and colleagues. Aim/Method: In this study, we assessed the response of five different human ES lines (H1, hSF6, BG01, BG02, BG03), originated in three different laboratories, to hematopoietic inducing conditions, by phenotypic and functional in vitro approaches. Results: Characterization of surface marker expression (i.e. CD34, CD45, glycophorin A) and assessment of hematopoietic progenitor clonogenic ability indicated that despite the expression of CD34 in all cell lines and an appreciable level of CD45 expression in H1, hSF6, and BG02, only H1 and BG02 gave rise to hematopoietic clonogenic progenitors following embryoid body (EB) culture. Their clonogenic potential was associated with generation of non-adherent cells, which were mainly CD45+ and a rich source of hematopoietic progenitors, from H1 and BG02, whereas hSF6, BG01, and BG03 were devoid of both non-adherent cells and hematopoietic progenitor emergence. Expansion of non-adherent cells with a cocktail of growth factors (IL-3, IL-6, SCF, Flt-3L, G-CSF, EPO, TPO, FGF, VEGF, transferrin) resulted in a drastic shift in the composition of colonies from granulocytic/monocytic lineages towards erythroid lineage in H1, but not in BG02. Erythroid cells generated under these conditions produced mainly embryonic (. and .) and fetal (.) globins and only a small proportion of them expressed adult . globin. Early exposure to hematopoietic growth factors during EB development dampened the generation of hematopoietic progenitors by non-adherent cells and subsequent erythroid development. Conclusions: Taken together, our data suggest: a) Different ES lines differ in their ability to undergo hematopoietic differentiation, which can not be predicted by the surface markers studied. b) Non-adher-ent cells emerged from EBs are rich in hematopoietic progenitors and can be expanded to generate highly enriched erythroid colonies only in H1 line. c) BFU-E-derived cells from H1 produce mainly embryonic and fetal globins. The predominant expression of both embryonic and fetal globins among the same population of erythroid cells is unlike the clean transition from embryonic to fetal globin pattern seen in vivo.

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EVALUATION OF GENETIC DEFECTS UNDERLYING FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS: PRELIMINARY RESULTS

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Background: Familial hemophagocytic lymphohistiocytosis (FHL) is a rare, autosomal recessive, if untreated, inevitably fatal disorder of early childhood associated with persistent fever, hepatosplenomegaly, pancytopenia, liver dysfunction, coagulation abnormalities, overproduction of inflammatory cytokines, hyperactivation of T lymphocytes and macrophages the latter frequently involved in hemophagocytosis in bone marrow, lymph nodes, liver and spleen. The disease is genetically heterogeneous; pathogenic mutations have been described in Perforin 1, Munc13-4, Syntaxin 11 genes, and a gene not-yet-identi-fied has been mapped to the long arm of chromosome 9. However, genetic defects in these genes only partially explain the molecular pathogenesis of all FHL cases. Aims: The purpose of this study is to gain further insights into the molecular basis of this disorder by evaluating the genetic defects underlying FHL in Turkish patients. Methods: We have investigated a total of 16 unrelated families of consanguineous marriages each with one or more affected children. Linkage analysis was performed using 5-10 microsatellite markers selected from chromosomal regions harbouring Perforin 1 (10q21-22), Munc 13-4 (17q25), Syntaxin 11 (6q24.2) genes and an unidentified gene (9q21.3-q22). The complete coding sequences of the Perforin 1 gene (exon 2-3) was screened for mutation by direct sequencing. Results: Homozygosity with the markers tightly linked to the Perforin 1 gene has been observed in 2 patients. Sequencing analysis of Perforin 1 gene revealed that a homozygous G to A substitution at codon 149 (GGC>AGC) resulted in the replacement of Gly with Ser (G149S) in one of these patients, while no mutation in coding sequences of the gene was detected in the other patient. Consanguineous common haplotype has been observed in parents of another 2 patients whose DNA were not available for the study. Heterozygous G to A transition at codon 374 (TGG>TGA) leading to the substitution of Trp with a stop codon (W374X) was identified in both parents of one of these cases. This nonsense mutation in homozygous state was most probably the cause of the disease in the affected child. While a heterozygous C to A substitution at codon 91 (GCG>GTG) leading to transition of Ala with Val (A91V) was detected in the father of the second patient, neither this nor any other mutation was detected in the mother. Regarding the

remaining patients Perforin 1 was excluded by linkage analysis, homozygosity has been detected in five and one patients for Munc13-4 and Syntaxin 11 gene loci respectively. Summary/Conclusion: Previous reports indicated that Perforin gene mutations account for significant portion of both Turkish and all FHL cases worldwide (about 30%). However, the preliminary results of this study suggested that molecular pathologies in Munc13-4 gene, but not in Perforin, may be the major cause of the FHL in Turkish population. First two authors contributed equally to this work.

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SEVEN TURKISH PATIENTS WITH NIJMEGEN BREAKAGE SYNDROME: CLINICAL CHARACTERISTICS AND MUTATION ANALYSIS

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Nijmegen breakage syndrome (NBS) (OMIM 251260) is a rare autosomal recessive disorder characterized by growth retardation, microcephaly, developmental delay, distinctive facial appearance, immunodeficiency, and predisposition to malignancies. Most of the previously identified patients have belonged to Slavic populations, such as Poland, Czech Republic, and Ukraine. Mutations in the NBS1 gene were first found to be associated with this syndrome in 1998. Almost all of patients from Slavic origin were found to carry a homozygous five base pair deletion (657del5) in the 6th exon of this gene. A conserved haplotype associated with this deletion was demonstrated and suggested that this mutation had a single origin in Slavic populations. A combined carrier frequency of this deletion in three Slavic countries, Czech Republic, Poland and Ukraine, was reported to be 1/177. Recently a Turkish patient with NBS have been reported and who was found to be homozygote for 657del5. In this paper, we reported seven patients with NBS from three different families in Turkey. These families denied any relationship with Slavic populations. All probands in these families were phenotypically diagnosed as having NBS based on growth retardation, microcephaly, developmental delay and facial features in addition to lymphoreticular malignancies. Cytogenetic and immunological investigations also supported the diagnosis. All the seven patients were found to be homozigote for 657del5. Four of seven patients were female. There was consanguinity within all families. One patient had acute leukemia, two patients had B-cell lymphoma, one had T-cell lymphoma. These four patients were death from malignancy. One patient had autoimmune lymhoproliferative syndrome. She is surviving with steroid treatment. Other two patients surviving and have no malignancy. This study demonstrated that NBS has not been rarely diagnosed in Turkish population. This paper gives the description of the first case of autoimmune lymhoproliferative syndrome diagnosed in patients with NBS in literature so far.

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INCIDENCE AND CLINICAL RELEVANCE OF COMPLEX CHROMOSOMAL ABERRATIONS IN A SERIES OF 88 CHILDREN WITH ETV6-AML1 POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA

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Background and aims: Cryptic translocation t(12;21)(p13;q22) which give origin to the hybrid gene ETV6-AML1(RUNX1) can be detected in a 20-25% of children with B precursor ALL as the most frequent specific aberration. Despite of the fact that according to the most authors this finding is associated with a favorable outcome, ETV6-AML1 positive children can have late relapses. One of the reasons could be high instability of the genome of leukemic cells, which is manifested on chromosomal level by additional aberrations and/or complex chromosomal rearrangements. Genetic changes that are most frequently associ-

ated with t(12;21) are the deletion of the wild type ETV6 alelle, trisomy of chromosome 21 and/or duplication of the ETV6/AML1 fusion gene. Also non-specific structural and/or complex chromosomal rearrangements could be detected. The aim of the study was to evaluate the significance of complex chromosomal aberrations for prognosis of chidren with ETV6-AML1 positive ALL. Methods: For the assessment of ETV6-AML1 fusion gene RT-PCR and/or double target interphase FISH with locus-specific probe (Abbott-Vysis) were used (200 interphase nuclei analysed, cut-off level 2.5% tested on controls, standard deviation 0.5%). Karyotypes were analyzed by conventional and molec-ular-cytogenetic methods. Structural and/or complex chromosomal aberration were proved by FISH with whole chromosome painting probes (Cambio, Cambridge, UK) and/or by mFISH with the "24XCyte" probe kit (Meta-Systems GmbH, Altlussheim, Germany). Results: We performed a retrospective study of 88 children with ALL and ETV6-AML1 fusion gene proved by RT-PCR and/or I-FISH. Patients were diagnosed between 1995 and 2005 in four haematological centers in Czech Republic, with an age ranged between 15 months and 16,6 years (mean 5 years). Most of them are living in the first or second complete remission. Relapse appeared in 17 children (19,5%). Three patients died (two because of relapse and one for treatment complications). In 53 children (60%) we found except t(12;21)(p13;q22) additional chromosomal aberrations, the most frequently trisomy or tetrasomy of chromosome 21 (14 cases), deletion of non-translocated ETV6 allele (14 cases) and/or deletion of 6q (6 cases). Complex karyotypes were identified in 40 children in total (45,5%). In nine of them variant translocations of chromosomes 12 and 21 with other partners were observed. Analysis of event-free survival (EFS) revealed significantly shorter survival in patients with additional structural and/or complex aberrations in ETV6-AML1 positive cells (p=0,005). Conclusions: In our cohort of patients with ETV6-AML1 positive ALL complex karyotypes were indicator of poor prognosis. Finding of complex chromosomal aberrations of leukemic cells is accompanied by higher risk of relapse even in those cases where is primarily present prognostically positive aberration. Supported by grants MSM 0021620813, GACR 301/04/0407 and IGA NC7490.

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11Q23 REARRANGEMENTS IN HEMATOLOGICAL MALIGNANCIES: A STUDY ON 34 TUNISIAN PATIENTS

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Structural rearrangement of the chromosome band 11q23 is one of the most common cytogenetic abnormalities seen in haematological malignancies, especially in acute leukemias. Molecular genetic investigations have revealed that these abnormalities often lead to rearrangement of the mixed lineage leukemia gene (MLL). In our study we report 34 patients diagnosed with 11q23 abnormalities. There were segregated into cytogenetic subgroups for comparison of presenting clinical and biological features, response to induction therapy, and long-term outcome. Between January 1994 and June 2003, 34 patients with haematological malignancies were diagnosed with 11q23 abnormalities by conventional cytogenetic analysis at diagnosis and prior to treatment. Chromosome studies were done at Laboratory of Cytogenetics, Farhat Hached Hospital and were performed on short-term culture (16, 24 and 48 hours) of the cells from bone marrow or peripheral blood by the R banding technique. To establish the involvement of MLL by Fluorescence In Situ Hybridization Analysis (FISH) we used LSI MLL (11q23) dual color DNA probe (5`MLL spectrum green -3` MLL spectrum orange). The median age of these patients was 25 years (range from 3 months to 67 years).62 % were female. 20 patients had an AML, 11 had an ALL and 3 had a MDS. The median white blood cell count (WBC) was 65,5x109/ l. 11q23 rearrangements were apart from a complex Karyotype in 11 cases. They were the sole chromosome abnormality in 13 cases. Twenty four patients showed an apparently simple reciprocal translocation. The most frequent chromosome partner sites were 4q21 (7 cases) and 9p22 (5 cases). We report rare translocations, such as t(11;17)(q23;q12) and t(10;11)(q22;q23), each in one case of acute monoblastic leukaemia. A del(11)(q23) were observed in 6 cases, a der(11)(q23) in 3 cases and an ins(4;11)(q21;q11q23) in one case. FISH analysis was possible in 25 cases. Twenty of them showed MLL rearrangement. Investigation of hematologic and clinical characteristics of each cytogenetic subgroup showed that t(9;11)(p22;q23) is strongly associated with acute monoblastic leukemia. The t(4;11)(q21;q23) and the rearrangement of MLL

are both associated with a splenomegaly, an extreme hyper-leukocytosis and a shorter complete remission duration. The median overall survival and event free survival were respectively of 8 months and 4 months. The age (<1 year and >50 yeras) and the WBC (>30.109 / l) are unfavorable predictive prognostic factors. Translocation t(4; 11) is associated to the worst clinical outcome. In conclusion, 11q23 rearrangements in hematological malignancies are associated to heterogeneous clinical features and treatment outcome according to the cytogenetic subroup.

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ROUTINE DIAGNOSTIC REAL TIME PCR MESUREMENT OF LYMPHOMA GENE SIGNATURES IN POLYA CDNA

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Microarray gene expression profiling has identified certain "Indicator" genes predictive of outcome in DLBCL and follicular lymphoma (FL). However, such methods rely on relatively large amounts of fresh starting tissue, and, therefore, measurement of Indicator genes in routine practice is difficult. To test the use of Indicator genes as a diagnostic tool for lymphoma, we have developed a simple, clinically practical polyA PCR based method for analysis of Indicator profiles in DLBCL and FL, specifically for use in very small tissue samples. PolyA RT-PCR, an extremely sensitive technique enabling global mRNA amplification from ng amounts of RNA, was applied to RNA extracted from 67 archived human frozen lymph nodes (LN). The resultant cDNA was analysed by TaqManTM real-time PCR for 36 candidate Indicator genes (from Shipp et al & Alizadeh et al) using specific 3` directed primers. The expression level of each gene was quantified against human DNA, and the data normalised using the mean expression of four housekeeping genes (IF2-b, Gap, Rbs9 and beta actin). The results demonstrate ability to distinguish between reactive and neoplastic LNs and between DLBCL and FL. Specifically, ACTA expression was reduced in neoplastic compared to reactive LNs ($p<0.03$), whilst Urokinase ($p<0.00$) and KIA0233 ($p<0.01$) distinguished between DLBCL and FL. Preliminary analysis also indicates correlation with length of survival. Specifically, expression levels of EAR2, 5HT2B, HSF1, HSP27, ID2, KIA0233 & PKCG (all at $p<0.03$) were correlated with interval to death in FL, and BSP1 with interval to death in DLBCL ($p<0.04$). These results validate a simple, sensitive and robust method for analysing lymphoma Indicator genes. Crucially, initial amplification using polyA PCR makes the technique applicable to very small clinical samples, such as needle core biopsies or fine needle aspirates, facilitating routine clinical application.

