27% for prevalance and 0-52% for incidence. Our rates were in this limit. Thanks to this project, inhibitor positive patients can be treated earlier with specific hemostatic agents as recombinant FVIIa and APCC for their serious bleedings.

Abstract: 162 Oral: 162

FVIII GENE MUTATION PROFILE OF HIGH RESPONDER HEMOPHILIA A PATIENTS IN TURKEY

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FVIII replacement therapy is ineffective in hemophilia A patients who develop alloantibodies (inhibitors) against factor VIII (FVIII). The type of FVIII gene mutation, genes in the Major Histocompatibility Complex (MHC) loci and also other proteins participating in the presentation of antigens are the major predisposing factors for inhibitor formation. A national collaborative effort was undertaken to identify the FVIII gene mutations and investigate the correlation of the type of mutation to antibody formation in high responder inhibitor patients. A total of 26 severely affected patients with known inhibitor levels of >5 Bethesda U/ml were included. The strategy followed was to initially screen the samples for intron 22 inversion mutations by Southern blotting and then for intron 1 inversion mutation by PCR. The exonic regions corresponding to A2, C2, and A3 domains that include functional binding sites of the ligands of the FVIII protein were then sequenced in inversion negative patients. Complete sequencing of the FVIII gene was performed in those patients without a change in the former exonic regions. About 54% of patients (14 of the total) had inversion mutations mostly involving intron 22, and 26% (7 of the total) had point mutations that involved 3 single nucleotide and 2 dinucleotide deletions, and 2 C>T and 1 G>A transitions in exons corresponding to A2, C2 and A3 domains leading to either nonsense or frameshift and termination mutations, all resulting in protein truncations. One patient had a A>T change resulting in a splicing error in intron 16. One patient had a large deletion involving exons 20-25. The remaining 4 patients are suspected to have variable length large deletions. In conclusion, large genomic rearrangements and termination mutations all leading to a defiency of the FVIII protein were the prominent cause of the inhibitor development in this group of high responder hemophilia A patients

Abstract: 163 Oral: 163

ACQUIRED INHIBITORS IN NON HEMOPHILIACS: A SURVEY OF IRANIAN HEMOPHILIA CENTER

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Introduction: Acquired hemophilia is a rare sever bleeding disorder, caused by auto antibodies to factor VIII, with an incidence of approximate 0.2-1 per million per year. Characterized by a spontaneous, sudden onset of server hemorrhagic complications which are fatal in 8-22% of the patients. The majority of deaths occur within the first few weeks after presentation. Conditions that may be associated with acquired hemophilia are autoimmune disease (SLE, rheumatoid arthritis, asthma) lynphoproliferative disorders, malignancies and pregnancies. In 50% of cases the condition is idiopathic. Management of these patients still represents a major therapeutic challenge. Bleeding arrest and inhibitor eradication are the two major objectives of acquired hemophilia treatment. We evaluate the clinical problems related to the acquired hemophilia in our hospital at Iranian hemophilia center. Results: Between 2000 and 2005: 24 patients, 17 females age 13-72 and 7 males age 53-82 were registered. All patients had a life threatening bleeding. 30% of cases were idiopathic and 70% associated with different clinical conditions. The most frequent sites of bleeding were skin, muscles, mucosa and urogenital tract. 2 patients didn`t require treatment for bleeding. In 22 treated patients 32 bleeding episodes were controlled successfully. One patient died because of uncontrolled bleeding. The immunosuppressive therapy used to suppress the inhibitor in 22

patients. The final results of indication are complete remission in 16 cases, partial remission in two and 4 treatment failures. The majority of patients who achieved C.R received steroids. Discussion: Treatment of patients with FVIII auto antibodies still represents a major therapeutic challenge. The hemorrhagic manifestation in acquired hemophilia is more dramatic and life threatening as compared to bleeds associated with alloantibodies. The severity of bleeding is not proportionally related to the inhibitor titer. The choice of treatment for acute bleeds should be based on the clinical presentation rather than on the level of inhibitor titer. According to recent studies lethality is reduced to 8% may be attributable to the availability of bypassing agents (such as APCC and rFVIIa concentrate).

Abstract: 164 Oral: 164

REVIEW OF BLEEDING EPISODES AND COMPLICATIONS IN HEMOPHILIACS WITHOUT PRIMARY PHROPHYLACTIC REPLACEMENT THERAPY FROM ROMANIA

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Background. Primary prophylactic replacement therapy, recommended by the WFH and EHC for hemophilia patients with severe disease, is not yet applied in our country, and on demand therapy is frequently insufficient. Under these circumstances, bleeding episodes are very frequent and diverse, with a high risk for immediate and late severe complications. Aims. Evaluation of the frequency, clinical aspects and associated risks and complications of different bleeding episodes, correlated with the severity of the disease and patients age, in hemophiliacs receiving almost only on demand treatment, without primary prophylactic replacement therapy. Methods. The study was conduced on a cohort of 212 hemophilia patients treated in the Hemophilia Center Timisoara (about one fifth of the diagnosed hemophiliacs in our country), in the period between 1990-2005, 180 with hemophilia A, 32 with hemophilia B, 148 with severe and moderate-severe forms of disease, aged between one month - 24 years. On demand replacement therapy was mainly represented by fresh frozen plasma and cryoprecipitate. Secondary prophylaxis was used for chronic synovitis or after ileopsoas hematomas. Results. From the total number of 1964 registered bleeding episodes, 87.22% in patients with severe hemophilia, hemarthroses (HA) and hematomas (HM) represented 75.97%. The real number of bleedings was probably higher. Hemorrhagic anemia appeared in almost all cases of intraperitoneal and gastrointestinal bleedings. Hematomas were responsible for 58.62% of hemorrhagic shock complications, retroperitoneal, iliopsoas muscle, liver, and spleen HM being the most dangerous (one death). In young patients, HM of the head and neck were more frequent, the majority with dangerous locations (79.35%). Iliopsoas HM, encountered in older patients (more than 14 years in 85.71% of cases) frequently relapsed (64.10%), and had a tendency to blood cysts formation. Intracranial hemorrhage (8.49% of patients) was related to birth trauma in 33.33% of cases, with consecutive severe neurosensorial sequelae in 72.22% of these. Gastrointestinal bleedings were associated with H.pylori infection in 62.50% of patients. Repeated hematuria was associated with a very high frequency of urinary tract infections. All these explain the high median hospitalization duration (between 10.6-5.1 days). Conclusions. The impossibility to apply the primary prophylaxis is the main cause of the extremely diverse spectrum of bleeding episodes, frequently associated with severe complications and late sequelae in our patients, compared with the nearly normal life of patients under prophylaxis. Key words: hemophiliacs, bleeding episodes, complications.

Abstract: 165 Oral: 165

THE PROGNOSTIC SIGNIFICANCE OF VEGF-A, VEGFC AND COX-2 EXPRESSIONS IN NON-HODGKIN`S LYMPHOMAS

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Background and aim: Angiogenesis is the fundamental step in carcinogenesis. Lymhangiogenesis is another step in this pathway. Cyclooxygenase system is also essential in tumorigenesis due to the its inhibitor effect in apoptosis, promoter effect in angiogenesis and increased tumor cell invasive effect. Non-Hodgkins lymphomas (NHL) are heterogenous tumors with highly variable biology and clinical outcome. Here vascular endothelial growth factor A (VEGF-A), vascular endo-

thelial growth factor C (VEGFC), and cyclooxygenase-2 (Cox-2) expressions were evaluated in cases with NHL and the expression profile of these biological factors were compared with known prognostic and clinical parameters. Patients and methods: One hundred seventy eight biopsy samples taken from NHL cases were uased as study material. Immunohistochemistry was used for this aim. More than 10% staining was accepted as positive for VEGF-A and C, Cox-2 staining was scored according to the stained percentage and intensity of the tumor cells. Pearson SPSS 12.0 student t test or ANOVA, Mann-Whitney U test or Kruskal-Wal-lis tests were used for statistical analysis. Results: Patients` ages were between 16 and 82, female/male ratio was 74/104, histologically 73 cases had low grade NHL (Group I), while 90 had aggressive (Group II) and 15 had very aggressive subtype (Group III). VEGF-A and VEGF-C expressions were found in 61.2% (109/178) and 34.8% (62/178) of the cases, respectively. Cox-2 expression higher than score 50 was detected in 56.2% of the cases. VEGF-A was not found to be correlated with histologic subtype (p=0.731), PS (p=0.505), stage (p=0.098), sex (p=0.922), extranodal involvement (p=0.209), ß2M (p=0.721). VEGF-C was not found to be correlated with histologic type (p=0.366), PS (p=0.901), stage (p=0.976), extranodal involvement (p=0.226), ß2M (p=0.569), sex (p=0.065) and age (p=0.33). There was an important correlation between VEGF-A and VEGF-C (p=0.000). There was not an important correlation between Cox-2 and age, stage, extranodal involvement, B symptom and sex, but there was a statistically significant correlation between histologic subtype and Cox-2 expression: Higher Cox-2 expression was seen in more aggressive NHL subtypes (p=0.027). Although VEGF-A and VEGF-C positive tumors tended to have higher Cox-2 score, this association was statistically nonsignificant (p=0.082 and 0.089). Conclusion: Angiogenesis and lymphangiogenesis is important in lymphomagenesis. The positive association between Cox-2 expression and aggressive histology and also correlation between VEGF-A- VEGF-C and Cox-2 and Cox-2 with aggressive tumor suggest that there is a common pathway between angiogenesis and cyclooxygenase pathways. Targeted therapy for vessel formation and Cox-2 system will be novel therapeutic approaches in NHLs as seen in many other malignant tumors.

Abstract: 166 Oral: 166

ANALYSIS OF FLAVOPIRIDOL EFFECT ON CELL CYCLE AND APOPTOTIC PROTEINS IN MANTLE CELL LYMPHOMA

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Typical Mantle Cell Lymphoma (MCL) and morphologic variants are a distinct group of B-cell Non-Hodgkin`s Lymphomas (NHLs) associated with over-expression of the cell cycle protein cyclin D1 following translocation between the IgH and Bcl-1 genes. Due to the important functional interaction between cyclin D1 and cyclin dependent kinases (CDKs), pharmacologically developed cyclin dependent kinase inhibitors (CDKIs) such as Flavopiridol are under consideration for treatment of patients with MCL. The present study investigated the in vitro effects of Flavopiridol on the MCL cell line (JeKo-1). Flavopiridol at a dose of 10 nmol/L induced apoptosis in MCL cells by 6 hours of treatment as noted by flow cytometric analysis, morphologic examination and Western blotting. The cleavage of proCaspase-3 and PARP and the decrease of Flavopiridol-induced apoptosis by pan-caspase inhibition suggested that the caspase pathway serves an important role in the apoptotic process. Furthermore, MCL cells exposed to Flavopiridol showed down-regulation of key cell cycle proteins acting at the restriction point control between the G1 and S phases. The onset of Flavopiridol-induced apoptosis also coincided with the down-regulation of Mcl-1, an important anti-apoptotic Bcl-2 family protein. Cell viability studies further demonstrated that the combination of Flavopiridol and Bortezomib causes significant cytotoxicity in MCL cells. Collectively, our data indicates that Flavopiridol may have significant therapeutic potential when used synergistically with drugs targeted against complementary cellular pathways.