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IN VITRO DRUG RESISTANCE IN CHILDHOOD ALL AND EFFECT ON PROGNOSIS

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Risk-adapted therapy in acute lymphoblastic leukemia (ALL) of childhood relies on traditional risk factors such as age, white blood cell count, immunological subtype, chromosomal aberrations and response to treatment although different risk factors was used at different protocols. The aim of this study is to assess the predictive value of in vitro cellular drug resistance and compare with these prognostic factors. PATIENTS AND METHODS: In vitro drug resistance was measured using the 4-day methyl-thiazol-tetrazolium (MTT) assay at bone marrow samples in 198 children (100 female, 98 males) at initial diagnosis. The patients had been treated according to two different protocols in either the CCG or BFM-based and followed from 1 to 144 months (median 25 months) We evaluated 5 drugs (VCR, PRED, L-ASP, DEXA, ETO) and calculated the 50% lethal concentration (LC 50) of each one. Nonparametrically distributed median LC 50 values were analysed with the Mann-Whitney U test for statistycal analysis RESULTS: Large individual patient differences in cellular drug resistance were found, Patients with high leukocyte count (> 50.000/mm3) were 5 fold more resistant to PRD. Cases with CD 34 positive ALL also were 2 and 5

fold more resistant to VCR and ARA-C respectively. There were also prednisolon resistance in T cell leukemia and prednisolon sensitivity in Calla positive ALL (3 fold). Patients with t (9,22) and t (4,11) were 3.6 and 2.2 fold more resistant to PRD respectively. Patients with t (12,21) were 2 fold more resistant to DEXA. There was no statistically significant difference between LC50 values and age and response to treatment. Patients who long term survive were highly sensitive to PRD and DEXA CONCLUSION: Childhood ALL has a heterogeneous in vitro resistance profile, with relatively sensitive and resistant cases. CD 34 positive and T cell samples, however, are generally more resistant compared to other immunophenotype In vıtro drug resistance might help explain the poorer prognosis of high risk leukemia. This research was partly supported by the İstanbul Unıversıty Research Fund. Project Number:T-1137/18062001

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THROMBOPHILIA SCREENING IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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BACKGROUND: Thromboembolism commonly occurs in leukemic patients and the occurence of thrombosis complicates the patient management. The etiology of thrombosis in malignacy is multifactorial and mechanisms include the release of procoagulants by tumor cells, as well as predisposing factors such as infection and anticancer drugs. L-asparaginase and corticosteroids have been successfully used in induction therapy in ALL, but several complications including thromboembolic events have also been attributed to these drugs. AIMS: The aim of this retrospective study is to investigate the thrombosis incidence and the effect of inherited hypercoagulable abnormalities on thrombosis in children with ALL. METHOD: The records of 71 children with ALL were reviewed. The patients received CCG-106-B, CCG-1952, CCG-1961, and CCG-2552 protocols. Thrombophilia screening was performed in patients with thrombosis. The laboratory evaluation of inherited hypercoagulable abnormalities included functional anticoagulant assays for protein C, protein S, antithrombin III(AT III), factor VIII and genetic analysis of the factor V Leiden, prothrombin 20210 G-A, and MTHFR 677 mutation were performed before induction therapy. Patient characteristics, thrombotic processes (catheter related thrombosis, stroke, pulmonary embolism, deep venous thrombosis) were evaluated. RESULTS: The median age of patients with thrombosis was 6,5 years (range: 1,4-17 years). Thromboembolic events were observed in 10 cases (4 cerebrovascular accidents, 2 deep vein thrombosis, and 4 catheter related right atrial thrombosis). Thrombophilia screening was performed in patients with thrombosis (protein C, protein S, AT III levels in all, genetic analysis of the factor V Leiden and prothrombin 20210 G-A mutation in seven, MTHFR 677 mutation in two, factor VIII level in three, and factor IX level in two). Elevated factor VIII level and factor V Leiden mutation were associated with cerebrovascular accident in a patient. And elevated factor VIII, factor IX level and MTHFR 677 mutation were associated with catheter related right atrial thrombosis in another patient. Postphelebitic syndrome was seen in one patient with deep vein thrombosis 7 years after thromboembolic event. CONCLUSION: Thrombophilia screening tests should be performed in all ALL patients. ALL treatment is one subject of discussion on the increased thrombotic risk due to gene polymorphisms. The children with thrombosis should be followed for development of late complications.

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IMMUNOPHENOTYPING AND CYTOGENETIC CHARACTERISTICS OF NEWLY DIAGNOSED CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) AT KING HUSSEIN CANCER CENTER (KHCC)

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Introduction: Jordan has a population of 5 millions, childhood cancer accounts for 6% of all cancer. Acute leukemia accounts for 36% of all childhood cancer, 75% of them are acute lymphoblastic leukemia. 60-70% of all childhood cancer are treated at the newly established King Hussein Cancer Center Immunophenotypic and cytogenetic charachteristics of children with acute lymphoblastic leukemia is lacking in Jordan and the

area as well. Differences in these charactereistics among children population from different parts of the world may shed light on the pathogenesis of acute lymphoblastic leukemia, and explain differences in clinical behavior. Materials and Methods: Since January 2003, 101 Jordanian children were diagnosed to have acute lymphoblastic leukemia. M;F ratio 1.3 (57 M, 44 F). Age distribution as follows: <1 year: 4, 1-4 years: 47, 5-9 years: 27, 10-15 years: 21, >15 years: 2. Median age was 4 years, and peak age 3-4 years. Immunophenotypes: precursor B: 83 (82%). T cell: 13 (13%), B cell: 3 (3%), bilineage 1 (1%) unclassified 1 (1%). Cytogenetics: Routine cytogenetics, and fluorescence in situ hybridization (FISH) were successfully performed on only 57 patients. Specific tranlocations detected were as follows: t(9:22), Philadelphia chromosome in 8 (14%), t(12:21) in 5(9%), t(4:11) in 3 (5%), and t(1:19) in 3 (5%), trisomy 21 was present in 3 (5%), and monosomy 7 in 1 (2%). Conclusion: Age and immunophenotypic characteristics of Jordanian children with acute lymphoblastic leukemia are similar to those reported worldwide, however there is increase in the incidence of Philadelphia chromosome positive ALL 14% vs 3% reported from western centers, and decrease incidence of t(12:21); 9% in our patients vs 20% in the west. These differences are highly significant if this trend persists.

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THE OUTCOME OF CHILDHOOD ALL IN A SINGLE CENTER FROM TURKEY: THE RESULTS OF 11 YEARS\` EXPERIENCE WITH ALL BFM 90 AND 95 TRIALS

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Background: Dramatic progress in the treatment of childhood ALL has been achieved during the last two decades in Western countries where the 5 year event free survival rate (EFS) has risen from 10 to 80%. However, this high cure rate has not been always occurred in every center in developing countries due to limited sources. The Aim: In this study we evaluted the treatment results of ALL BFM 90 and 95 chemotherapy protocols used between 1993 and 2004 in our center. Methods: A retrospective analysis of 150 children diagnosed as ALL (M/F: 92/58; mean age: 6.2±3.7 years) was performed. The overall (OS) and event free survival rates(EFS) according to age, sex, initial leucocyte count, chemotherapy response and risk groups were analysed by Kaplan Meier survival analysis. Children received BFM -90 chemotherapy protocol between 1993-1996 and BFM-95 were given afterwards. Methotrexate (MTX) dose was reduced as 1 g/m2 in the protocol II of BFM 90 (n:37; 25%) whereas children in BFM 95 protocol (n:113; 75%) received the orginal dose of MTX (5 g/m2). MTX could not be given to 7 children due to their loss during the induction phase. Results: EFS and OFS were not significantly different according to age, sex and initial leucocyte count ($p > 0.05$). However, the children who had good response to prednisolone in the first 7 days of treatment achieved significantly better OS and EFS ($p<0.05$). Patients showing good response in BM aspirates to chemotherapy at 15 days of induction and also the ones achieving complete remission at day 33 had also significantly better OS and EFS than the others($p<0.0001$). Children in the standart and median risk groups obtained better survival rates comparing to the patients in high risk group ($p<0.05$). Five years EFS and OS in children given reduced dose of MTX (BFM-90) were 65% and 76%, in respectively. Although the children who received orginal dose of MTX (5g/m2-BFM-95) had better 5 years EFS (78%) and OS (80%) than the others, the difference between the two was significant ($p<0.05$). Death occurred in 33 patients out of 150 (22%). None were lost due to MTX toxicity. Conclusions: BFM 90 and 95 protocols in our center were successfully applied with no severe MTX toxicity causing death. In our study, an improved outcome was obtanied with BFM-95 treatment protocol of which 5g/m2 MTX was given.

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ACUTE CHILDHOOD LEUKEMIAS AND EXPOSURE TO ELECTRO-MAGNETIC FIELDS GENERATED BY VERY HIGH VOLTAGE OVER-HEAD POWER LINES

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Many investigators have studied the effects of Electro Magnetic Fields (EMF) generated by ordinary domestic power lines, as a risk factor in the

pathogenesis of acute leukaemias in children, but there is little such study on very high voltage overhead lines. Children living(usually in slums) in industrializing major cities in developing countries, sometimes live very close to very high voltage (i.e.; 132000 - 230000 volts) power lines, by a negligence on housing safety standards. In this study we have analyzed 60 consecutive patients with acute leukaemias, and 59 matched controls living in a provincial capital city in North - Western Iran. The cases consisted of 58 patients (pts) with ALL, and 2 with AML, 35 males (58%) and 25 females (42%), 56 alive, and 4 died, with a mean age of 12.3 years. After a written consent, a detailed quationary was filled in, by the help of mothers as the main interviewees. A visit to the present (and for the cases, the previous) residential areas of the study groups was arranged. The locations of the high voltage power lines were detected in each area, if present, and their distances from the houses under study were detected. The expected intensity of the EMF generated was calculated in microTeslas (μT) having the mean intensity of the electrical current (I = 1000 amperes, HZ=50) by means of the relevant equations. The distances .2000 meters regarded potentially safe (=0 μT). Fourtteen pts(23.5%) in the case group were living near the power lines in distances equal to or less than 500 meters(range 67 - 500) experiencing constant mean EMF intensity of 0.6 microteslas (range 1.7 - 0.2). In the control group the figure was 2 patients (3.5 %). On statistical analysis, residency near very high voltage power lines in distances equal to or less than 500 meters significantly increased the likelihood of acute leukaemias in children.(95% CI=10.6 - 29, Odds ratio=6.88, P value=0.001). (E-mail: hpourfeizi@tbzmed.ac.ir)

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IS THE IMPOSSIBILITY TO TRANSFUSE BLOOD DERIVATIVES AN ABSOLUTE CONTRAINDICATION TO GIVE FIRST LINE CHEMOTHERAPY FOR ACUTE LYMPHOBLASTIC LEUKEMIA? TREATING JEHOVAH´S WITNESSES

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Combination chemotherapy(CC) has dramatically changed the prognosis of acute lymphoblastic leukemia (ALL) in the last decades with a high rate of complete remissions(CR) and long event free survivals (EFS) particularly in children; less so for adolescents and adults. However, it requires intensive support with blood derivatives(BD) to fight the complications of cytopenias due to both, the ALL and the CC. Patients like Jehovah`s Witnesses(JW) who refuse BD for religious reasons are usually given only symptomatic treatment and their prognosis is poor.The advent of stimulant agents of hematopoiesis(SAH) - erithropoietin, filgrastim, IL-11- open the possibility to use first line CC(FLCC) without necessarily transfusing blood derivatives. We hereby present our experience with 7 cases of ALL, 5 males and 2 females aged 6-25 years. The patient`s data are shown on the Table. The cases with L3 morphlogy showed no t{8:14}-. Five had high risk LLA. Informed consent was signed by the patients or their parents if they were minors. The induction treatment(ITx) included prednisone 60 mg/m2/d x 28d PO, vincristine 1.5-2 mg/m2/w IV x4, bleomycin 10U/m2/w x4 IV, daunorubicin 40 mg/m2/w IV x4 and L-Asparaginase 10,000U/m2/ x 10 SC. SNC prophylaxis with MTX 10 mg/m2, hydrocortisone 100 mg +/- Ara-C 30mg IT was given on days 1, 14 and 28. Supportive treatment included EPO 4,000-24,000U/d, filgrastim 150-300ug/24-48 hrs and IL-11 2.5 mg/d, high-dose IV iron and folic acid as needed. No important bleeding disorders were recorded. Infections were controled with conventional antibiotics. Four patients (cases 1,2,3 and 6) received the full ITx. Of the other three, two (cases 4 and 7) received only prednisone and one dose of vincristine. One dose of bleomycin and 3 of L-Asparaginase were added to the third one (case 5). The severity of the anemia precluded any other chemotherapy and they died of profound anemia. Among these cases were one adolescent with the lowest Hb and a 25yr man with the highest WBCC.The four patients who completed the ITx achieved CR and 3 of them were then accepted by social security institutions to continue their treatment. Case N° 1 has an EFS of 32 months, N° 2 relapsed after one year and died. N° 3 has an EFS of 9 months and N° 6 completed his first month in CR. These results show that it is possible to give FLCC and achieve CR in a substantial proportion (57%) of ALL patients, including high risk ALL, with the aid of an appropiate supportive treatment with SAH. The strategies used in these cases can certainly be improved and warrant further

trial. They open, however, new paths in the use of CC for ALL in patients like JW who positively refuse BD and are thus, doomed to death in a very short term for the reluctance of most institutions to offer them FLCC without the support of adequate conventional BD.

Abstract: 194 Poster: 101

SEQUENTIAL THERAPY RASBURICASE AND ALLOPURINOL IN THE TREATMENT OF HYPERURICEMIA IN LEUKEMIAS AND LYMPHOMAS

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Background Hyperuricemia and tumor lysis syndrome (TLS) are life threatening metabolic complications that can occur in patients affected by hyperleukocytosic leukemias or other haematological malignancies with a large tumour burden and rapid cell turn-over. Hyperuricemia, hyperphosfatemia, hyperkaliemia and hypocalcemia are the main metabolic abnormalities of TLS. Hyperuricemia is caused by the shedding of purinic basis from the tumour following spontaneous or chemotherapy-induced cell tumour lysis. It is well known that that blood U.A. > 8 mg/dl and white blood cell count > 50 x 103/ microliter are correlated to a high early death risk. There is a high incidence of TLS in tumours with high proliferative rate tumor burden such as acute lymphoblastic leukaemia and Burkitt`s Lymphoma. Usually profilaxis and treatment of hyperuricemia consists of hydratation, alkalinization and administration of Allopurinol. Allopurinol, a Xanthine-Oxydase inhibitor, acts by inhibiting the synthesis of uric acid, but it does not eliminate the already accumulated uric acid. Therefore, Allopurinol has a slow action, it is administered only per os and can lead to nefropathy with urolithiasis due to high blood concentrations of xanthine. Rasburicase, urate-oxydase from recombinant DNA, is a new therapeutic weapon able to catalyze the oxydation of uric acid in allantoin and to allow its elimination. It may represent an effective alternative drug in the treatment of hyperuricemia. Rasburicase administered i.v. (0.20mg/Kg/day) induces a rapid decrease in U.A. levels, improves the patient`s electrolyte status and can reverse renal insufficiency. The sequential administration of the two drugs could be an effective strategy for the treatment of hyperuricemia and to prevent uratic nefropathy. Aim: we report our preliminary results after the use of sequential Rasburicase and Allopurinol therapy in the treatment of hyperuricemia in 20 patients with Acute leukemias and aggressive NHL. Patients and methods: Twenty patients, 12 males and 8 females, mean age 56 years (range: 17-84), affected by haematological malignancies and high U.A. levels (2 ALL, 10 hyperleucocytosis AML and 10 aggressive NHL) were treated with Rasburicase (0.20 mg/kg iv) started the day before or day 1 of chemotherapy for two days and subsequently with Allopurinol (300 mg/os) from day + 4 until the end of chemotherapy. U.A. blood levels were measured 8 hours after Rasburicase injection, then daily during treatment. Blood U.A. determination was performed maintaining the test tubes at 4°C to block Rasburicase in vitro activity. Results: All patients responded to Rasburicase showing a fast and constant decrease of blood U.A. within 8 hours after the first i.v. administration. Plateau was reached after 36 hours of therapy (1-2 mg/dl U.A. levels) even in the presence of chemotherapy administration. No patients exhibited increased U.A. and creatinine levels during chemotherapy and two patients, who had increased creatinine levels at the diagnosis, showed a decrease after chemotherapy. Conclusion Rasburicase+Allopurinol sequential therapy might be the treatment of choice to control U.A and to prevent uratic nefropathy and TLS in adult patients with acute leukaemia or aggressive NHL.

Abstract: 195 Poster: 102

MANAGEMENT OF HYPERLEUKOCYTOSIS AND PREVENTION OF TUMOR LYSIS SYNDROME WITH LOW-DOSE PREDNISONE CONTINUOUS INFUSION IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background and Aim: The standard management of childhood acute lymphoblastic leukemia (ALL) with hyper-leukocytosis is unclear and its treatment has focused on prompt leukocytoreduction. Leukocytoreductive therapies have been used for

the prevention and management of tumor lysis syndrome (TLS), but the outcomes have been variable and their benefit has not been proven in controlled clinical trials. This problem needs much more investigations and effective and uncomplicated therapy strategies. Methods: In the present prospective trial, 15 children with ALL and hyperleukocytosis were treated with intravenous low-dose prednisone continuous infusion (LPCI) (6 mg/m2/24 hours) as the remission induction therapy. Results: The mean initial white blood cell (WBC) count of the patients were 218±184 x109/L (range, 101 to 838 x109/L). The mean WBC count reduction achieved by this treatment was 34.4% at first day, 56.9% at second day, and 76.6% at third day. The treatment was well tolerated. None of the 15 patients developed life-threatening metabolic derangement or required dialysis after the start of LPCI. Conclusions: When comparing this approach to exchange transfusion and leukapheresis we found it to be very economic, safe and effective. Our data suggest that LPCI treatment may be used for the patients presenting with especially WBC counts between 100 to 400x109/L.

Abstract: 196 Poster: 103

IS THERE ANY IMPORTANCE OF DIFFERENT BCR-ABL TRANSCRIPTS IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Leukemia specific fusion genes can be used for exact diagnosis, classification, detection of MRD during follow-up and for targeted therapy. These fusion genes can also be a target for PCR. One of the well-defined leukemia specific fusion gene transcript is BCR/ABL. In t(9;22), there are two main types of breakpoint cluster regions with BCR-ABL fusion genes. These are BCR-ABL p190 which is almost exclusively occurs in ALL and BCR-ABL p210 which is found in all CML and some ALL. The aim of this study was to compare lightCycler and nested-RT-PCR results and to evaluate the transcript of BCR-ABL in patients with ALL and the effect of different transcripts on clinical presentation and prognosis. Twenty-one ALL patients were included into the study. After total RNA extraction real time PCR (Lightcycler-LC-Roche) and inhouse nested RT-PCR (p190 and p210) was performed in all patients. BCR/ABL was found positive in 10 ALL patients with LC (47,6 %). P190 was found positive in 8 patients (80%). [5 patients had only e1-a2 transcript (381 bp), 2 patients had e1-a3 (207 bp) and one patient had both e1-a2 and e1-a3 transcript]. p210 was also studied in these patients and two patients (20%) who had negative p190, revealed positive p210 [b2-a2 (285 bp), b2-a2 and b3-a (285 bp and 360 bp)]. Neither of the patients were demonstrated both transcripts. When we analysed these transcripts according to clinical presentation (splenomegaly, leucocyte count, haemoglobin, platelet count) and prognosis we found comparable results and only two of them were alive who had p190 transcript with e1-a3 and e1-a2. Because of p210 positive ALL cases were only 2 patients we didn`t perform statistical analysis. We can suggest that both of them has bad prognosis and probably the difference in transcripts do not have any importance in patients with ALL.

Abstract: 197 Poster: 104

CAN RENAL LEUKEMIC INFILTRATION CAUSE HYPERTENSION IN CHILDREN?

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Out of 334 children with acute lymphoblastic leukemia who were treated with St Jude Total XI and Total XIII chemotherapy protocols were investigated and 21 (6.3%) were hypertensive. The incidence of tumor lysis syndrome was higher in the hypertensive group than in the non-hypertensive group (28.6 % vs. 11.5%) (p = 0.035). There were no differences between patients treated with high-dose methylprednisolone and prednisolone St Jude Total XI and Total XIII, St Jude Total XIII LR and St Jude Total XIII HR groups in respect of the abovementioned parameters. Central nervous system involvement, skeletal system involvement, abdominal lymphadenopathy, elevated LDH and leukocyte count, FAB types and immunophenotypes were not found to be statistically significant to the development of hypertension (p>0.05). We found that renal leukemic infiltration is a risk factor in hypertension development (p = 0.04) and hypertension is a risk factor for renal parenchymal disorder in the follow-up period (p = 0.0001). However, only 15.6% of patients had renal leukemic infiltration and suffered from hypertension.

This low ratio shows that renal leukemic infiltration alone is not a cause of hypertension.

Abstract: 198 Poster: 105

GRANISETRON HCL IMPROVES THE LEFT VENTRICULAR SYSTOLIC FUNCTION AND PASSIVE DIASTOLIC FILLING OF BOTH VENTRICLES IN CASES WITH HEMATOLOGIC MALIGNANCIES

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Background:Five-HT3 antagonists are commonly used in hematology/oncology clinics. Aim:Investigating the hemodynamic effects of granisetron HCl in terms of ventricular function utilizing tissue doppler and conventional echocardiographic parameters. Methods:We included 22 (6 ALL, 3 AML, 13 MDS, 8 females) patients into this study. All patients were given anthracycline lacking chemotherapy. All of them were studied with both conventional and tissue doppler echocardiography before and after administering 3 mg iv granisetron HCL within 12+/- 5 hours. The mean age of the group was 40.66±15.84 years, height and weight were 1.6±0.08 meters, 59.35±13.24 kg respectively. All patients underwent a complete two-dimensional transthoracic echocardiographic and doppler study in the left lateral decubitus position from multiple windows. All studies were performed with Vingmed system V (GE, Horten, NORWAY) echocardiograph and a 2.5 MHz transducer. Echocardiographic measurements were performed according to recommendations of the American Society of Echocardiography. Left ventricular dimensions were obtained using the parasternal short-axis view at the level of the papillary muscle. All variables were expressed as mean±standard deviation. All statistical analysis were completed utilizing paired samples t test and $p<0.05$ were considered as statistically significant. Results:They are shown on the table. Conclusion: We can conclude that granisetron, which is used as an antiemetic agent, slightly improves the left ventricular systolic function and passive diastolic filling of both ventricles in cases with hematologic malignancies. alev-erikci@yahoo.com

Abstract: 199 Poster: 106

SURVIVAL ANALYSIS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA- SINGLE CENTRE EXPERIENCE

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Acute lymphoblastic leukemia (ALL) is the most common malignancy diagnosed in children with a current cure rate of about 80%. Aim of this study is to perform a retrospective analysis of survival of children with ALL in the setting of contemporary intensive therapy. Patients and methods: Study population was 122 children (71 male and 51 female) with median age 4.74 years (range 3 months - 15 years), who were treated according to the BFM 90 and BFM 95 protocol in the 2nd University Department of Pediatrics at AHEPA Hospital from January 1992 until December 2004. Results: Hepatosplenomegaly, higher LDH values (.1000ÉU/L), higher white blood cell counts (.50x109/L) and high risk NCI (National Cancer Institute) classification were significantly more common among patients with T-ALL than those with BALL. Incidence of splenomegaly (.3 cm below umbilical line) was significantly higher among patients with WBC count .50x109/L. Higher leukocyte counts (.50x109/L) were significantly associated with higher LDH levels (LDH.1000IU/L). Among all patients of our study the event-free survival (EFS) rate was 83.48%(±5.2) and the overall survival rate was 81.67%(±5.3) with a median observation time of 78 months. Relapse occurred in 14 patients and 9 patients died of complications that were more closely related to treatment. 99 patients are today in continuous complete remission. T-cell immunophenotype, white blood count .50x109/L, LDH.1000IU/ L, hepatosplenomegaly, were significantly unfavorable for event-free survival (p log-rank<0.05). White blood count.50x109/L emerged as the strongest adverse prognostic variable. Our results appear to be in concordance with other reports from international study groups.

This can be attributed to the high compliance to standardized protocol-based treatment.

Abstract: 200 Poster: 107

EXPRESSION OF THE ONCOPROTEINS BCL-2,C-MYC,AND P53 IN RELATION TO THE CLINICAL OUTCOME IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA OF T-PHENOTYPE

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Acute lymphoblastic leukemia of T-cell phenotype (T-ALL)accounts for 10-13% of childhood ALL.OBJECTIVES: To study the prognostic significance of the expresion of oncoproteins bcl-2, c-myc,and p53 in childhood T-ALL. STUDY DESIGN:The study included 30 children, 11 females and 19 males,aged 3-16 years (mean 7.6+/- 3.1 years) diagnosed as T-ALL and treated in the Pediatric Hematology/Oncology Unit,Children`s Hospital, Ain Shams University, Cairo, Egypt.Patients were subdivided into Group(ND):18 newly diagnosed,and Group (R):12 T-ALL in bone marrow relapse.Controls (Group C) were 10 healthy age and sex matched children.METHODS: Intracellular detection of bcl-2 and p53 were done by flow cytometry while c-myc detection was done by immunocytochemistry.Samples from peripheral blood and bone marrow were examined on days(0) and (28) of therapy in ALL patients.Patients were followed clinically for one year and oncoproteins expression were correlated to laboratory and clinical response criteria.RESULTS:Compared to controls: bcl-2 and p53 at D0 were significantly higher in ND group(P< 0.001 and P< 0.05;respectively); and in (R) group (P< 0.001 and P< 0.01;respectively).The mean p53 expression at D0 and D28 were significantly higher in R group compared to ND group (P< 0.01 and P< 0.05),with no significant difference in bcl-2 (P>0.05).In both ND and R groups,bcl-2 and p53 were significantly lower in D28 compared to D0(P< 0.0001 and P< 0.0001, for both groups). In both ND and R groups,D28 bcl-2 and p53 were significantly lower in complete remission (CR) versus partial remission patients (P< 0.01).The mean value of bcl-2 at D0 was higher in patients who failed to achieve complete remission compared to patients in CR,when both groups ND and R were collectively examined (P< 0.05).T-ALL patients in ND group with intermediate thymocyte phenotype had higher bcl-2 values at D0 compared to late thymocyte phenotype (P< 0.05).Concerning the c-myc expression in both ND and R groups, 50% had strong positivity and 50% had mild to moderate positivity compared to controls; with no significant difference between the ND and R groups at both D0 and D28.However, there was significant decrease in c-myc positivity in D28 compared to D0 in both ND and R groups(P< 0.05). There was a significant relation between p 53 expression and survival in R group only(P< 0.05).Kaplan-Meier analysis failed to reveal any effect of bcl-2,p53, and c-myc expression in ND and R groups on survival difference. CONCLUSION:Although our preliminary results need to be confirmed in a larger group of patients, analysis of c-myc,bcl-2,p53 proteins, the indicators of cell alterations, may help to identify high risk patients requiring intensive therapy.

Abstract: 201 Poster: 108

NEUTROPHIL ALKALINE PHOSPHATASE IN HEMATOLOGIC DISORDERS IN CHILDREN

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Background:A diagnostic role of neutrophil alkaline phosphatase (NAP) in hematologic disorders in children has not been fully elucidated. Aims:The aim of this study is to define whether the NAP score can be used as a useful marker for the differential diagnosis of pediatric hematologic disorders. Methods: The NAP activity was determined cytochemically according to the method of Tomonaga et al. After the staining, the degree of the enzyme activity in each neutrophil was rated from 0 to V on the basis of the number of precipitated blue granules in the cytoplasm. From April 2000 to March 2004, 58 patients with various hematologic disorders (12 leukemia, 26 ITP, 18 anemia, and 2 neutropenia) were admitted to our department at their initial onset, and all of these cases were enrolled. 53 children admitted for medical examination were served as controls. Results:(1) The mean of the NAP score in controls was 228 (s.d. 44). Calculating the mean plus/minus 2 s.d, I have temporarily set the normal range, 140~316, in the present study. (2) 3

patients with CML and 3 patients with AML having t(8;21) had low NAP scores (<100). Following successful treatment, NAP scores have been normalized in these patients. Among them, 3 patients experienced a relapse, but their NAP scores remained within the normal range. In contrast, 6 ALL patients had normal NAP scores. (3) In 7 out of 12 patients with chronic ITP, NAP scores exhibited below the lower limit of controls. In 4 out of 14 patients with acute ITP, the NAP scores were over the upper limit. (4) Four patients (50%) with iron deficiency anemia showed low NAP scores, whereas 3 out of 4 patients with aplastic anemia and 2 out of 3 patients with hemolytic anemia had high NAP scores. Conclusion: The present study has, for the first time, presented a diagnostic role of the NAP score in pediatric hematologic disorders. Measurement of the NAP score is helpful, at least in part, for the differential diagnosis of hematologic disorders;AML or CML vs ALL, chronic vs acute ITP, iron deficiency anemia vs aplastic or hemolytic anemia.

Abstract: 202 Poster: 109

SUCCESSFUL TREATMENT OF VINCRISTINE INDUCED CRANIAL POLYNEUROPATHY WITH PYRIDOXINE AND PYRIDOSTIGMINE

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Vincristine (VCR) is a commonly used chemotherapy drug for different malignancies, although its toxicity is frequent. It can cause different neuropathic symptoms and signs, in which cranial nerve palsies are infrequent1. We describe a 5-year-old girl showed recovery of VCR induced cranial polyneuropathy with pyridoxine and pyridostigmine treatment. A 5-year-old girl was diagnosed preB cell ALL (FAB L1 subtype). She received chemotherapy according to the previously described modified St. Jude total therapy studies XIII. Five days after the fourth dose of vincristine, she presented with bilateral ptosis. Neurological examination revealed bilateral ptosis, and complete external opthalmoplegia with normal pupillary and corneal reflexes. She received 3.8 mg (6mg/m2) cumulative dose of vincristin before development of ptosis. She did not receive further doses of vincristin. A neuroprotective and neuroregenerative treatment attempt with pyridoxine (150 mg/m2 p.o. BID) and pyridostigmine (3 mg/kg p.o. BID) was initiated. The bilateral ptosis markedly improved after 7 days of pyridoxine and pyridostigmine treatment and completely resolved after two weeks. The both agents were given for 3 weeks and were well tolerated without any side effects. During the follow up of 2 months we did not observe residue or recurrence of the ptosis. In animal studies pyridoxine has been demonstrated to induce some neuroprotection during intrathecal administration of lethal doses of vincristine6. Pyridostigmine or analogues have been used for enhancement of intestinal motility in patients with atonic bowel due to various causes, and reduction of gastrointestinal motility represents one of the major symptoms of VCR-induced neuropathy7. This treatment was very well tolerated by a 5-year old girl with no documented side effects. We suggest that pyridoxine and pyridostigmine are safe and effective treatment options in vincristin-induced cranial neuropathy.

Abstract: 203 Poster: 110

THE mRNA EXPRESSION OF ANTI-APOPTOTIC PROTEIN AVEN IN ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDHOOD

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Apoptosis or programmed cell death distinct from necrosis and it is involved in every homeostatic and pathologic process in the body. Aberrations in the mechanism of apoptosis may lead to tumourogenesis. Cysteine proteases enzymes, called caspases, appear to be involved in both initial signaling events. The caspases inhibitor aven is a recently described member of the family of inhibition of apoptosis protein (IAP). In this study, we investigated the expression of AVEN mRNA in the bone marrow blasts in childhood ALL by using RT-PCR method. Twenty five cases with ALL at the ages of 1-14 years are included this study. The age distribition of the control group varied 116 years consisted of 15 children. The average value of AVEN mRNA expression in the patient group was 458,80 copy/10000 cells. The comparison of the average values of the ALL patients and control group have shown important difference statistically (p<0,01). We found statistical correla-

tions between the expression of AVEN mRNA avarage values during diagnosis and CD20 (r: -0.56; p<0,01), CD41 (r: 0.48; p<0,05) lymphocyte surface markers. The expression of AVEN mRNA value wasn`t find to be important for the expected survival time. The antiapoptotic protein, aven could have not to be found as a prognostic parameter in childhood ALL. However, aven is thought to be an important role in the apoptosis pathway in childhood ALL.

Abstract: 204 Poster: 111

EVALUATION OF CENTRAL NERVOUS SYSTEM RELAPSE FREQUENCY FOR PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA TREATED BY CALG-B TREATMENT PROTOCOL IN ANKARA NUMUNE EDUCATION AND RESEARCH HOSPITAL

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Introduction and Goal: Involvement of central nervous system (CNS) is rarely seen during the diagnosis of acute lymphoblastic leukemia (ALL). In this study, we evaluated relapse frequency for ALL patients treated by CALG-B protocol between March 2002 and April 2005. Materials and Methods: Fifty eight patients was evaluated (16 to 60 years of age, 28 women, 31 men). Initial white blood cell (WBC) counts ranged from 443 to 308,000/ µL. 24 patients had poor prognosis according to their cytogenetic evaluation and initial WBC counts. Cytogenetic evaluation could not applied to 10 patients due to dry tap occurrence on their bone marrow aspiration and peripheral pancytopenia. Patients received cyclophosphamide, daunorubicin, vincristine, prednisone, and L-asparaginase as remission induction treatment and then early intensification, CNS prophylaxis and interim maintenance, late intensification, and prolonged maintenance treatment steps was performed for 24 months. Results: Two patients died while undergoing remission induction treatment, and one patient in remission died due to infection. Forty eight (82,75%) of the patients achieved a remission on 28th day. Of these patients, 12 patients (25%) had bone marrow relapse, 11 patients (22,91%) had CNS relapse, 4 patients (8,33%) had CNS and bone marrow relapse, 1 patient (2,08%) had CNS, bone marrow and extramedullary relapse, and 1 patient (2,08%) had only extramedullary relapse. Relapse rates ranged from 1 to 17 months (mean: 5,2 months). 16 patients(33,33%) had CNS relapse with the rate ranged from 1 to 12 months (mean: 4.8 months). As another treatment protocol Hyper-CVAD performed on the patients who had relapse. Discussion: Since CNS relapse occurs frequently on early period, high dose treatments (such as high dose ARA-C, methotrexate) is convenient for the patients in poor prognostic group without allogeneic transplant donors and with high initial WBC.