Abstract: 167 Oral: 167

PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA IN IMMUNOCOMPETENT PATIENTS IN THE SOUTH OF THAILAND: INCIDENCE, CLINICAL MANIFESTA-

TIONS, RADIOLOGIC FEATURES AND OUTCOMES

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Background: Primary central nervous system lymphoma (PCNSL) is a separate entity of non-Hodgkin`s lymphoma. It confines to the craniospinal axis without systemic disease. Over the last decade, the incidence in the immunocompetent population reported in several studies has risen. The causative factors accounting for this phenomenon is not explained by the improved ability to diagnose and still remain unresolved problems. Because of many differences in the natural history and management of acquired immune deficiency syndrome (AIDS)-associated and non-AIDS-associated PCNSL, we review in immunocompetent patients only. Aims: In our study, we present an incidence, clinical manifestations, radiologic features and outcomes of PCNSL patients underwent radiotherapy in a single university hospital in the southern Thailand. Methods: We retrospectively reviewed 20 immunocompetent patients diagnosed with biopsy-proven PCNSL between 1993 and 2002 at Songklanagarind hospital, Thailand. We described presenting symptoms, histological subtypes and treatment outcomes. Preoperative computerized tomography (CT) and magnetic resonance images (MRI) were analyzed by a radiologist (Fig. 1). SPSS for window version 12.0 was used for statistical analysis. Results: The increasing trend of incidence was observed in the last three years from 0- 2.13 in 1993-1999 to 2.05-3.88 cases per 100 newly-diagnosed NHL in 2000-2002. The median age was 58 years (range: 34-71 years). At diagnosis, hemiparesis was the most common presenting symptom (40%). According to Working Formulation Classification, diffuse large B-cell was the most common subtype (61%). CT and MRI revealed the majority of patients (60%) had solitary lesion and most of them located in supratentorial area. Pre-contrast media administration (CM), CT demonstrated 70% of lesion appearing hyper- or isodensity. In contrast to MRI study, T1-weighted images demonstrated only 30% with hyper- or isointensity. Post-CM, CT and MRI revealed homogeneous enhancement as shown in figure. Regarding to treatment, only 18 patients received radiation therapy which was the sole treatment. Post radiation, 11 patients were recovery without interference of daily activity. The median survival time for our study was 15 months Summary: Although the incidence of PCNSL is low, the increasing is observed now even in the immunocompetent patients. Currently, a combination between chemotherapy and radiotherapy reveals better. Figure 1. Brain MRI, Post-gadolinium administration

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Abstract: 168 Oral: 168

EFFECT OF PRIOR RADIATION THERAPY ON AUTOLOGOUS PERIPHERAL BLOOD STEM CELL (CD 34+ CELLS) COLLECTION IN PATIENTS WITH RELAPSED OR REFRACTORY DIFFUSE LARGE CELL LYMPHOMA AND HODGKIN`S LYMPHOMA

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Background Factors effecting stem cell collection are poorly understood. Many factors effect stem cell collection including type of lymphoma, age, stage, previous chemotherapy, previous XRT and mobilization regimen Aims To check the effect of prior radiation therapy on autologous peripheral blood stem cell (CD 34+ cells) collection in patients with relapsed or refractory diffuse large cell lymphoma (DLCL) and Hodgkin`s lymphoma (HD) Methods 127 consecutive patients with relapsed or refractory DLCL and HD who received ESHAP followed by BEAM and APBSCT were reviewed. Characteristics of these 127 patients are: 68 males: 59 females. DLCL 49: HD 78. Median age at ASCT 26 years, prior XRT treatment 48 patients (24 / 49 DLCL and 24 / 78 HD patients), median prior chemotherapy cycles 6. Median XRT dose was 3600 cGy (range 1500 to 4500 cGy). The majority of patients received involved field XRT, only 8 / 48 had extended field and 5 / 48 with pelvic area XRT. Results 37 / 48 (77%) patients with prior XRT and 56 / 79 (71%) patients with no prior XRT required only one apheresis (p= NS). Median total CD 34+ cells / kg collected were 6.9 x10*6 for the whole group; for no XRT group (79 patients) 7.48 x10*6, with XRT (48 patients) 5.7 x10*6 (p = 0.12). Patients with HD and no XRT (54 patients) 8.17 x10*6 and with XRT (24 patients) 6.8 x10*6 (p = 0.64). DLCL patients and no XRT (25 patients) 5.6 x10*6 and with XRT (25 patients) 5.05 x10*6 (p = 0.125). Different XRT doses did not

seem to effect CD 34+ cells collection (p = 0.64). In XRT group, CD 34+ cells collection in patients 30 years or younger (27 patients) (8.6 x10*6) vs > 30 years (21 patients) (4.9 x10*6) (p = 0.046). No XRT group showed the same trend for age (8.77 x10*6 vs 5.4 x10*6) respectively (p = 0.043). In XRT group, CD 34+ cells collection in patients required one apheresis (37 patients) (6.9 x10*6) vs >1 apheresis (11 patients) (3 x10*6) (p = 0.016). No XRT group, one apheresis (56 patients) (9.9 x10*6) vs >1 apheresis (23 patients) (5 x10*6) (p = 0.011). Multivariate analysis to check the effect of histology, gender, age at transplant, chemotherapy cycles <=8 vs > 8, response to initial therapy and number of apheresis required (1 vs > 1) was performed. In XRT group, age (p = 0.038) and number of apheresis (p = 0.023) and in no XRT group, histology (p = 0.017), age (p = 0.005) and number of apheresis (p = 0.003) were significant factors. Conclusion: In these uniformly and minimally pretreated patients with DLCL and HD, effect of prior XRT use showed a trend towards inferior CD 34+ cells collection as compared to no XRT group but the effect failed to reach the statistical significance. Prior XRT use resulted in significantly lesser CD 34+ cells cell collection in patients older than 30 years and if patient required > 1 apheresis. Same observation related to age and apheresis was found in patients with no prior XRT.