Abstract: 205 Poster: 112

THE RETROSPECTIVE ANALYSIS OF PEDIATRIC HEMATOLOGIC MALIGNANCY CASES BETWEEN 19802003: HACETTEPE EXPERIENCE

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Background Leukemia constitutes 25-30% of all pediatric malignancy cases. The epidemiologic and demographic characteristics of this group of patients are important not only for determination of the prognostic factors, but also the risk factors. Aims In this study, the patients who were diagnosed with hematologic malignancies in Hacettepe University, Pediatric Hematolgy Division are analyzed retrospectively. Besides the epidemiologic characteristics including age, sex, geographic distiribution; the type of disease, clinical presentation, physical examination and laboratory findings on admission and the survival and prognosis relationships are aso evaluated in order to determine the disease properties of our country. Methods Between January 1980-July 2003, 739 hematologic malignancy cases are analyzed retrospectively using the hospital records. Results The study group includes 548(74.1%) ALL, 135(18.2%) AML, 13(1.8%) chronic myeloid leukemia(CML) ve 43(5.8%) myelodysplastic syndrome(MDS) cases. Of the 739 patients 444 (60.1%) are male and this male predominance is obvious among each four groups. The median age at diagnosis is 62 months for ALL and 108 months for AML. ALL is more common among 1-5 year old group; AML is

more common in adolescence. The incidence of hematologic malignancies shows an abrupt increase in 1997 and then decline later. The cases who have been admitted to our clinic is most commonly living in the northern and southeastern parts of Turkey. Approximately 50% of ALL and AML presents with the complaints of fever and pallor. Bone pain is significantly more common in ALL, rash is more common in MDS cases. Lymphadenopathy is present in almost half of ALL and AML patients at diagnosis. Hepatomegaly(72.4% vs 50.4%) and splenomegaly(53.8% vs 36.3%) are more commonly observed in ALL then AML patients($p<0.001$). Hepatomegaly is present in 76.9% and splenomegaly in 92.3% of CML patients.The central nervous system(CNS) involvement is present in 5.8% of ALL and 5.9% of AML patients. The most common cytogenetical abnormality in ALL is hypodiploidy, t(9;22) in CML and monosomy 7 in MDS. 25.4% of ALL and 43.7% of AML patients have relapsed subsequently. Four MDS patients have evolved to ALL or AML. CNS relapse is more common in ALL then AML cases(31% vs 4%). Fatality rates of ALL, AML, CML and MDS are 20.1%, 56.3%, 46.2% and 30.2%, respectively. The fatality rates of AML and CML are significantly higher then ALL and MDS. The CNS involvement and sex have no influnce on the fatality rates; however the presence of relapse for ALL and AML and L3 subtype, being less then 1 year old for ALL have a negative effect. Summary/Conclusions The collection of the cancer data throughout the country is crucial for the determination of the distribution and risk factors. The best way of cancer data collection is development of cancer recording systems and analyzing these data for the determination of distribution and risk factors of patients.

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HEPATOTOXICITY IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background and Aims: Hepatotoxicity with elevation of aminotransferase levels is common during the treatment of acute lymphoblastic leukemia (ALL). Hepatotoxicity is resulted from the chemoterapeutic agents especially methotrexate (Mtx), 6-Merkaptopurin (6-MP), and Vepeside. In addition to the chemotherapy, hepatitis B and C infections and antibiotics which were used during febrile neutropenia periods were also responsible for hepatotoxicity. In our country the prevalance of hepatitis B infection is 6.6-8%. Hepatitis B infection is more common in children with ALL because of the transfusions during the treatment. The aim of the study was to evaluate the prevalance of the toxic hepatitis, hepatitis B and C infections in children treated for ALL. Material-Methods and Results: Between March 1991 and January 2001, 196 children were treated for ALL in Hacettepe University Faculty of Medicine Pediatric Hematology Unit. Of these 196 children with ALL, 85 (43.4%) were girls and 111 (56.6%) were boys. The mean age of the children was 6.45±3.87 years (range: 0.5-15 years). One hundred and four patients (53.0 %) were received St Jude Total XI protocole, whereas 92 patients (46.9 %) were received St Jude Total XIII protocole and the follow-up period was 51.22±23.5 months for all of the patients. Of these 196 children with ALL, 82 children (41.8%) were vaccinated for hepatitis B infection during maintanence chemotherapy, 27 children (13.7%) were vaccinated before the diagnosis of ALL. Among 196 patients treated for ALL, 29 patients (14.7%) had hepatitis B infection, 3 patients (1.5%) had hepatitis C infection, and 36 patients (18.3%) had toxic hepatitis. In patients with toxic hepatitis, the mean aspartate aminotransferase (AST) level was 388.15±284.46 U/L and alanine aminotransferase (ALT) level was 424.0±316.56 U/L. The high AST and ALT levels were decreased during follow-up period. Conclusion: Methotrexate and its poly-glutamates, 6-thioguanine nucleotides, the major cytotoxic metabolites of 6-mercaptopurine, and methylated metabolites of 6-mercaptopurine generated by thiopurine methyltransferase in competition with the formation of 6thioguanine nucleotides were suggested to be responsible for hepatotoxicity in children with ALL. The hepatotoxicity resulted from chemoterapeutic agents were reversible and does not result in chronic liver disease. However hepatotoxicity resulted from hepatitis B and C infections can cause chronic liver disease and they should be followed up for this respect. The prevalance of hepatitis B infection in our series (14.7%) is higher than those in our country. The prevalance of hepatitis B infection may be decreased with strict vaccination programme in children with ALL.

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CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA: A SINGLE CENTER EXPERIENCE

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Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy, where the survival rates increased up to 80-90% over the last decade. Aim: The aim of this study is to analyze the pediatric ALL data of a single pediatric university center between 1987-2005 retrospectively. Method & Results: A total of 372 cases [220 male (60%) and 151 female (41%)] were diagnosed and treated in our center between 1987-2005. The age distribution was as follows: 7% patients under 2 years, 68% between 2-10 years, 25% above 10 years of age. At diagnosis, 76% patients had a hemoglobin level <10gr/dl, 56% WBC >10.000/mm3, 74% platelet <100.000/mm3. Primary CNS involvement was positive in 2.5%, mediastinal mass in 8% of all patients. The morphological subtypes were as follows: L1 64%, L2 32%, L3 4%. Immunophenotypic results revealed T ALL in 22%, mature B ALL in 8% and B ALL (common, pre B, proB) in 70%. All patients received CCG modified BFM protocol (80% of all patients) until 1999. Since then high-risk patients were treated with the augmented BFM protocol and L3 patients BFM NHL 95 Protocol, while standard risk patients continued to get the CCG modified BFM protocol. The remission rate at day 33 was 97.8%. Eighty-one patients relapsed (68% patients isolated bone marrow, 16% bone marrow + extramedullary, 9% CNS, 7% testes). Relapse time was 66±57 months (1-216 months) after the initiation of the therapy. After a follow up of 72±59 months (1-300 months) 58% of patients are alive & well, 28% were lost to follow up and 14% patients succumbed to death. Overall survival (OS) for 60&120 months follow up was 83%, 83% and event free survival (EFS) 72%, 70% respectively. Our remission rates and OS, EFS are similar to the literature. Conclusion: Eighty to ninety percent of all childhood ALL patients can be cured, but there are still a group of patients with unfavorable outcome, therefore treatment of all ALL patients still remains a challenge.

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COGNITIVE PERFORMANCE AND MAGNETIC RESONANCE IMAGING FINDINGS AFTER THE CESSATION OF THERAPY IN PEDIATRIC ALL PATIENTS

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Background: Despite improved outcome in patients with ALL, long term neurotoxicity is a frequent complication of combined chemotherapy and radiotheapy. Aim: Neuropsychological and neurological data were documented to asses the long term effect of treatment modalities in children with ALL who were in remission, a prospective study was undertaken. Results: Between September 2003, and May 2005 thirty concecutive ALL patients who came to Hacettepe University Pediatric Hematology Department for routine control were eligible for this study. Median age of patients was 48 (18-168) months. There were 23 male and 7 female. Ninetheen patients were classified as high risk ALL, 3 of them were having CNS leukemia at the time of diagnosis.. Immunophenotype examination revealed 14 CALLA positive B cell ALL, 5 mixed phenotype ALL, 2 B cell and 2 T cell ALL. On admission median WBC was 7800 /mm3 (1400-100000). Treatment protocols were St Jude Total XI in 13 (43.3%) and Total XIII in 17 (56.7%) patients. Eleven patients with low risk had received chemotherapy alone and 19 (63.3%) had received chemotherapy plus radiotherapy. Standardized neuropsychologic testing and magnetic resonance imaging (MRI) investigations were performed to determine whether chemotherapy protocols based on high-dose methotrexate and radiotherapy results in cognitive and/or magnetic resonance changes during the follow up period.. Neurocognitive evaluation using WISC-R were performed in all patients median 67 (6.9-175.8) months after cessation of treatment. Verbal IQ of the study group was mean 93.78 (SD±18.80), performance IQ was mean 103.74 (SD±20.14) and overall IQ was 98.91 (SD±18.53). There is no effect of the radiotherapy in patients on overall IQ (p=0.268). MRI and MR spectroscopy applied to all patients. Gliosis in 11, caverneus formation in 8 patients and increase in white matter intensity in 8 patients were most frequent MRI abnormalities whereas eight (26.7%) of them were normal. When the patients with

abnormal MRI findings compare with the patients with normal MRI there was not significant difference for their neuropsycologic tests. Conclusion: This study suggest that patients with ALL have MRI abnormalities and that patient group should be evaluated in long term period.

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NITRIC OXIDE AND FAS/FASL SYSTEM AS PREDICTORS OF APOPTOSIS IN LEUKEMIC CELLS

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The role of nitric oxide (NO) and Fas/Fas Ligand (Fas/ FasL) system in various disease states has become apparent. Apoptosis is an important mechanism by which NO and Fas/FasL system may contribute to the pathogenesis of many diseases; tumors and leukemia. Objective: Was to evaluate the role of nitric oxide and Fas/FasL in children suffering from acute lymphoblastic leukemia (ALL). Patients and Methods The present study was conducted on 36 children diagnosed as ALL who were divided into: group I; 24 children in remission and group II; 12 children who were either de novo or in relapse. In addition to 30 healthy children served as control group. Serum NO, sFas and sFasL were assayed in all ALL cases and controls using Enzyme Linked Immunosorbant Assay (ELISA) technique. Results A significantly high levels of (NO), sFas and sFasL were detected in ALL cases (group I and group II) when compared to normal controls ($p < 0.001$). Interestingly, this increase was more pronounced in group II when compared to group I ($p < 0.001$). Acute lymphoblastic leukemia cases who suffered from lymphadenopathy or hepatosptenomegaly showed a significant increase in serum levels of NO, sFas and sFasL when compared to non-suffered ALL cases ($P < 0.01$). A positive correlation was established between serum levels of sFas and sFasL in group I and II ($P < 0.01$). Conclusion: It is concluded that, the role of NO and Fas/FasL system in ALL is obvious. Further assessment and better knowledge of the mechanisms governing anti-versus pro apoptotic processes provide insight into creating a new therapeutic modality using nitric oxide synthase (NOS) inhibitors, cytotoxic drugs and cytokines together with modulation of Fas-system.

Abstract: 210 Poster: 117

SKIN LESIONS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Background: Skin lesions in patients with hematological malignancies are divided into two: skin lesions due to infiltration of skin by leukemia cells or by the cells of any hematological malignancy (leukemia cutis, LC), and nonspecific benign skin lesions (NBSL). It is reported that LC is associated with poor prognosis and the presence of extramedullary disease. Aims: In our study, we determined the features of our patients with LC. In addition, we compared the clinical features of patients with LC with those of patients with NBSL. Methods: We evaluated 53 skin lesions that developed in 48 patients (33 males, 15 females, mean age:58.8±16.1) between January 2002 to May 2005. The diagnoses of the patients were: AML (13 patients), ALL (5 patients), CLL (4 patients), CML (3 patients), lymphoma (12 patients), prolymphocytic leukemia (PLL) (3 patients), rest of the patients had either myelofibrosis or MDS (Table 1). Forty-three lesions were diagnosed by histopathological examination of a skin biopsy specimen. The diagnosis was reached clinically in 10 skin lesions. The patients` clinical features, laboratory data, medical therapies were recorded down from hospital files. Skin lesions were divided into three as NBSL, LC, and secondary skin malignancy (SSM). Results: There were 8 patients with LC: 6 had AML, one had ALL and one had PLL. Of 10 cases with SSM, 4 had Kaposi sarcoma and 3 had basal cell carcinoma. There were 35 patients with NBSL: 5 had leucocytoclastic vasculitis. The median time for the appearance of LC lesions in LC patients was shorter than the time for the appearane of NBSL ($p=0.015$). Hemoglobin, leucocyte and neutrophil values in the LC group were lower ($p=0.02$, 0.016 and 0.002, respectively) than in the SSM group. The median age ($p=0.027$) and leucocyte count ($p=0.011$) in patients with NBSL were lower than those in patients with SSM, and the lesions were associated with chemotherapy more frequently ($p=0.026$). Although not significant, mortality rate and median survival in the LC group were different from the other groups ($p>0.05$) (Table). Conclusions: We must consider

NBSL in the period after chemotherapy, LC at the initial presentation of the patient or during early disease course, and SSM when the patient is old. Although it is not significant, LC seems to be associated with an unfavourable survival.

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INVESTIGATION OF TEL/AML1 AND BCR/ABL FUSION GENES IN PATIENTS AFFECTED BY ACUTE LYMPHOBLASTIC LEUKAEMIA PATIENTS USING INTERPHASE FLUORESCENCE IN SITU HYBRIDIZATION

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A total of sixty patients affected with acute lymphoblastic leukaemia (ALL), including thirty children and thirty adults were studied by the conventional cytogenetics and fluorescence in situ hybridization (FISH) techniques. The TEL, AML1, ABL, and BCR probes were applied on interphase cells prepared from patients` bone marrow samples. The signal distribution and the presence of the fused genes, together with the clinical features were statistically analysed. The age ranged from 16 to 42 years (mean 23±7.3) in adults and 2 to 15 years (mean 6.9±3.9) in children. The most common chromosome aberration was found to be hyperdiploidy in both child (60%) and adult (47%) patients. The most common chromosomal aberrations included gain of chromosomal material (70%) and structural alterations of chromosomes (in 77% and 79% of patients). In children 46% had an abnormal FISH pattern, including 23% having fused ABL/AML1, 3% and 7% with deletion and gain in TEL gene respectively; 3% and 10% having deletion and gain in AML1 gene respectively. In adults, out of eight (27%) patients had abnormal FISH pattern, of those only 3% presented the fused TEL/AML1 gene, and the distribution of signal patterns was the as same as found in children. There was a significant inverse correlation between the increased age of ALL children and a higher survival time ($P<0.05$). A direct correlation were also found between the presence of fused TEL/AML1 genes and decreased WBC ($P<0.05$), however this was not significant in adults. The adults with more than 50000 WBC had a significantly lower survival period ($P<0.05$), of whom, the patient 9 had the fused TEL/ AML1, accompanied by additional materials, including TEL, ABL, and BCR genes with 43000 WBC and survival time of 55 months. The combination of conventional karyotyping and molecular cytogenetics (FISH), and a long time follow up study could provide the clinicians the useful information leading to plan the more affective therapeutic management for the ALL patients.

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DETECTION OF MINIMAL RESIDUAL DISEASE IN ACUTE LYMPHOBLASTIC LEUKEMIA USING AN ALLELE SPECIFIC OLIGONUCLEOTIDE PCR ASSAY

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Background: Immunoglobulin chains (Ig) and T-cell receptor (TCR) gene rearrangements are excellent patient specific PCR targets for MRD detection in complete remission (CR) after about 4 weeks of chemotherapy with ASO-PCR and Clonotypic techniques. MRD monitoring in acute lymphoblastic leukemia patients has demonstrated high prognostic value for predicting subsequent relapse. Aims: To evaluate the kinetics of molecular response achievement with use ASO-PCR and Clonotypic techniques in ALL patients after therapy or BMT. Materials and Methods: PB samples were obtained at the time of diagnosis, after treatments, and in CR every month for up to 18 months. The presence of clonality in leukemia blasts is searched in PB sample of 30 patients obtained at diagnosis, using different combinations of FR1 familyspecific primers and J primer flanking the unique CDR3 region. When clonal IgH or TCR-δ gene rearrangements were present in leukemia cells, one-sized PCR signal was obtained which was used to monitor MRD with these primers (Clonotypic method). For ASO-PCR method, PCR products from 10 patients were sequenced and ASO primer for the CDR3 region of each specific clone was designed. The ASO primer was then used in conjunction with FR1 primer in a PCR amplification to detect leukemia-specific rearrangements in subsequent samples

obtained from the same patient and then compares residual disease levels estimated by ASO-PCR and Clonotypic. Results: Monitoring the kinetics of MRD during the first year of therapy permitted identification of two MRD-based risk groups of patients with low (stable or falling MRD) and high risk (rising or persistently high level of MRD) of relapse. We compared two methods with periodic and frequent monitoring of MRD level and we could anticipate the patient`s clinical outcome. The ASOPCR (with sensitivity of 10-4 to 10-6) and Clonotypic (with sensitivity of 10-2 to 10-3) methods were able to detect residual malignant cells with the same pattern in PB samples from patients who were considered to be in CR. Conclusion: In Comparison of residual disease levels estimated by ASO-PCR and Clonotypic, the application of the ASO-PCR method of immunoglobulin heavy chain (IgH) and T-cell receptor (TCR) gene rearrangement regions provides a sensitive and highly specific detection with potential clinical relevance of MRD in ALL patients. Monitoring the kinetics of MRD with these techniques provide important prognostic information on the in vivo effectiveness of treatment in ALL, and with the increase of the MRD level enable to anticipate impending relapse.

Abstract: 213 Poster: 120

THE EXPRESSION OF mRNA OF SURVIVIN IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Apoptosis, an active suicidal response to different physiological or pathological stimuli, plays an important role in a variety of biological events, including morphogenesis, homeostatic maintenance of diffferent tissues and removal of harmful cells. Aberrations in the mechanism of apoptosis lead to autoimmune diseases, neurodegenerative disorders and carcinogenesis. Survivin is a recently described member of the family of inhibition of apoptosis proteins. It inhibits apoptosis induced by chemotherapeutic agents, Fas, and caspases. It is shown that it is overexpressed in the most common human cancers. In this study, 25 cases with ALL at the ages of 2-14 years are included, the average age was 7,7 years in the patients. The age distribition of the control group varied 1-19 years, the average age was 8,4 years consisted of 13 children. The average of survivin mRNA expression was 652,2 copy/10000cells at the diagnostic aspiration material from bone marrow samples of the patients. The statistical comparison of the average values of the ALL patients and control group have shown important difference statistically (p=0,013). Survivin mRNA expression value was found to be important for the expected survival time. As a result, survivin is thought to be an important prognostic parameter in hildren with ALL.

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CARDIAC FUNCTION WITH MYOCARDIAL PERFORMANCE INDEX AND QT DISPERSION IN CHILDHOOD LEUKAEMIA

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Background: Childhood leukaemia treatment contains multiple chemotherapeutic agents with high doses and it can cause severe toxic effects on heart and other vital organs. In this respect patients under cancer chemotherapy are followed for these adverse effects. Myocardial performance index (MPI) was reported as a new method of combined systolic and diastolic function for both adults and children, calculated as isovolumic relaxation time plus isovolumic contraction time divided by ejection time. In addition, it has been postulated that increased interlead differences in QT interval (QT dispersion) may be associated with an increased risk of cardiac death. Aims: The aim of this study was to determine the probable immediate and late adverse effects of childhood leukaemia treatment on heart by using MPI and QTc (corrected QT) dispersion. Methods: MPI and QTc dispersion in 55 children with leukaemia and MPI in 38 healthy controls with age and sex matched were evaluated. Results: There was no statistically significant difference between MPI values of patients and controls (20.7 ±13.1 (1-59.4) and 16.1 ± 13.5 (0.3-77.5), p: 0.1, respectively). Also, there was no significant difference in MPI and QTc values between patients under active treatment and completed the therapy and between the patients given a cumulative dose of anthracycline of lower and upper than 250

mg/m2. Summary/Conclusions: There were no observed clinical and subclinical cardiotoxicity in our children with leukaemia treated with protocols of ALL BFM 95 and TRALL 2000 (Modified BFM in Turkey), but further monitoring and evaluation over a longer period of time are needed.

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CEREBROSPINAL FLUID LEVELS OF METHOTREXATE IN PATIENT TAKING HIGH DOSE METHOTREXATE

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Methotrexate (Mtx) is widely used in malign childhood tumors. Its structuraly analogus to folinic acid, its antimetabolic chemotheropeutic effects are by binding to dihydrofolate reductase enzyme. In this study, we planned to design a handbook for those patients with central nervous system relaps and also those who are taking high dose Mtx treatment and its proportion of penetration into the Cerebrospinal Fluid (CSF). In this study we included 4 patients with osteosarcoma and 6 leukemic patient with T-marker positivity. Patients with osteosarcoma were given 12 gr/m2 high dose Mtx (iv) for 10 hours on 13 occassions, leukemic patients were given 5 gr/m2 high dose Mtx (iv) for 12 hours on 15 occassions. After Mtx infusions, folinic acid was given iv for 4 hours. From the start of high dose Mtx infusion to the end of 12 th hour, lumbar puncture was performed and CSF (Cerebrospinal Fluid) sample was obtained. Simultaneously serum levels and CSF levels of Mtx were measured. During this treatment protocol, non of our patients died. Three of our patients (%10.7) CSF levels of Mtx were below 1.0 umol/L while 24 patients (%89.3) had levels above 1.0 umol/L and higher. Lastly, we conclude that in patients with CNS and bone marrow infiltration, high dose Mtx treatment protocol is enevitable and should be considered for relaps therapy.

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OUTCOME OF CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA ON THE BASIS OF CYTOGENETIC STUDY

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Acute lymphoblastic leukemia (ALL) is the most common type of leukemia in children. In the present study, 30 children with ALL attending Hematology and Oncology unit, Pediatric Department, Tanta University Hospital were studied. Flow cytometric, immunophenotypic and cytogenetic analysis by Fluorescence in Situ Hybridization (FISH) were done for each patient. Flow cytometric analysis revealed that 22/30 patients (73.33%) were assigned to B-lineage, which included early pre B (14 cases), pre B (4 cases) and mature B (one case) while 8/30 patients (26.66%) expressed T cell lineage markers. Structural chromosomal aberration was detected in 12/30 patients (40%) of them the commonest was t(12,21) found in 5 cases (16.66%), t(l,19) in 3 cases (10%), t(9,22) in 2 cases (6.66%) and t(4,ll) was detected in 2 cases (6.66%). Some chromosomal abnormalities in. ALL have prognostic significance suggesting that these factors should be added to ALL risk classification schemes e.g. t(12,21) was found to be associated with good prognosis while t(9,22), t(l,J9) and t(4,ll) were associated with bad prognosis. There were significant differences regarding disease free survival and the overall survival between patients with no chromosomal aberrations (group I) together with patients with t(9,22), t(4,ll), and t(l,19) and patients with t(12,21). There was no significant difference regarding the overall survival between 1(12,2]) and children with no chromosomal aberrations (group I). We concluded that cytogenetic study of leukemic blast cells at initial diagnosis should be an integral part of diagnosis, treatment and prognosis of ALL.

Abstract: 217 Poster: 124

BONE MINERAL DENSITIES AND BONE RESORPTION IN ACUTE LEUKEMIAS IN CHILDHOOD

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Background: The effects of treatment on bone resorption in children with established diagnosis

of acute leukemia whose treatment were completed were investigated by measurement of bone mineral density and levels of dyprydinoline (Dpd) The effects of treatment on risk of development of osteopenia/osteoporosis, and impact of chemotherapy and radiotherapy on excretion of Dpd, and persistence of therapeutic effects on BMD and Dpd with passing of time and increasing age were investigated. Material and Method. Our study group consisted of 29 cases of acute leukemia who completed their course of therapy. The patients were divided into 2 groups Group I (n= 19) and Group II (n=10) were investigated 1.00 and 43.36 months after cessation of treatment. BMD control group consisted of 52 healthy children, while urinary Dpd control group had 20 pediatric subjects. BMDs and urinary Dpd values were measured in cases who completed their treatment schedules and healthy controls. Results. Mean BMD values for two groups combined (Groups I and II) and those of 0.17 g/cm2, 0.58±0.09 g/cm2, Groups I, II and controls were not different (0.66 0.73±0.25 g/cm2 and 0.65±0.16 g/cm2 respectively) (p>0.05). Osteopenia/ osteoporosis was detected in 10 (34.48 %) of 29 cases. Dpd values for all groups combined and for Groups I, II and controls were not different 13.74 nmol/mmol Cr ve 13.74 nmol/mmol Cr, 39.02±15.37 nmol/mmol Cr, 32.92 (32.92 13.48 nmol/mmol Cr respectively) (p 30.15>0.05). In two groups combined (Group I-II) and in Group II a significant negative correlation was revealed between BMD and Dpd values. (r: -0.488, p≤0.01; r: -0.712, p≤0.05 respectively). A positive correlation was detected between Groups I and II with respect to age at the time of diagnosis and BMD (r: 0.700, p≤0.01; r: 0.736, p≤0.001; r: 0.822, p≤0.01). Only in Group II, a negative significant correlation (r: -0.822, p≤0.01) was detected in terms of age at the diagnosis and Dpd. In Group I age influenced BMD significantly (p≤0.05). In Group II age and time passed since termination of treatment effected BMD significantly (p≤0.00001, p≤0.05 respectively). BMD and Dpd were not 18.09 0.15 g/cm2, 31.29 different between those receiving (Group I-II (0.60 0.20 nmol/mmol Cr) or not receiving radiotherapy and Dpd (Group I-II (0.67 0.05). In Group I a significant 11.91 nmol/mmol Cr) (p g/cm2, 37.29 (p&0.74) ≤;0.05). difference between BSDS of patients receiving (1.04 0.80) cranial radiotherapy and those treated with or not receiving (-0.19 0.93) was revealed.. In Group I cranial extracranial radiotherapy (-1.36 radiotherapy effected BSDS negatively, while in Group II this effect was not detected. Conclusion. If evaluated together with bone formation rate, measurement of urinary Dpd will yield better results. It was observed that in patients who completed their treatment of leukemia BMDs normalized and bone resorption didn`t progress further.

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AVASCULAR NECROSIS OF THE FEMORAL HEAD AS THE FIRST MANIFESTATION OF ACUTE LYMPHOBLASTIC LEUKEMIA

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Avascular necrosis of bone is a well-described complication of cancer chemotherapy containing corticosteroids, and has been observed in lymphomas and acute lymphoblastic leukemia (ALL). We report the case of a young male patient in whom avascular necrosis of right femur head was the presenting feature of acute lymphoblastic leukemia. The temporal association in this instance implicates a pathophysiologic relationship between the development of ALL and necrosis.

Abstract: 219 Poster: 126

ORGANIC BRAIN SYNDROME DURING CHEMOTHERAPY IN A CHILD WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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An 11-years-old boy who had complaints of appetite loss and skin bruising was diagnosed as T cell acute lymphoblastic leukemia. On admission, his neurologic examination was normal and he seemed to be more intellegent than other children in his same age group. He had no central nervous system involvement. ALL BFM 95 high risk group treatment was given beacause of hyperleukocytosis. During the induction therapy, he asked many questions about his disease and he wanted to know his diagnosis. The patient was informed about his disease and treatment and was told that he will be healthy again without declaring the name of the disease clearly. He showed depressive symptoms during his hospitalization and a

pediatric psychiatry consultation was needed. Pediatric psychiatrists explained his findings as depressive type adaptation disorder secondary to his organic disease and started escitalopram treatment. On the last day of the first high risk group block therapy, suddenly he complaint from blindness. His neurologic examinations were normal and there were no pathologic findings on ophtalmology consultation. Cranial MRI, cranial diffusion MRI and cerebrospinal fluid findings were normal. Few hours later, the patient complained about deafness and told that his vision became normal time to time. He saw his mother and nurses as dismorphic, horrible figures and developed further hallucinations, fear, and agitation. On the following days, he told that his brain received orders. He made plans to follow this orders and he draw figures that were describing those plans in detail. During that time, his person, time, and place orientations were defective. He also developed tonic-clonic seizure-like contractions with urinary incontinance but his video electroencephalogram was normal during those contractions. The diagnosis of acute organic brain syndrome (delirium) due to chemotherapeutic agents was thought by the pediatric psychiatrists. Risperidon 2 mg/day was added to his therapy and his symptoms resolved. On follow-up, delirium symptoms started again with intrathecal chemotherapy administration and they were taken under control easily. Recently, he is recieiving maintenance therapy and has no complaints. The case is presented since organic brain syndrome during chemotherapy is not usual and paintings he made during his severe psychiatric symptoms are very interesting.

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THE SYNDROME OF INAPPROPRIATE ANTIDIURETIC HORMONE SECRETION IN THE CHILDREN TREATED WITH VINCRISTINE FOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The syndrome of inappropriate antidiuretic hormone secretion (SIADH) is a rare neurotoxic side effect of vincristine (VCR) therapy. It`s a serious but reversible adverse event. Aims: To describe tree pediatric cases developed SIADH during chemotherapy for acute lymphoblastic leukemia (ALL), to evaluate the value of various clinic symptoms alert the physicians for SIADH in pediatric patients treated with vincristine. Methods: SIADH was documented in three children (ages 6, 7, and 12 years) who received induction chemotherapy for ALL, which included prednisolone, vincristine (VCR), daunorubicin, l-asparaginase (BFM protocol). We evaluated retrospective review of clinical and laboratory data of our patients with SIADH presenting within a 9 week interval. Results: After the repeated weekly doses of VCR (at least third dose), SIADH was manifested as frequent vomiting, abdominal pain, drowsiness, headache, and fatigue in all tree patients. They had normal serum sodium levels at the onset of the symptoms. Within 7-10 days after the presentation of clinic symptoms, examinations revealed decreased serum sodium concentrations (117 mEq/l in two and 119 mEq/l in one), continued urinary excretions of sodium (> 30 mEq/L) and elevated antidiuretic hormone levels. These findings yielded a diagnosis of the SIADH. One patient developed seizures due to hyponatremia. In that patient, brain computed tomography found no abnormality. All symptoms subsided within 48 hours either by fluid restriction alone in two patients or by additional anticonvulsant and infusion with saline plus 5% glucose in one patient. Their serum sodium levels became normal within 1-2 weeks. Recovery was complete in all patients and there was no recurrence on repeated use of vincristine in any of our case. Summary/Conclusions: Although the overall reported rate of SIADH associated with vincristine is very low, some data suggest that Asian patients may be at increased risk of hyponatremia and/or SIADH associated with vincristine use. It may be also more common in Turkish children than others. The physicians who treated pediatric leukemiac patients should be aware of this potential adverse effect of VCR and alert with symptoms such as vomiting, abdominal pain, drowsiness, headache, and fatigue unexplained with any other reason. SIADH was prevented easily by careful monitoring of the serum sodium level and prophylactic fluid restriction.