Abstract: 169 Oral: 169

RESPONSE ADAPTED RADIOTHERAPY IN THE MANAGEMENT OF EARLY STAGE HODGKIN`S DISEASE

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Background and Aim: Combined modality treatment with 4-6 courses of chemotherapy and involved field radiotherapy (IFRT) is becoming the standard treatment in recent years in the management of early stage Hodg-kin`s disease. In order to reduce the risk of late complications, ongoing randomized trials are seeking for minimal effective radiotherapy dose in addition to chemotherapy intensity. This prospective study was undertaken in order to find the efficacy of 3 courses of doxorubicin, bleomycin, vinblastine, dacarbazine (ABVD) chemotherapy and response adapted IFRT dose. Methods and Materials: Fifty patients with supradiaphragmatic CS I-IIA Hodgkin`s disease without bulky mediastinal lymphadenopathy were enrolled into this prospective study between September 1997 and 2004. Patients were treated with 3 courses of ABVD chemotherapy followed by involved field irradiation. Radiotherapy dose was 20 Gy in complete responders and 30 Gy in partial responders. The median age was 37,5 years (range 19-58). Eighteen patients (36%) were female and 32 (64%) were male. The majority of the patients were CS II (68%). Twenty-six patients (52%) were with mixt-cellularity (MC) histology, while 18 were with nodulary sclerosing and 6 were with lymphocyte-predominant (LP) histology. Results: Median follow-up was 57 months (range 8-91). At the time of this analysis 47 patients (94%) were without any evidence of disease recurrence. Three patients developed recurrence, one in the abdomen, one in the contralateral neck and the other in the previously involved neck. Time to recurrence was 11 and 31 months in patients with abdominal or contrlateral neck recurrence, while the patient with in field recurrence developed lymphadenopathy 85 months after the end of radiotherapy. The 5-year relapse-free and overall survival rates were 95% and 98%, respectively. None of the prognostic factors as stage (I vs II), histology, gender, age(<40y vs .40y), presence of bulky peripheral lymphadenopathy, ESR(50 mm/h vs >50 mm/h), number of the involved sites (2 vs >2), response to CT (partial vs complete) were found as statistically significant. Six patients developed treatment related complications. One patient, who received neck and 30 Gy mediastinal radiotherapy developed ischemic heart disease and five other patients developed subclinical hypothyroidism. The patient with ischemic heart disease had a history of smoking for more than 10 years and had an increased serum cholesterol levels before rdiotherapy. Conclusion: Despite the limited number of patients, 3 courses of ABVD chemotherapy and response adapted XRT seems to be successful in terms of disease control and low complication rates.

Abstract: 170 Oral: 170

DOWN-REGULATION OF NOTCH-1 EXPRESSION DECREASES PU.1 MEDIATED MYELOID DIFFEREN-

TIATION IN ACUTE MYELOID LEUKEMIA

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Background Notch-1 mediates a series of signal transduction pathway to control cell fate, self-renewal and differentiation during the development process of human. In the hematopoietic system, the Notch signaling is considered to maintain the stem cell pool and the self-renewal capacity of hematopoietic stem cells. Recent study showed that in normal hematopoietic system, Notch-1 up-regulates the expression of a myeloid-dif-ferentiation related transcriptional factor, PU.1, and promotes myeloid cells differentiation. We therefore assume that decreases Notch-1 expression may involve in the leukemogenesis of acute myeloid leukemia (AML) through down-regulation of myeloid differentiation process. Aims To investigate the role of Notch-1, PU.1 transcriptional factor and downstream differentiation signal pathway in the leukemogenesis of acute myeloid leukemia. Methods The CD34+ stem cells harvested from five healthy donors were used as a control in this study. Notch-1 expression of leukemic cells from 54 AML patients and 7 AML cell lines (CTV, KG1, RPMI1640, MOLM13, THP1, GDM1, ML2) were analyzed by real-time quantitative PCR (RQPCR). Western blot analysis was used for evaluating the Notch-1, PU.1 and downstream molecule (MCSFR) expression in a part of AML patient samples and cell lines, and PQ-PCR of PU.1 was done in all AML patient samples for confirmation. Co-immunoprecipitation was performed for evaluating the interaction between Notch-1 and PU.1. To investigate the mutational status of Notch-1 and PU.1 in AML, PCR single strand conformation polymorphism (SSCP) for Notch-1 (ANK and PEST domain) and PU.1 (PEST and DBD domain) was performed. Results Significantly decreased Notch-1 mRNA expression was found in AML patients and cell lines compared with CD34+ stem cells (Figure 1A). Western blot confirmed the phenomenon of down-regulated Notch-1 expression in AML (Figure 1B, 1C). Regarding the downstream differentiation signaling, decreased PU.1 and MCSFR protein expression was found in AML cell lines and patients, and the expression level of Notch-1 was correlated with PU.1 and MCSFR expression. RQ-PCR of PU.1 also demonstrated decreased PU.1 expression in AML. No mutation could be detected on certain domains of Notch-1 and PU.1. Immunoprecipitation study showed that Notch-1 intracellular domain bound to PU.1 directly. Conclusions Notch-1 regulates the differentiation-related transcriptional factor PU.1 expression through expression level and direct binding. Down regulation of Notch-1 expression decreases PU.1 mediated differentiation signal pathway, and it may participate in the leukemogenesis of AML. Figure 1. Down-regulation of Notch1 Expression in AML cells and patients.

Abstract: 171 Oral: 171

DETECTION OF TELOMERE LENGTH IN PATIENT WITH ACUTE PROMYELOCYTIC LEUKEMIA (APL) REFLECTS RESPONSE TO TREATMENT WITH ARSENIC TRIOXIDE

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Introduction: The telomeric DNA together with its associated proteins protects the chromosome ends from degradation or aberrant recombination. The length of telomere in cancer cells depends on a balance between the telomere shortening at each cell cycle and the telomere elongation resulting from telomerase activity. In leukemias and in some solid tumors, a correlation between decreasing telomere length and an increasing severity of disease has been described. Telomere reduction was previously demonstrated in acute and chronic leukemia. Acute promyelocytic leukemia (APL) characterized by a specific chromosomal translocation t(15;17) that form a PML-RAR. fusion gene. Arsenic trioxide (As2o3) is able to induce complete remission in t(15;17)-positive APLs. Arsenic trioxide treatment promotes telomere shortening and apoptosis. Methods: 300 peripheral blood samples were taken from 30 APL patients before, during and after therapy with Arsenic Trioxide. Leukemic blasts were isolated by ficoll- gradient, and then genomic DNA extracted by salting out protocol from those samples, and NB4 cells. Genomic DNA was digested with Rsa1 and Hinf1 restriction enzymes; electrophoresis was performed in 0.8% agarose gels. Finally telomere length was determined by south-

ern analysis. Results: We studied telomeric DNA in APL leukemic cells from patients as well as NB4 cell line as a human APL model. Marked differences were observed in the sizes of the telomeric repeats in the normal blood cells and APL leukemic cells. The leukemic cells of 30 patients with APL showed a variable reduction in the length of telomeric DNA, ranging from 2.0 to 7.0 kb, while the telomere length in PB mononuclear cells obtained from the same patients during complete remission was 9.0 to 10 kb. Conclusion: Arsenic therapy leads to telomere shortening, growth arrest, and leukemic cell death (by apoptosis). Longer telomeres were found in APL patients after induction by arseninc treatment compared with those found in diagnostic specimens. Most likely this was due to the loss of the leukemic clone (with shorter telomeres) and the emergence of normal hematopoietic cells (with longer telomeres) after induction therapy. These data indicate that telomere length shortening in APL patient treated with arsenic can be used as a marker to monitor disease condition and response to therapy.

Abstract: 172 Oral: 172

A RANDOMIZED, CONTROLLED STUDY OF EMA VERSUS EMA-CYA IN PATIENTS WITH RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA

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Background: A potential mechanism of chemotherapy resistance in acute myeloid leukemia (AML) is the multi-drug resistance (MDR-1) gene product P-glycoprotein (P-gp), which is often over expressed in myeloblasts from refractory or relapsed AML. EMA, consisting of etoposide, mitoxantrone, and cytarabine, is a timed-sequential chemotherapy (TSC) regimen and an efficacious option for induction treatment of acute myeloid leukemia (AML). The objectives of this study were to compare the efficacy and toxicity of the combination regimens of EMA with EMA plus cyclosporine-A (CyA) and to assess any benefit of cyclosporine (CyA) as a multidrug resistance modulator in relapsed/refractory AML patients. Patients and Methods: A randomized, controlled study was performed using CyA plus EMA (n=19) versus EMA (n=20) to treat patients with relapsed or refractory AML. Results: For the EMA-CyA versus EMA arms, complete response (CR) was achieved in 63% versus 25% of patients, respectively (ki-square= 4.32, P= 0.038). But, the median disease-free survival (DFS) in those achieving CR was similar in the two arms (12.4 versus 10.1 months) as was the patients` overall survival (OS) (15.2 versus 13.8 months). Greater hematologic toxicity was observed with EMA-CyA resulting in an increased number of infections. However, non-hematologic toxicities included cardiotoxicity, stomatitis, and reversible increases in serum creatinine and bilirubin levels were similar in the two treatment arms. Two patients experienced treatment-related deaths from pneumonia, and cerebral hemorrhage in EMA-CyA arm, and there is one treatment-related death because of septic shock for EMA arm. Conclusion: CyA combined with EMA is relatively well tolerated. The use of EMA-CyA regimen may improve the response rates of patients with relapsed or refractory AML. Since CyA had not any effect on DFS and OS, allogeneic stem cell transplantation should be considered in early post-remission period for those patients complete remission achieved.

Abstract: 173 Oral: 173

STUDY OF TH1 AND TH2 CYTOKINE PRODUCTION BY T-CELL FROM ACUTE MYELOID LEUKEMIA (AML) PATIENTS

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CD 4+ T-helper cells are an integral part of the effective immune response against various malignancies; however in tumor-bearing patients they are frequently functionally unresponsive. Some sets of data indicate on the important role of T-helper 1 subset in the control of tumor growth. In order to assess the influence of T-cell-response polarization on the course of the disease intracellular cytokines (IFN-gamma, IL-4) were investigated in T-cells of AML patients. Methods. Th1 and Th2 cytokine production was detected in CD3+CD8-(consided as CD4) and CD3+CD8+ lymphocytes from AML patients by flow cytometry analysis. Lymphocytes were isolated from the whole blood by Ficoll-Hypaque density centrifugation, activated with PMA (phorbol2-myristate-

13-acetate, 50ng/ml) and calcium ionophore (250 ng/ml) for 12 h in the presence of Golgi Plug (brefeldin A) and stained for surface CD3, CD8 antigens and intracellular IFN gamma, IL-4. Samples from AML patients were collected at diagnosis (n=12), duration of remission (n=17, samples=35), and at relapse (n=4, samples=6). Ten healthy donors constituted the control group. Statistical data were computing by program "Statistics for Windows 5.5". Results. There were CD3+CD8+IFN-gamma 37,6±12,2%, CD3+CD8-IFN-gamma 14±3%, CD3+CD8+IL-4 5,4±4,4%, CD3+CD8-IL-4 5,4±1,8% in 9 healthy donors and there were 37,9±22%, 31,9±18,5%, 5,1±3,6%, 3,5±1,7% in 10 AML patients at diagnosis respectively. There were 42,7±16,3%, 27,5±9,3%, 5,9±4,5%, 6,4±3,4% in 14 AML patients at remission respectively and there were 38,1±18,7%, 18,8±5,1%, 11,9±6%, 13,6±2,2% in 4 AML at relapse respectively. The percentages of IFN-gamma producing CD3+CD8+ cells did not differ much in AML patients and in donors. The percentages of IFN-gamma-producing CD3+CD8-T cells in AML patients were similar at diagnosis and in remission and exceeded such counts in healthy donors ($p<0,02$, $p<0,0001$). The amount of IFN-gamma-producing CD3+CD8-cells decreased at relapse. Simultaneously prior to relapse and at relapse the increase of IL-4 producing cells, both CD3+CD8+ and CD3+CD8-, was observed ($p=0,05$, $p<0,0001$). Conclusion. The activation of pro-inflammatory cytokine response (Th1) was detected in AML patients at diagnosis and during remission. Increased level of Th2 cytokine IL-4 `was registered before and at AML relapse. The data provide the evidence of altered cytokine secretion by T-cell subsets in AML patients at different time points of the course of acute leukemia treatment.