Abstract: 221 Poster: 128

ANEMIA IN CHILDREN WITH ALL TREATED BY BFM90 PROTOCOL

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Anemia, defined as the presence of blood hemoglobin (Hb) below the normal range for the patient`s age, is very common in children with Acute lymphocytic leukemia (ALL). Bone marrow replacement by malignant cells determines the reduction of hematopoietic cells, resulting in a lower Hb level and clinical signs of anemia. Anemia related to bone marrow infiltration is primarily more frequent at diagnosis or when relapse occurs, while decreased erythropoietic activity caused by chemotherapy occurs throughout treatment. The severity of anemia in children is influenced by several factors, including the extent of malignanat disease, and the chemotherapy regimen and intensity. Aims. To analyze the pathogenesis of anemia as a complication in children with LAL, the clinical impact, the influence of protocol BFM-90 over the anemia, and possible options for the treatment of cancer-related anemia. Methods and results: We retrospectively evaluated data of 64 (34 male and 30 female) newly diagnosed patients with ALL treated by ALL-BFM-90 protocol between January 1977 and January 2003.The percentage of patients treated by age were <1 year: 1,5%; 1-10 years: 74,5%; 11-16 years: 24%. Anemia at the diagnosis, before starting cytotoxic treatment, was observed as mild in 14%; moderate in 19%, severe in 22%; and life threatening in 31% of patients. At least one transfusion of red blood cells (RBC) was administered to 97% of children during Protocol I. This high frequency of transfusions has been related to intensity of treatment. Initial hemoglobin level wasn`t significantly associated with transfusions units. Almost 94% of patients treated by Protocol II, 32% treated by Protocol M and 6, 6% of patients undergoing maintenance treatment received RBC transfusions. Transfusion of packed red cells is generally prescribed when the Hb level was <= 8, 5 g/dl. There were other clinical conditions for witch keeping the Hb level at more normal levels was desirable. Transfusion of RBC was prescribed at higher Hb levels in the presence of severe thrombocytopenia and leucopenia with potentially life-threatening consequences. Patients with platelet count less than 50•109 and high risk of sudden massive bleed-ing received platelet transfusion. Febrile neutropenia was treated by antibiotics and G-CSF. Conclusion. Our study confirms that children with ALL frequently develop anemia both from the disease and from chemotherapy. The most frequent treatment employed for anemia in children with ALL treated by BFM-90 protocol was RBC transfusion. The use of RBC transfusions increased as the intensity of therapy increased (Protocol I and Protocol II). RBC transfusions increased Hb values and improved outcomes in children with LAL treated by BFM-90 protocol. Because of the risks associated with transfusions a new safe and reasonable therapeutic options are needed in the future.

Abstract: 222 Poster: 129

ACUTE LYMPHOBLASTIC LEUKEMIA IN A PATIENT WITH WILSON DISEASE TREATED WITH D-PENICILL-AMINE

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Wilson disease (WD) is a rare inherited disease of copper metabolism, characterized by liver and central nervous system dysfunction. It is transmitted through a recessive gene located on chromosome 13. It has been coexisted with some other disease such as autoimmune disorders and malignancies. We hereby report a case with diagnosis of acute lymphoblastic leukemia which was observed in the course of WD under the treatment of D-penicillamine. Twenty five years old female was diagnosed WD ten years ago with hepatic presentation with the laboratory findings of abnormal liver function tests and Kayser-Fleischer rings detected in eye examination. Liver biopsy revealed the diagnosis of WD. Therapy was started with D-penicillamine, zinc sulfate and a low copper diet was given. She has been in remission for the last ten years. She was admitted to the hospital with complaints of pallor, fatigue, fever and ecchymoses in May 2005. She was diagnosed as acute lymphoblastic leukemia (ALL) with splenomegaly and peripheral blood and bone marrow findings. Flow cytometric analysis and bone marrow biopsy have confirmed the diagnosis of ALL. Chemotherapy regimen including L-asparaginase was immediately started and liver function tests have been in normal range. There is no data about the relationship between WD and ALL. And also, whether D-penicillamine treatment has any inducing effect on ALL has not been elucidated yet. Further studies are needed to clarify the genetical and clinical bases of coexistence of these disorders.

Abstract: 223 Poster: 130

ACUTE LYMPHOBLASTIC LEUKEMIA PRESENTED WITH PRIAPISM

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Priapism is defined as prolonged and persistent erection of the penis without sexual stimulation. It is associated with excessive hyperleukocytosis (e.g. in acute or chronic leukaemia); however, this complication is rarely seen in young population. We report a 17-year-old boy suffering from acute leukemia presenting with persistent erection. During the evaluation of priapism his first complete blood count revealed WBC 990.000/mm3, hemoglobin 8.6gr/dl and 62.000 platelets. We performed leukopheresis for 5 days and his leukocyte count dropped to 76.000/mm3. On the third day of leukopheresis priapism diminished. This case demonstrates the importance of identifying the underlying cause of priapism, as it directly impacts on both initial and ongoing management.

Abstract: 224 Poster: 131

EFFECT OF INDUCTION CHEMO AND RADIOTHERAPY ON CERTAIN CEREBROSPINAL FLUID ENZYMATIC ACTIVITIES IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Lactate dehydrogenase (LDH) and aspartate transamlnase (AST) enzyme activities in cerebrospinal fluid (CSF) as well as in sera were estimated in 15 children with Acute Lymphoblastic Leukaemia (ALL) categorized into 3 stages, when newly diagnosed, after induction of remission and after CNS prophylactic therapy. The CSF enzymes showed insignificant changes in ALL patients in the first and second stages in comparison with the reference group, but their levels s flowed significant increase in third stage. Such changes in CSF enzymes might be due to the biochemical and functional changes in blood brain barrier as a result of the effect of"mtrathecal chemotherapy in addition to cranial and spinal irradiation. Serum enzymes LDH and AST of ALL patients showed significant ele\`atlon at diagnosis in comparison with the reference group. After induction of remission and CNS prophylaxis, serum LDH showed insignificant changes. After CNS prophylaxis therapy there was significant decrease in serum AST than before therapy, but still higher than the reference group. Ther was a positive correlation between CSF enzymatic levels and total leukocytic count. On the other hand there was no correlation between serum and CSF enzymatic values. Also there was no correlation between CSF enzyme levels and CSF protein. So these CSF enzymatic activities could not be used as a reliable index of CNS involvement or remission in ALL

Abstract: 225 Poster: 132

BREAST AND OVARY RELAPSE IN A CASE OF ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background:Extramedullary relapse of acute lymphoblastic leukemia (ALL) outside the testes and central nervous system (CNS) are rare. Aims: To report an unusual case of extramedullary relapse of ALL with both involvement of the breast and ovarium. Case Report: A 27-year-old woman patient who was diagnosed as pre-B ALL 1.5 years ago presented with weakness, pallor and fatigue. She was given remission induction protocol. Induction therapy consisted of intravenous cyclophosphamide, doxorubicin, L-asparaginase, vincristine, and oral prednisone. After achievement of complete remission, central nervous system prophylaxis was given as 2400 cGy cranial irridation plus inthrathecal methotraxate. Maintenance therapy consisted of daily 6-mercaptopurine and weekly methotrexate. But, 1.5 year later It was hospitalized with abdominal pain and a painless mass in the left breast. On admission, performance statue of patient was good. Physical examination of the skin and conjunctiva was normal. Physical examination revealed painless mass of 5 cm dimater in the left breast and 2 cm diameter lymphadenopathy in the left axillary region. His temperature was 37.50C, the pulse rate 120/min and the blood pressure 145/90 mmHg. Neurological examination was normal. A complete blood count showed: Hb; 13g/dl, WBC;

7.6x109 /l (PNL; 70%, lymphocytes; 25%, eosinophyls; 1%, monocytes; 4%), platelets; 250x109/l. Erythrocyte sedimentation rate was 25 mm in the first hour. Serum biochemistry was normal except for elevated LDH (1200 U/l). The bone marrow aspiration showed an normocellular marrow without any blast excess. Chest x-ray scan was normal. Magnetic resonance imaging (MRI) of the abdomen revealed two large circumscribed mass with cystic region, max 85.1 x 69.7 mm, in the area of the left ovary, compressed the uterus. Also, there were a few lymphadenopaty next to mass (Figure 1). MRI Mammography revealed covered with sclerosed structure of all left breast tissue and increased density fibroglandular tissue at the site of the lesion (Figure 2). The patient underwent fine-needle aspiration biopsy. Histopathologic examination of the specimen showed infiltration of breast tissue by medium-sized lymphoid cells with scant cytoplasm suggesting diagnosis of ALL. Karyotypic analysis was 46XX. It was started combined chemotherapy with Cytocine-arabinoside (2g/m2, 1-4 day) and Etoposide (500mg/m2,1-4 day). The patient has as yet received first day of chemotherapy, she sudden died. It was wanted for autopsy, but relatives of the patient did not accepte. Conclusion: In medical literature we didn`t meet any case of ALL who has both breast and ovarian involvement. Our case represents an unusual case of extramedullary relapse of ALL with both involvement of the breast and ovarium. Figure 1. MR image show enlarged ovary due to diffuse leukemic infiltration

Abstract: 226 Poster: 133

A CASE OF RUBELLA WITH CLINICAL PRESENTATION RESEMBLING LEUKEMIA

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Rubella is a contagious disease of chilhood characterized by mild constitutional symptoms, rash resembling measles or scarlet fever, enlargement and tenderness in post-occipital, retroauricular and posterior servical lymph nodes. 6-year-old boy admitted to our clinic with fever, abdominal pain and leg pain which occured 3 days ago and followed by reddish purple colored rash in legs that appeared 2 days after onset of leg pain. In physical examination, he had fever of 37°C (axillary) and 2 tender lymphadenopathies (1x1 cm), in right posterior servical region. Oropharynx, respiratory and cardiovascular system examinations were normal. There were a few reddish purple colored petechial rashes in abdomen. He had 2 cm splenomegaly but no hepatomegaly. He had petechial and purpuric lesions at lower extremities. There was tenderness in right testis by palpation. Leukocyte count was 3500/mm3, hemoglobin level was 12.3 gr/dl, and platelet count was 45000/mm3. There was lymphocyte dominance in peripheral blood smear. Sedimentation rate was 80 mm/hr, CRP was negative and biochemical tests were normal. Hepatit markers and monotest were negative; rubella IgM and IgG were positive and other TORCH parameters were negative. Bone marrow aspiration smear was normal, no blasts were seen. In this case, we wanted to emphasize that rubella can rarely present with splenomegaly, leucopenia, trombocytopenia, petechiae, purpura and testicular pain; without occurrence of typical maculopapular rash and can be easily be misdiagnosed as leukemia; therefore rubella has to be kept in mind in differential diagnosis of leukemia. Fortunately, differential diagnosis is very easily made by bone marrow aspiration and serological tests.

Abstract: 227 Poster: 134

INCREASED EXPRESSION OF MULTI DRUG RESISTANCE 1 (MDR1) GENE AT RELAPSE IN A CHILD WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Modern treatment protocols lead to complete remission in a high proportion of patients with childhood acute lymphoblastic leukemia (ALL). However, a large number of them show a relapse of the disease. Treatment failure in these patients is mainly attributable to de novo or acquired re-

sistance to a wide variety of cytotoxic drugs, which is called multi drug resistance (MDR). Expression of multi drug resistance 1 gene (MDR1), is implicated in the drug-resistance mechanism. A 15-month old girl with newly diagnosed CALLA positive pre-B acute lymphoblastic leukemia, was admitted to the Hematology-Oncol-ogy Department with 90% leukemic blast infiltration of the bone marrow. According to her clinical and labora-tory characteristics upon admission she was stratified to standard risk group and started chemotherapy according to BFM 95 treatment protocol. Although, she was characterised as prednisone good responder with complete remission on day 33 of the induction therapy, bone marrow examination before protocol II showed a relapse of the disease with 15% blast infiltration of the bone marrow. Due to this fact we evaluated the expression of MDR 1 gene by reverse transcriptase-polymerase chain reaction (RT-PCR) in the bone marrow sample using the appropriate primers at initial diagnosis and upon relapse. The expression of the house keeping gene of b-actin was used as an internal control. PCR products were submitted to 1.5% agaroze gel electrophoresis with ethidium bromide and band intensity was analysed using a computerized densitometer. According to the results a five-fold stronger intensity of the PCR product of MDR1 gene upon relapse compared with diagnosis was found after normalisation with the b-actin gene. This finding supports the hypothesis that the initial MDR1 positive leukemic clone was sensitive to chemotherapy and that a resistant subpopulation was emerged when relapse occurred. The participation of the MDR1 gene in the drug resistance mechanism should be confirmed in other patients at relapse before any definitive conclusions are made.

Abstract: 228 Poster: 135

P-GLYCOPROTEIN POSITIVITY AND QUANTITATION OF MRNA OF MULTIDRUG RESISTANCE-RELATED PROTEIN, MULTIDRUG RESISTANCE-ASSSOCIATED PROTEIN, LUNG RESISTANCE PROTEIN IN PATIENTS WITH ACUTE LEUKEMIA

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The purpose of this study, was to determine the incidence of P-glycoprotein (P-gp) positivity and quantitation of mRNA of Multidrug resistance-related protein (MDR1), multidrug resistance-asssociated protein (MRP), lung resistance protein (LRP) and relationship each others in patients with acute leukemia. In 26 new diagnosed leukemic patients (18 ANLL, 8 ALL), P-gp positivity by flow cytometry, MDR, MRP ve LRP mRNA levels by real time (RT) PCR were detected in bone marrow samples. For analysis of P-gp, mouseIgG2a PE, P-gp (170 kd) PE ve CD45 Percp monoclonal antibodies were used. For RT-PCR, RNA isolated from bone marrow blasts and cDNA obtained from RNA by using Roche 1st cDNA Synthesis kit for RT-PCR(AMV) and RT- MDR1, MRP, LRP were detected by Roche LightCycler System by using Metis Biyotechnology primers and probes for MDR, MRP ve LRP quantitation. P-gp positivity was detected in 65% of 26 leukemic patients, in 56 % of 18 ANLL, in 88 % of 8 ALL. MDR-1 expression was 0,004 ± 0,001 in ALL patients, 0,005 ± 0,001 in ANLL patients, 0,000 ± 0,000 in control group. MRP expression was 0,018 ± 0,007 in ALL patients, 0,014 ± 0,004 in ANLL patients, 0,002 ± 0,004 in control group. LRP expression was 0,008 ± 0,001 in ALL patients, 0,007 ± 0,002 in ANLL patients, 0,096 ± 0,092 in control group. Our findings indicate that there are P-gp positivity, increased MDR-1, MRP mRNA levels in patients with acute leukemia but LRP does not and there was no cor-relationship among P-gp positivity, increased MDR, MRP mRNA levels.

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EXTRAMEDULARRY LEUKEMIA IN THE FORM OF ORBITAL GRANULOYTIC SARCOMA ASSOCIATED WITH ACUTE MYELOYTIC LEUKEMIA IN TURKISH CHILDREN

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Extramedularry leukemia (EML) in the form of orbital granuloytic sarcoma (OGS) associated with Acute Mylocytic Leukemia (AML) is an uncommon finding in the Western World. However, 27.2 % of Turkish children with AML showed orbital tumor in our previous series and classified a "high risk" subgroup of AML. The etiology of EML as

OGS still remains obscure. In order to clarify underlying mechanisms, the following studies were initiated in recent 10 cases: Cytogenetic (G-banding technique), immunophenotypic analyses (flowcytometry) and tissue adhesion molecules (CD 44, CD 54, CD 56, CD 11) by APAAP method and some cytokines in serum (IL-2, IL-2R, TNF- .) by Elisa technique have been studied. Cytogenetic analyses revealed the presence of t (8:21) (q22:q22) in 4 of 10 AML with OGS and in 3 of 9 AML without (no significant difference). In addition, double Ph chromosome and recently t (1;11)(p36;q23) karyotypic abnormality have been shown in EML (OGS) (+) AML case: Immunophenotypic analysis revealed the presence of stem cell markers (CD 34 and HL-DRA) more frequently in EML (+) group than simple AML. Expression of adhesion molecules such as CD 44 and CD 56 expressions were found to be positive in blast cells in some cases with EML whereas no positivity existed in the 10 cases of AML representing control group. Serum TNF . and IL-2R serum levels were shown to be elevated in all 5 EML cases so studied, as compared to normal levels found in 7 AML without EML (OGS). These interesting findings altogether may indicate that EML in the form of OGS associated with AML, represents a biologically different subtype of AML in Turkish Children, and should be treated accordingly.