Abstract: 174 Oral: 174

REAL-TIME PCR COMPARISON OF GENE EXPRESSION PROFILES IN PARALLEL BONE MARROW AND PERIPHERAL BLOOD SAMPLES IN ACUTE MYELOID LEUKAEMIA

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Accurate and rapid diagnosis and monitoring of haematological malignancy is vital for optimal treatment. Recent microarray studies have identified Indicator genes that may provide more precise prognostication (Golub et al, 1999). Bone marrow (BM) aspiration is painful and not inexpensive and it would be preferable if these genes could be measured in peripheral blood (PB) samples. We have developed a quantitative gene expression-profiling method which we have used to compare expression of these Indicator genes in human BM and PB samples. Parallel whole bone marrow aspirate and peripheral blood samples were obtained from 19 patients with AML and mononuclear cells (MC) isolated from both sample types by density gradient centrifugation. The mRNA was then globally amplified using a PolyA RT-PCR method and the expression profile of the 17 top ranked genes from Golub et al (1999), were measured by real-time PCR. All values were calibrated against control standards and normalised to the mean of three housekeeping genes (IF2-beta, GAPDH and human ribosomal protein S9) and obtained data were statistically analysed and compared using SPSS software. The results demonstrate similar expression levels between BM and PB for some genes (Leptin receptor, fumarylacetoacetate, CD33, Adepsin, Proteoglycan 1, MB-1, Cyclin D3, hSNF2b, RBAp48, Proteasome iota, HKrT-1 and E2A) indicating the possibility of their routine use in monitoring disease activity in PB samples rather than BM; conversely there was significant difference in expression in BM and PB samples for C-myb, Hox-A9, LYN, Cystatin c and LTC4 ($P<0.05$) and these genes would not be good PB markers.

Abstract: 175 Oral: 175

EFFECTS OF THALIDOMIDE AND RECOMBINANT HUMAN GM-CSF ON CARBOHYDRATE METABOLISM IN PROMYELOCYTIC LEUKEMIA HL-60 CELL LINE

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Background: Since virtually all malignancies are associated with defects in differentiation and most cancer cells utilize anaerobic glycolytic way to compensate their faster metabolism, reestablishing differentiation programs or blocking energy

metabolism may lead to the cessation of tumorigenic self-renewal and elimination of the malignant clone. Combining clinically applicable cell cycle inhibitors with myeloid growth factors was shown to induce terminal differentiation of resistant myeloid leukemias. Aim: To investigate effects of the single and combined use of thalidomide, a cell cycle inhibitor, and recombinant human granulocyte macrophage colony stimulating factor (rhGM-CSF) for 2 and 5 days on the rate of energy metabolism of promyelocytic leukemia HL60 cells while inducing terminal differentiation. Methods: Seven cultures for each normal leukocytes and promyelocytic leukemia HL-60 cells were prepared and each HL60 cell culture was separated into 8 groups: one for the determination of initial protein and glycogen amount, one for the control, and 6 for the single and combined application of thalidomide (50 μM) and rhGM-CSF (200 U/ml); one set for 2 days and one set for 5 days. All cell cultures except the ones for the determination of initial protein and glycogen amounts were incubated with radiolabelled glucose (D-[6-C14]Glucose) for 4 hours at 37 °C. Then, glycogen consumption and amounts of radiolabelled carbon dioxide (CO2, collected in scintillation vials via nitrogen gas), and lactate (collected in scintillation vials by anion-exchange chromatography) produced by the cells were calculated in pmol glucose per μg protein for each hour. Results: Principally anaerobic glycolysis was effective in promyelocytic leukemia HL-60 cell lines, in contrast to normal leukocytes in which aerobic glycolysis was dominant. While single use of rhGM-CSF for 2 days increased glycogen consumption and further decreased the rate of aerobic glycolysis, single use of thalidomide or combined use of two drugs for 2 days had no significant effect on energy metabolism of HL-60 cells. However, the single or combined use of these drugs for 5 days caused significant shift in energy metabolism from anaerobic to aerobic glycolysis (Table 1). When compared to rhGM-CSF, thalidomide was more effective in reduction of glycogen consumption and anaerobic glycolysis rate, and in increase of aerobic glycolysis rate ($p<0.05$ for each). Combined use of two drugs was more effective than single use of either drug in these regards (Table 1). Combined use decreased glycogen consumption by 50.37%, and increased CO2 production by 94.03%. Conclusion: The results of this study indicate that single or combined use of thalidomide and rhGM-CSF mediates promyelocytic leukemia HL-60 cells into aerobic glycolysis. Further investigations are required for the understanding of the mechanisms as well as clinical significance of this effect.

Abstract: 176 Oral: 176

PHOSPHATIDYLINOSITOL-3 KINASE/AKT/MTOR SIGNALING CASCADE PLAYS A CRITICAL STEP IN HEPATIC AND ADIPOCYTIC DIFFERENTIATION OF HUMAN BONE MARROW MESENCHYMAL STEM CELLS

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Background: The mesenchymal stem cells (MSCs) were clonal, plastic adherent cells from bone marrow capable of differentiating into osteoblasts, adipocytes, chondrocytes and, as demonstrated more recently, other tissue lineages including myoblasts, hepatocytes and possibly even neural tissue. Because human bone mar-row-derived MSCs are easily accessible and expandable in culture, there has been much interest in their clinical potential for tissue repair and gene therapy. MSCs provide a promising source for autologous fat graft and cells for liver transplantation. As a result, numerous studies have been carried out demonstrating the migration and multi-organ engraftment potential of MSCs in animal models and in human clinical trials. However, the signaling pathways or networks involved in mediating such differentiation have yet to be clarified in MSCs. Aims: To investigate whether PI3/Akt/mTOR cascade plays a role in the differentiation of MSCs towards adipocytes and hepatocytes. Methods: MSCs were purified from bone marrows of human donors with informed consent and cultured in expansion medium. These cells were induced, in the absence or presence of specific signal inhibitos, into adipocyte differentiation by exposure to 1-methyl-3-isobutylxanthine, dexamethasone and indomethacin in time course, and hepatic differentiation by exposing to HGF (hepatocyte growth factor) and bFGF (basic fibroblast growth factor) for 7 days followed by Oncostatin M (OSM) for 4 weeks. Real-time PCR, ELISA, Western blotting and functional assays were used for detection of gene expression and protein production of the adipocytes and hepatocytes. Results: Under in vitro induction with bFGF and HGF, human MSCs from bone marrow can be differentiated into hepatocytes.

These differentiated cells acquire a cuboidal morphology and are indeed able to express liver marker genes, present functional characteristics of hepatocytes such as albumin production, glycogen storage, urea secretion, uptake of low-density lipoprotein, cytochrome P450 activity in vitro, and also express an antigen normally expressed on the bile canaliculi formed between adjacent hepatocytes. All these criteria have been listed as required to define a functional hepatocyte. Once activated upon initiation of adipocyte differentiation, the level of perixisome proliferators-activator receptor (PPAR) gamma mRNA, the lipid content, and the lipogenic activity as measured by leptin and adiponectin secretion in medium were markedly increased in a time-dependent manner. Interestingly, the protein levels of phospho-Akt, phosphor-4EBP1, and phosphorp70S6k were also increased along with the maturation of adipocytes and hepatocytes, implicating the activities of PI3K were activated. The increase of PPAR gamma transcription, the secretion of leptin and adiponectin and the lipid content during adipocytic differentiation induction, and the albumin production and expression of hepatocyte markers were blocked by LY294002, the PI3-kinase inhibitor but strengthened by PD98059, the p42/44 MAPK inhibitor. The results have led to the proposal in which the PI3-kinase/Akt pathway plays a critical role in the hepatic and adipocytic differentiation of MSCs from bone marrow. Conclusions: We conclude that the intracellular PI3 kinase/Akt/mTOR signaling pathway appears to play a critical role in inducing the hepatic and adipocytic differentiation process in bone marrow-derived MSCs.

Abstract: 177 Oral: 177

DEVELOPMENTAL CELL FATE OF PRECURSOR FOR CARDIOMYOCYTE AND HEMANGIOBLAST IS ACCOMPANIED BY THE PRESENCE OR ABSENCE OF EXPRESSION OF PDGFR-ALPHA AND FLK-1 AT THE DIVERGENT POINT

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Background Both of hemangioblasts and cardiomyocytes are thought to be derived from lateral plate mesoderm of early embryo, but little is known about the phenotypic specification in these two lines of differentiation at the divergent point through development. Pluripotency of embryonic stem (ES) cells provides a powerful tool for dissecting the cellular and molecular mechanisms regulating germ layer fate determination and tissue formation. Utilizing the in vitro two dimensional culture system for ES cell differentiation on appropriate supportive cell layers, significant progress was achieved to clarify the biological mechanism involved in embryonic hematopoiesis and vasculogenesis. Aims Utilizing the in vitro differentiation system of murine ES cells in the presence of stromal cell layer, and gene expression data in the microarray analysis, we determined a set of cell-surface molecules as specific markers, which enabled efficient enrichment of hemangioblasts or cardiac myocytes. Methods Murine embryonic stem cell line, EB5 was cultured on gelatin-coated plates for mesodermal differentiation after withdrawal of LIF and Blastcidin. The expression of surface molecules related to mesodermal differentiation was evaluated with flowcytometry or quantitative RT-PCR techniques. The cell population defined by combination of the candidate surface molecules was sorted at day 5 and seeded on PA6 or OP9 stromal cell layers until day 12 of differentiation. Differentiational direction of each culture sample was evaluated by the expression of genes or membrane molecules specific for tissue lineages with quantitative RT-PCR or flowcytometric techniques. The cardiogenic potential was also assessed by scoring beating colonies co-cultured on stromal cells. Results In combination with cell surface expression of Flk-1 (VEGFR2), which is known to be a restricted marker for mesodermal tissues during early embryonic development, upregulated expression of Platelet-Derived Growth Factor (PDGF) receptor alpha on cell surface was detected more frequently in the fraction containing pre-cardiomyocytic cells. In contrast, negative fraction for PDGFRalpha contributed to efficient formation of hematopoietic burst colonies growing together with endothelial cells engulfing DiI-acetylated LDL. We also cofirmed that these suspended hematopoietic cells were spurting out of hemangioblast-like compartment in time-lapse imaging analysis. Conclusions Our present data indicates that up regulated expression of PDGFRalpha on cell surface is detected more frequently in the fraction containing pre-cardiomyocytes. In contrast, negative fraction for PDGFRalpha contribute to efficient formation of hematopoietic clusters growing together with endothelial cells engulfing DiI-acetylated LDL. Our findings may provide a new experimantal

window to analyze the common mesodermal precursors for hemangioblasts and cardiomyocytes in vitro.

Abstract: 178 Oral: 178

GENE EXPRESSION PROFILING OF SINGLE HAEMATOPOIETIC PRECURSOR CELLS DURING HAEMATOPOIESIS

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Disruption of normal transcriptional control has emerged as one of the central mechanisms underlying the development of leukaemia, for understanding of which it is essential to establish expression patterns associated with normal haematopoiesis. Expression profiling of haematopoietic cells is hampered by the heterogeneous nature of haematopoietic tissues and the absolute rarity of early-unrestricted progenitors. To overcome these restrictions the expression profile of lymphoid and myeloid associated genes (LEF-1, Mb-1, EBF, CD19, Sox4, B29, CD45, c-fms, Lysozyme, PU.1 and CD5) were measured by real-time PCR in; 40 single cell PolyA cDNA samples representing eight different stages of mouse haematopoietic hierarchy from multipotential to committed single lineages, 20 PolyA cDNA samples representing ten different maturing/mature mouse haematopoietic colonies and populations, 7 mouse bone marrow haematopoietic fractions and 11 differentiating FDCP mix cells. Analysis 40 single hematopoietic precursors failed to detect any expression of LEF-1, EBF, CD19, and CD5 in the myeloid cells despite detection in lymphoid cells. The lymphoid associated genes Sox-4 and B29 were detected with a peak of expression in granulocyte/macrophage precursors. These findings suggest a potential role for Sox-4 and/or B29 in early myelopoiesis and preliminary results indicate that single cell cluster analysis may provide a means of identifying gene hierarchies and characterising individual cells on the basis of their expression profiles. This study has also determined baseline expression values for a range of myeloid and lymphoid lineage markers during mouse hematopoiesis that can be used to study lineage hierarchy and relationships.