Abstract: 230 Poster: 137

IN VITRO ANTHRACYCLINE RESISTANCE IN CHILDHOOD ALL

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Anthracyclıns are the main drugs in the treatment of childhood ALL. In this study, celluler drug resistance was assessed among different anthracyclins and evaluated sensitivity pofile at important prognostic factors as age, sex, leucocyte count, translocations, response to treatment, relaps rate and survival. PATIENTS AND METHODS: In vitro drug resistance was performed by 4-day methyl-thiazol-tetrazolium (MTT) test for DNR (n:181), ADR (n:117), and IDA (n:69) between 1996 and 2005 in multicenter study. In vivo, clinical and laboratory parameters were evaluated such as age, sex, risk factor (RF), leukocytes (WBC)) at diagnosis, blast count day 8, the percentage of blast cells in bone marrow at day 15 (BM15) and at day 33 (BM33), and leukocyte surface antigens CD3, CD4, CD5, CD8, CD10, CD19, CD20, HLADR, precence of translocations (t (4,11), t(9,22) and t (12,21). Man Whitney-U test was used for statıstıcal analysis. RESULTS: There was significant correlatıon between ADR resistance and precence of hepatomegaly, splenomegaly and CD20 positivity. DNR resistance was also close correlation with CD 22 positivity and the percentage of blast cells in bone marrow at day 33. Although there was no correlation between sensitivity of DNR,ADR and IDA and above mentioned prognostic factors, infant ALL and age more than >10 years seems to be more resistant to ADR, age more than >2 years to DNR. Interestingly, age of more than ten years seems to be IDA more sensitive than other anthracyclines. Patients with t(4,11)translocation were the most resistant to ADR. Translocation with t(4,11) was the most sensitive to ADR. Translocation with t(9,22) was more sensitive to ADR, resistant to IDA. Translocation t(4,11) was sensitive to IDA, resistant to ADR. Blast count >1000 at 8. day was more resistant to ADR. There was no statistically significant correlatıon between ADR resistance and age, sex, hepato-splenomegaly, leucocyte count, hct level, CD10, CD3, CD5, CD7, CD 19, CD 20, CD 22, CD13, CD14, CD33, CD34 and response to treatment. Relaps rate was not correlate with ADR,DNR and IDA CONCLUSION: Young patients were more sensitive to ADR, also >10 years patients was sensitive to IDA. Age might be important factor for in vitro drug resistance to different antracyclins. This research was partly supported by the İstanbul Unıversıty Research Fund. Project Number:T-1137/18062001

Abstract: 231 Poster: 138

SOLUBLE VEGF/SFLT1 RATIO IS AN INDEPENDENT PREDICTOR FOR AML PATIENT`S OUTCOME

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Angiogenesis is the formation of new blood vessels and is controlled by a balance between positive and negative angiogenic regulatory factors. Soluble vascular endothelial growth factor receptors 1,2 (Flt-1, KDR) are the negative counterpoint to the vascular endothelial growth factor (VEGF) signaling pathway, which has been characterized

as one of the most important endothelial regulator in human angiogenesis. In the present work, we tested the differential prognostic relevance of soluble vascular endothelial growth factor (VEGF), their receptors 1 (Flt-1), 2 (KDR), and the ratio between sVEGF/sFlt-1 in 43 patients with acute myeloid leukemia (AML). sVEGF and its soluble receptors were assessed using an ELISA. Soluble VEGF, sFLT-1 and sKDR concentration levels were significantly higher in AML patients at diagnosis when compared to the levels in normal controls. sVEGF, sFlt1 and the sVEGF/sFlt1 ratio were significantly higher in non responders when compared to responders (P<0.001 for all). However, there was no significant difference regarding sKDR levels (P>0.05). sVEGF, the sVEGF/sFlt1 ratio but not sFlt1 and sKDR levels were significantly elevated in those who did not survive, when compared to survivors. sVEGF, sFlt1 levels were significantly correlated to WBC counts (R=0.93,P=0.000,R=0.5 6,P=0.000, respectively); bone marrow blast cell counts (R=0.92,P=0.000;R=56,P=0.000, respectively); peripheral blood blast cell counts (R=0.91,P=0.000;R=0.52,P=0.00 0, respectively); sKDR was only correlated to peripheral blood blast cell counts(R=0.37,P=0.014). Cox regression analysis results with sVEGF, sFlt1, sKDR, sVEGF/sFlt1 ratio suggest that the most important predictor for AML outcome is the sVEGF/sFlt1 ratio. In conclusion, sVEGF/sVEGF ratio is independent predictor of AML patient out come, and its significance should be assessed when considering antiangiogenic therapy.

Abstract: 232 Poster: 139

ASSOCIATION OF CERTAIN HLA ANTIGENS WITH ACUTE MYELOID LEUKEMIA

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Attempts have been focused on HLA asscociations with leukemias as well as other disease associations recently. This study investigates possible relations of HLA antigens with acuete myeloid leukemia (AML). HLA Class I (HLA - A, HLA - B) and HLA Class II (HLA DRB1/3/4/5) alleles in Turkish AML patients diagnosed at several different centers were tissue typed by PCR-SSP method. A total of 374 AML patients with an average age of 30.9 ± 13.3 and 100 volunteer donors as controls from İstanbul Medical Faculty Bone Marrow Bank with an average age of 36.0 ± 10.3 were taken in this study. HLA - A* 24, HLA - B* 27 and HLA DRB1* 13 were found to have statistically significant associations with AML with p= 0.012 OR=1.778, p= 0.019 OR= 2.092and p= 0.006 OR= 1.822 respectively. Significant reduction in the frequency of appearence of some HLA antigens suggested protection against AML occurance. These HLA antigens were HLA - A* 33 and HLA - B* 27 with statistically significance of p= 0.04 OR= 0.391 and p= 0.01 OR= 0.364 respectively. The heterogeneity of Major Histocompatibilty Complex genes and complex disease associations await larger number of AML patients to be studied in order to ascertain above associatons which may well yield important findings to be used in clinical settings.

Abstract: 233 Poster: 140

A NEW PREDICTIVE AND PROGNOSTIC MARKER FOR DE NOVO AML: PERIPHERAL BLOOD CD34 COUNT AT RECOVERY FOLLOWING REMISSION INDUCTION THERAPY

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Aim: In EORTC-LCG AML-10 study patients with low CD34 mobilization profile showed a better outcome (Leukemia, 2003, 17: 60-7.). There is always a strong correlation between peripheral blood CD34(+) cell count (pCD34) at the time of collection and total CD34 content of the harvest. We hypothesized that patients with low pCD34 at the recovery period after remission-induction therapy with de novo AML should have a better clinical outcome or vice versa and we launched this prospective study. Patients and methods: Between Jan 2002 and Oct 2004, forty patients with AML (22 male; 18 female) were enrolled to this study. Median age was 40 years (range, 17-60). Using flow cytometrical method and Ishage single stage platform CD34-expressing cells were measured in peripheral venous blood samples once WBC was between 1x10e9/L to 3x10e9/L at the recovery period after first remission-induction therapy (RI). Monoclonal antibodies-conjugated

with a flourochrome, CD45FITC and CD34PE (Immunotech, Coulter, France) were used in this study. Results: The median time for estimation of pCD34 was 23 days (range, 7-35) after RI. Hematological remission after first RI was obtained in 32/40 (80%) patients. Mean pCD34 count was 146.6x10e6/L±96.14. The pCD34 cell count was lower in patients responding to RI therapy (12.4x10e6/L vs 68.6x10e6/L, p=0.011). We observed a weak, but statistically significant positive correlation between CD34 expression of the blasts at diagnosis and pCD34 at recovery period (p=0.048, r2:0.322) (Table 1). We could not show any significant negative impact of CD34 expression of the blasts at diagnosis and response to first RI (12 % vs 44 %, p=0.059). Only two out of 8 patients who did not respond to the 1st RI achieved a complete remission after re-induction. The two-year probability of OS, LFS, relapse incidence and mortality rates in our patient`s population were 47.1%, 37.2%, 55% and 42.5, respectively. If we set up a cut-off value of 18x10e6/ L for pCD34, we did not observe any impact of pCD34 on relapse incidence but the mortality rate was significantly increased in patients with high CD34+ cell count (60% vs 25%, p=0.025). Conclusion: We were able to show a positive impact of pCD34 estimations after first RI in AML on remission and mortality rate. AML Patients with lower pCD34 after 1st RI tends to have a higher hematological remission rate and lower mortality. The impact of this easily obtainable, relatively cheap and standardized measurement as a predictive and prognostic marker in AML should be verified in large cohorts using multivariate analysis.

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QUANTITATIVE ANALYSIS OF WT1 GENE FOR MINIMAL RESIDUAL DISEASE DETECTION IN ACUTE LEUKEMIC PATIENTS BY REAL-TIME RT-PCR

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Introduction: WT1 gene encodes a transcription factor which is involved in differentiation and proliferation of Hemtopoeitic precursor cells as well as some other tissues like kidney, ovary, heart etc. It is also expressed in 80% of Acute Leukemia cases (AML, ALL) as determined by various qualitative and quantitative RT-PCR methods. It is proposed to be a useful marker in minimal residual disease (MRD) detection and leukemia management. Methods: To assess the relevance of this gene, sequential peripheral blood samples from 72 leukemic patients (62 AML and 10 ALL) were analyzed for the expression level of WT1 mRNA, using Real-Time Quantitative RT-PCR. Samples from patients obtained at the time of diagnosis, and during treatment (follow-up), in remission, relapse and after relapse. Results: Samples of diagnosis and relapse showed significantly higher WT1 expression levels (90%), compared to samples from patients in complete remission (CR) or healthy volunteers. No significant difference in expression levels was found between various AML subtypes. ALL patients showed lower levels of WT1 expression compared to AML ones. Our study revealed that rising of WT1 expression predicts a forthcoming relapse 1-6 months before overt hematologic or clinical relapse. A linear correlation between quantities of WT1 and PML-RARa fusion transcripts could be seen in APL patients treated with arsenic trioxide. Conclusion: There was a strong correlation between WT1 and specific fusion gene expression in leukemic patients, showing the significant potential of WT1 as a non-specific leukemia marker (NSLM) for monitoring of MRD and treatment approaches in leukemia.

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EVALUATION OF ARSENIC TRIOXIDE TREATMENT IN ACUTE PROMYELOCYTIC LEUKEMIA CELL LINE; NB4

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Introduction: APL accounts for 10% of adult acute myeloid leukemia and presents with coagulopathy and a specific chromosomal translocation, t(15;17), that fuses the promyelocytic leukemia (PML) and retinoic acid receptor . (RAR.) genes. As2O3 is able to induce complete remission in t(15;17)-positive APLs and has shown substantial

efficacy and low side effects in treating both newly diagnosed and relapsed patients, Although the exact mechanism of arsenic efficacy remains unknown, arsenic actions may result in the induction of apoptosis, the inhibition of growth and angiogenesis, and the promotion of differentiation. The aim of this study was to investigate the effect of As2O3 on APL cells, using NB4 cell line. NB4 is a human APL permanent cell line which harbors the (15; 17) translocation with the PML / RAR. fusion genes. Methods: The human APL cell line NB4 was cultured in presence of different concentration of As2O3. Maturation, differentiation, and apoptosis of cell line were evaluated at different time points by morphology, cell count, staining with trypan blue and MTT cytotoxicity assays. Apoptosis effect of As2O3 on NB4 model was evaluated by Fluorescent Microscopy and flowcytometery assays, using double-labelled PropidiumIodide(PI) /Annexin and Hoescht 33342/PI dye treatment. Results and conclusion: The therapeutic effect of As2O3 in the treat of APL was evaluated in NB4 cell line as an APL model. The treatment with different doses of As2O3 showed two pharmacokinetic behaviors. At low doses (0.125-0.25µM), As2O3 showed differentiation and cell maturation; these effects seem to be weaker than reported for ATRA. At high doses (0.75-2.0µM), As2O3 induced apoptosis and with increasing amount of As2O3 in cell culture higher and more rapid apoptosis occurred. Apoptosis in NB4 cells accompanied by down regulation and inhibition of telomerase activity, leading to accelerated telomere length shortening. This data could be beneficial in arsenic therapy and can be applied to the APL patient treatment in our research center

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ANAEROBIC GLYCOLYSIS IS THE MAIN PATHWAY FOR ENERGY GENERATION IN PROMYELOCYTIC LEUKEMIA HL-60 CELLS

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Background: In physiological conditions, normal cells use mainly the glycolytic aerobic pathway to provide energy. On the other hand, most cancer cells utilize anaerobic glycolytic way for energy generation. But the rates of metabolic pathways the cells use can be different although they function on some common ways in principal. Aim: To investigate the carbohydrate metabolic pathways of promyelocytic leukemia HL-60 cells for energy production. Methods: Seven cultures from each promyelocytic leukemia HL-60 cells and normal leukocytes were prepared and then each culture was separated into three groups: one for the aerobic culture, one for the anaerobic culture after potassium cyanide addition, and one for the determination of the initial glycogen and protein contents of the cells. Aerobic and anaerobic cell cultures were immediately incubated with radiolabelled glucose (D-[6-C14]Glucose) for 4 hours at 37 °C. Then, glycogen consumptions and the amounts of radiolabelled glucose catabolized into carbon dioxide (CO2, collected in scintillation vials via nitrogen gas) and lactate (collected in scintillation vials by anion-exchange chromatography) were calculated in pmol glucose per µg protein for each hour, thus the rates of aerobic and anaerobic glycolysis were determined. Results: The predominant end product of glycogen metabolism was CO2 in aerobic normal leukocyte cultures, whereas it was lactate in aerobic HL-60 cell cultures (Table 1). Normal leukocytes were capable of shifting their energy metabolism predominantly to anaerobic glycolysis in anaerobic conditions. In aerobic HL-60 cell cultures, when compared with aerobic normal leukocyte cultures, the glycogen consumption and amounts of labelled and total end product lactate were significantly higher and amounts of labelled and total end product CO2 were significantly lower (Table 1). The rate of anaerobic glycolysis was 93.8% in promyelocytic leukemia HL-60 cells in aerobic conditions and it increased to 96.6% while utilization of glycogen increased by 7.31% in anaerobic conditions. Conclusion: In conclusion, principally anaerobic glycolysis is effective for energy generation in promyelocytic leukemia HL-60 cells. Moreover, the rate of their anaerobic glycolysis gets more prominent in anaerobic conditions. Therefore a chemotherapeutic agent targeting anaerobic glycolytic pathway in promyelocytic leukemia cells may potentially have beneficial effect in the treatment. Table1. Consumption of glycogen and production of CO2 and lactate by normal leukocytes and promyelocytic leukemia HL-60 cells in aerobic and anaerobic cultures

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EUGENOL INDUCED APOPTOSIS IN HL-60 HUMAN PROMYELOCYTIC LEUKEMIA CELLS

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Background: Eugenol is a major component of essential oil isolated from the Eugenia caryophyllata (Myrtaceae), which has been widely used as a traditional medicine. In this study, we investigated the effects of eugenol on the cytotoxicity, induction of apoptosis, and the putative pathways of its actions in human promyelocytic leukemia cells (HL-60). Method: After applying eugenol on cultured HL-60 cells, changes in the mitochondrial membrane potential were examined by monitoring the cells after double staining with propidium iodide and rhodamin 123 and 2`,7`-Dicholorofluorescin diacetate was used to measure of levels of reactive oxygen species (ROS) Result: We show that eugenol is a potent inducer of apoptosis and that it transduces the apoptotic signal via ROS generation, thereby inducing mitochondrial permeability transition (MPT) and cytochrome c release to the cytosol. ROS production, mitochondrial alteration, and subsequent apoptotic cell death in eugenol-treated cells were blocked by the antioxidant N-acetylcystein (NAC). Conclusion: Taken together, the present study demonstrated that eugenol induces the ROS-mediated mitochondrial permeability transition and resultant cytochrome c release.