Abstract: 179 Oral: 179

THE EFFECT OF USING HUMAN HEMATOPOIETIC STEM CELLS (HSC) FROM AUTOLOGOUS BONE MARROW (BM) IN JAWS BONE AUGMENTATION

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Background: Guided bone regeneration (GBR) is the procedure necessary and useful in bone augmentation in jaws bone defects (trauma, cystectomy, impacted tooth, pre-implantology treatment). GBR process is depends on tetrad of: 1. growth factors, cytokines, i.e. BMPs bone morphogeneic factors 2., hematopoietic stem cells HSCs, 3., biocompatible, resorbable carrier, 4., the resorbable or non-resorbable barrier between formed epithelial and connective tissue. The described effects of human hematopoietic stem cells CD34+ on jawbones augmentation are not significant differ from effect of transplantation of bone marrow (BM). The plasticity phenomenon "mature " stem cells are still controversial. On the other hand it was found, that the effect of platelet rich plasma (PRP), isolated using COBE Spectra System, which are pure product contains high concentration of growth factors (PDGF, IGF, TGFbeta and others) on bone augmentation was more potent than CD34+ or BM. Differences between various biological systems seems to be depends on: cell type, their number, activity growths factors and their concentration also carrier as natural or synthetic substrate. Aims: The aim of study was to compare the effects of bone augmentation by the transplantation of combined transplants autologous hematopoietic cells (CD34+) on natural substrate- deproteinazed bovine chips, and on the synthetic beta-tri-calciumphosphate. Both carriers are resorbable. Methods: Seven males had a two-wall bone defects on maxillary bone. The population of mononuclear cells were isolated from 20 ml of autologous bone marrow, concentrated to 3 ml, and the CD34 cells were evaluated in the product The number of CD34 cells per transplant was included between 2.8x10(6) to 0.8x10(6). Mononuclear cells suspended in autogenic serum were mixed with the

carrier Bio-Oss (bovine bone mineral, Bio-Geistlich) or Cerasorb (synthetic tricalcium phosphate -Curasan). Against the epithelial tissue in growth into the bone defect, two barriers were used: autogeneic fibrin glue, and synthetic, resorbable Bio-Guide. The trabecular structure of regeneration bone was evaluated using Fourier analysis of radiograms. Results:. The cases of jaws bone defects regeneration were present. The higher and faster bone augmentation and maturation were not observed using relative CD34 cells in transplant. Proposal: higher concentration of CD34 + cells or CD34 + cells stimulated before transplantation by growth factors should be more effective. Preliminary data after using of HSCs allows for suggestion, that it could be a methodology useful in the treatment of bone deficiency, however the "plasticity phenomenon" of CD34 + cells were not fully confirmed in presented bone augmentation methodology (CD 34 needs differentiation factors and more time).

Abstract: 180 Oral: 180

POSTNATAL NEOVASCULARIZATION WITH IMPLANTATION OF AUTOLOGOUS BONE MARROW MONONUCLEAR CELLS FOR PATIENTS WITH RUTHERFORD GRADE II/III THROMBOANGIITIS OBLITERANS

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Objective: We investigated efficacy and safety of implantation of autologous bone marrow-mononuclear cells (ABMMNCs) in patients with ischaemic limbs due to Buerger`s disease. Methods: We conducted a clinical, prospective study to test cellular therapy with ABM-MNCs in patients with ischaemic limbs at the Univer-sity of Ankara School of Medicine. We investigated 25 patients (23M/2F) (age 43±10.8 years) who had critical limb ischaemia defined as ischaemic rest pain in a limb with or without non-healing ulcers but with preserved proximal arterial inflow. Patients were non-responders to previous Iloprost infusion and smoking cessation within six months and were not candidates for nonsurgical or surgical revascularisation. The study protocol consisted of pain score, ankle-brachial pressure index (ABI), peak walking time (PWT) and claudication onset time (COT), Intra-arterial Digital subtraction angiography (IA-DSA), pulse oximetry (SaO2), Vascular Quality of Life Questionnaire at baseline and 3,6-months follow-up. Results: We harvested bone marrow (639.1±100.4 mL) from the posterior iliac spine under GA. After red blood cell depletion and volume reduction we achieved 72.7±11.8 % depletion and concentrated ABMMNC to a final volume and concentration of 61±12.2 mL and 21.1±14.5x10e8 total nucleated cells, respectively. ABMMNC mplantation (mean 10.4±6.8x10e8) within three hours after marrow aspiration by intramuscular injection into the gastrocnemius muscle of ischaemic legs. Unilateral intramuscular administration of ABMMNC was not associated with any complications. Total healing of the most important lesion, was achieved in 18/20 (90%) patients. Change in PWT at 12 weeks, total relief of rest pain without the need of analgesics improved in 23 patients (92%). DSA studies before and 3 months after the ABMMNC implantation showed the presence of a new vascular collateral network across the affected arteries in 22 patients (88%). Comment: The results of the presented study are promising. Thus, bone marrow may be a potential source of cells for Buerger`s patients with end-stage limb ischaemia refractory to other treatment modalities.

Abstract: 181 Oral: 181

GENERATION OF HEMATOPOIETIC CELLS BY FIVE HUMAN ES LINES AND EXPRESSION OF UNIQUE GLOBIN PATTERNS BY ES-DERIVED ERYTHROID CELLS

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Background: Human embryonic stem cells are unique tools for understanding developmental and tissue differentiation and this knowledge may have therapeutic potential for human disease. Thus far, evidence of generating specialized cells for different tissues, e.g. cardiac, hepatic, pancreatic, and neural tissues, from human ES cells has been presented but hematopoietic differentiation was only shown in ES lines derived by Thomson