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SERA VALUES OF NO AND LDH CORRELATES WITH FAB SUBTYPES OF AML

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The malignant cell shows different metabolic profile, energy use, oxidative status and other membrane alterations in comparison to normal tissues. Based on this we investigated prognostic significance of sera Nitric oxide and compared their values with LDH in 40 AML patients, according different clinical parameters and FAB classification. This study included 22 man and 18 woman, age from 25 to 68 years (median values of 50). Bone marrow aspirate was analyzed with classic cytochemical staining and immunophenotyping of blast cells was performed by flow cytometry (Becton Dickinson) on bone marrow cells. The following panel of monoclonal antibodies was used: HLA-DR, CD 34, CD13, CD33, CD14, CD11c, CD15, glicoforin A, CD41/61 and MPO. Cytogenetic examination of bone marrow cells revealed a normal karyotype in 20 pts. In 1 pts t(15;17) was found, trisomy 8 in 1, del(9) in 1, hyperploidy in 1, del(16) in 2, inv(16) in 3 and Ph chromosome in 3 patients. Distribution of subtypes of AML was according to cytologic, immunophenotypic and cytogenetic findings as follows: M2 was present in 10 pts, M1 in 2, M3 in 1, M4 in 11 pts, M5 in 8, M0 in 7 and M7 in 1 pts. TNF was analyzed by ELISA essay and NO by biochemistry method at diagnosis. Results show that patients with AML have significantly different values of sera NO according FAB classification. AML patients in M0 groups have higher values of NO (27.17 mmol/L) that correlate with other poor clinical and cytological characteristics indicates undifferentiated type of AML. In comparison to M0, patients with M4 subtype show values of N0 of 5.45 mmol/L, which is statisticaly significantly different in comparison to M0 (Mann Whitney U test, p=0.009). In addition, sera NO in AML patients with M2 subtype showed values of 14.12 mmol/L which is significantly different in comparison with M4 (Mann Whitny U test, p=0.02), but no in comparison with M2 (Mann Whit-ny U test, p> 0.05). Lowest values of NO in M4 subtypes correlated with favorable cytogenetic findings (inversion and deletion of chromosome 16) and with good prognosis. Significant positive correlation between NO and LDH was found in this study. AML patients with sera LDH below 300 U/ml show at the same time lowest sera NO and AML patients with elevated LDH (over 1000 U/ml) have elevated sera NO. Correlation between percentage of blast cells in bone marrow and NO was also found. These data indicated that NO is one of indicators of metabolic disturbance in malignant cells and especially of undifferentiated cells types. We conclude that this parameter can be used simultaneously with other prognostic factors for analyses in AML patients.

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AML WITH T(15;19;17)(Q22;P13.3;Q21) AND LEUKEMIC CELLS SHOWING

SALMON-PNK CYTOPLASMIC GRANULES

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Background: There is increasing recognition of the importance of genetic events in the classification and therapy of the acute myeloid leukaemias. As a result, the WHO classification of the acute myeloid leukemias incorporates and interrelates morphology, cytogenetics, molecular genetics and immunologic markers in an attempt to structure a classification that is universally applicable and prognostically relevant. The t(15;17) is a special chromosome abnormality exclusively associated with acute promyelocytic leukemia (APL; M3 subtype according to French-American-British (FAB) classification). Aims: We report a case of acute myeloid leukemia (AML) harboring a complex translocation t(15;19;17)(q22 ;p13.3;q21) with leukemic cells with atypical morphology such as myelocyte-stage granulocytic precursors with salmon colored cytoplasmic granules and CD59 deficiency on granulocytes. Methods: A 32-year-old female was admitted to our hospital because of pancytopenia and was suspected to have paroxysmal nocturnal hemoglobinuria (PNH) with the symptom, morning hematuria. So we studied peripheral blood smear, bone marrow biopy, PNH study and cytogenetic and molecular study to confirm diagnosis. Results: Peripheral blood examination showed pancytopenia with left-shifted maturation. A standardized flow cytometric method for PNH showed the CD59 deficiency in about 62% of cells. The bone marrow aspirate showed 83.3% myelocyte-stage granulocytic precursors with salmon colored cytoplasmic granules. Blasts and promyelocytes were counted up to 0.6% and 1.6%, respectively. Cytogenetic study with G banding technique showed 46,XX,t(15;19;17)(q22;p13.3;q21)[17]/ 46,XX[3]. FISH with t(15;17) translocation DNA probe showed that 91% (192/200) interphase cells had one fusion, two PML and two RARA signals. Seminested reverse transcrip-tion-polymerase chain reaction analysis showed the long form PML/RARa chimeric transcript. The patient was treated with all-trans-retinoic acid and idarubicin, she experienced pericardial effusion, which might be attributed to retinoic acid syndrome. The 1st follow-up bone marrow study on day 38 revealed cytogenetic and molecular remission. However flow cytometric analysis for PNH still showed CD59 deficiency in about 9% of cells was detected after complete remission. Conclusions: This cytogentetic abnormality is the second report of t(15;19 ;17)(q22;p13.3;q21) with AML. This PNH clone might be the preleukemic clone because she had symptom of PNH before the diagnosis of leukemia and still have the CD59 deficient cells after remission. The atypical morphology of the leukemic cells and a pathophysiological association between PNH and AML in this patient are discussed.

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PERIPHERAL FLT-3 LIGAND AS A PATHOBIOLOGICAL PARAMETER DURING THE CLINICAL COURSE OF ACUTE MYELOID LEUKEMIA

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FLT-3 is a growth factor affecting the hematopoietic lineage and may reflect the prognostic nature of the disease course in acute leukemias. The aim of this study is to assess alterations in the circulating levels of FLT3 during the clinical course of acute myeloid leukemia (AML). Twent-four AML patients were enrolled to this study. Median age of the patients was 49.9 years (min 19, max 90) and the male-to-female ratio among the patients was 1:1. AML-M2 was the predominant French-American-British (FAB) subtype (n=14) in our group, followed by M4 (n=4), M1 (n=1) and M5 (n=1). One patient had biphenotypic acute leukemia and 3 had CML-blastic transformation. The effect of FLT-3 Ligand on the early hematopoietic lineage prompted us to assess peripheral cell-FLT-3 interaction. There should be also an association between FLT-3 and infection if the former interaction exists. Because of the chemotheraphy action on the early hematopoietic lineage, alterations of the FLT-3 level had also searched. We studied the association in the diagnostic period between FLT-3 and peripheral blood cells (erytrocytes, leukocytes, platelets) together with the serum electrolytes (Na, K, Cl, Ca and LDH). FLT-3 levels (pg/ml) during the aplastic period due to remission induction and consolidation were higher than the levels during the initial diagnosis. On the other hand, diagnostic and remission induction value of leukocytes and FLT3 showed an inverse

association. The results suggested that higher white cell counts may be together with lower FLT-3 levels. We found also a reverse association between FLT-3 and serum LDH level. However there was no association between FLT-3 and other serum electrolyte levels (Na, K, Ca, PO4, Cl). There were no associations between FLT-3 and infection or febrile state. We found also higher FLT-3 levels in male patients. Our study implied inverse proliferative actions of FLT-3 on the early myeloid lineage. There was no relation between FLT-3 level and red cell and platelet counts. FLT-3 represents a candidate laboratory marker as well as novel molecular targeting for the management of leukemia.

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IN VITRO EFFECT OF PLANT ALKALOIDS ON CYTOTOXCICITY AND DIFFERENTIATION OF HL60

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Background: Acute leukaemia is characterised by uncoupling of proliferation and differentiation. Cytotoxic and differentiation agents have been employed for treatment of leukaemia particularly APL. In iranian traditional medicine plant-derived agents have been used for treatment of cancer. Aim:The present study is evaluation of proliferation, cytotoxcicity and differentiation of several plant alkaloids such as Peganum Harmala, Harmine, Harmaline, Urtica Dioica and Chelidonium Majus on HL60 cells. ATRA has been used as standard agent. However, little study have been reported using these agents on this cell line, these components were initiated such as an investigation. Methods: HL60 cells were cultured and plant alkaloids added to cells and incubated for 5 days. Counting of cells, viability, MTT, morphology, NBT reduction and cytofluorometric analysis performed by FACS using PI for cell cycle and markers including CD11b and CD14 for myeloid differentiation. Results: These data showed that all agents in optimal dose caused cessation of proliferation in dose and time dependent manner.($p<0.05$). Optimal concentration of Peganum Harmala, Harmine, Harmaline, Urtica Dioica and Chelidonium Majus (10 micg/ml,1.6 micg/ml,10 micg/ml, 2.5 mg/ml and 0.1 mg/ml respectively) were chosen as anti-proliferative effect with good viability and low cell death. However, all agents in over optimal dose were cytotoxic. Treated cells with ATRA showed depletion of growth in optimal dose of 10-7 Mol. Cells accomulated in G1 phase using ATRA (81.5%) and Urtica Dioica (75%) but they arrested in S phase using Peganum Harmala, Harmine, Harmaline (52.7%) and Chelidonium Majus (54.5%). Cells induced by Harmaline 10 micg/ml showed some morphological changes and NBT positivity (28%) and increase in CD11b (24.3%) and CD14 (43.5%) ($p<0.05$) compared to ATRA (40% as NBT, 71% and 5.7% as CD11b and CD14). Conclusion: These preliminary data showed all plant alkaloids agents were effective in cessation of proliferation in optimal dose and had cytotoxicity effect in higher concentration as well. However, Harmaline caused some degree of myeloid differentiation. These result may open a new window in leukemic in vitro therapy.

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LEUKEMIC TRANSFORMATION OF MYELOFIBROSIS WITH FLT3/D835 MUTATION AND INVERSION OF CHROMOSOME 16

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Myelofibrosis is a clonal myeloproliferative disorder characterised by splenomegaly, abnormal deposition of collagen in the bone marrow, extramedullary haematopoiesis, dacriocytosis and leukoerythroblastic blood smear. Fms-like tyrosine kinase 3 (FLT3) is a member of class III receptor tyrosine kinase expressed on early hematopoietic progenitor cells and plays an important role in stem cell survival and differentiation. FLT3 is preferentially expressed on the surface of a high proportion of acute myeloid leukemia but no in myelofibrosis. Secondary leukaemic transformation may occur in 5 to 20% of patients which may affect any cell lineage. We present an atypical case of myelofibrosis and secondary leukamia FAB subtype M4 with inversion of chromosome 16 and mutation of the loop activation of the receptor second tyrosine kinase domain of FLT3 Asp835. In addition during the leukemic transformation bone osteolytic lesions developed accompanied with elevated TNF-alpha. The presence of these cooperative mutations reflects the progressive association of genetic lesions developing secondary leukemia with relatively benign course.

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CLASSIFICATION OF 404 LEUKEMIAS IN THE ONCOLOGY AND HEMATOLOGY INSTITUTE, CARACAS, VENEZUELA

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The objective of this study is to present the correlation between FAB and inmunophenotyping classification of 404 leukemic venezuelan patients. Materials and methods: the immunophenotyping analysis was done by Flow Cytometry analysis with the following antibodies: CD1, CD2, CD3, CD4, CD5, CD6, CD7, CD8, CD10, CD19, CD20, CD22, CD13, CD14, CD33, CD11b, CD45, CD34, CD1a, CD41w, Glycophorin A, HLA-Dr, CD14/45, CD 4/8, CD19/10, CD2/20, CD13-3, CD5/7, anti kappa light chain, anti lambda light chain, CD79b, CD11b, CD38, CD15, FMC7, MPO, TdT, anti-IgA, anti-IgM, anti-IgG, anti IgD. The FAB was done with Wright, PAS, SUDAN, acid phosphatase, NASDA, NASDA-F. Results: the leukemias were classified in 348 acute and 56 chronics. The immunophenotyping analysis detected 124 acute lymphoid leukemias B, 11 acute lymphoid leukemias T, 19 biphenotypics, 6 undifferentiated, 60 lymphomas in leukemic phase, 108 acute myeloid leukemias, 18 chronic myeloid leukemias in blast phase, 4 chronic myeloid leukemias and 54 chronic lymphoid leukemias. According to FAB classification 6 of the 124 classified by immunophenotyping as acute lymphoid leukemias B were acute lymphoid T due to the unipolar positivity of acid phosphatase in blast cells. Two of these patients had important mediastinal mass. Three of the patients with acute lymphoid leukemia T determined by immunophenotyping were classified as acute myeloid leukemias by FAB classification. Four of the acute lymphoid leukemic patients in aplasia post chemotherapy in treatment with GM-CSF presented an elevated expression of CD33 and CD14 like acute myeloid leukemias. Acute myeloid leukemias were classified by immunophenotyping as follows: Mo:5, M1:18, M2/M3:33, M4/M5: 46, M6:3; M7: 4. Of the 98 acute myeloid leukemias classified by FAB, 6 were actually acute lymphoid leukemias by immunophenotyping. The myeloid chronic leukemias in blast phase were classified as follows: 14 acute myeloid leukemias and 4 acute lymphoid leukemias. The chronic lymphoid leukemias were classified as: 2 chronic lymphoid leukemias T and 52 chronic lymphoid leukemias B. The lymphomas in blast phase were classified as: 12 T lymphomas, 45 B lymphomas and 3 and undifferentiated lymphomas. Conclusion: We have classified by immunophenotyping 348 acute leukemias and 56 chronic leukemias that were correlated with FAB classification. There was no correlation in 5.8% between both classifications. The unipolar positivity of acid phosphatase in blast cells is not conclusive to the definitive diagnosis of acute lymphoid leucemia T. Partial or total inhibition with NASDA-F can occur in lymphoid blasts correlated with a elevated expresión of CD19 or CD20. The use of GM-CSF elevates the myeloid cells population, which may induce errors during the classification of leukemias.

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ACUTE MYELOMONOCYTIC LEUKEMIA IN A BOY WITH LEOPARD SYNDROME (PTPN11 GENE MUTATION POSITIVE)

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LEOPARD syndrome (LS) (multiple lentigines syndrome) (MIM 151100) is a rare autosomal dominant condition with characteristic skin abnormalities, facial dysmorphism, hypertelorism, cardiac abnormalities, and occasional conductive hearing loss. The syndrome is an acronym of lentigines, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonary stenosis, abnormalities of genitalia, retardation of growth and deafness (sensorineural). More than 120 cases have been described so far and one review has been published. Mutations of the PTPN11 gene (located in 12q24.1) are described as the causal gene defects for the clinical features of Noonan syndrome (NS) (MIM 163950) and also for LS. For confirmation of the clinical diagnosis of multiple lentigines syndrome, the molecular genetic mutation analysis of the PTPN11 gene could be helpful. We present a case of a 13-year-old boy with diagnosis of both LS and acute myelomonocytic leukemia (AML-M4). PTPN11 gene mutation was present in our patient and his mother who was also diagnosed to have LS. AML-M4 had not been previously known to be associated with this syndrome. PTPN11 gene en-

codes for SHP-2 which is a member of a small subfamily of cytoplasmic src-homology 2 domain containing protein tyrosine phosphatases. It is required for hematopoietic cell development and participates in signal transduction of a number of cytokines, mediated at least in part by activation of the RAS/MAPK cascade. In addition a gain of functional mutations in PTPN11 have been reported in 34% of juvenile myelomonocytic leukemia (JMML) patients and in 10%, 4% and 5-6.3% of childhood myelodysplastic syndrome (MDS), acute myeloblastic leukemia (AML) and acute lymphoblastic leukemia (ALL) respectively, excluding a NS and LS in these patients. Recently, Tartaglia et. al. reported that among children with AML, PTPN11 mutations are frequently (4 of 12 cases) found in children with acute monocytic leukemia (AML-M5). According to other studies, in contrast to childhood MDS and AML, mutations in PTPN11 make little or no contribution to pathogenesis of adult MDS and AML. 98% of the PTPN11 mutations identified in JMML, childhood MDS and AML are clustered in exon 3. But in our patient PTPN11 mutation is found in exon 7. Cardiac myxoma, iris-retina-choroid coloboma and choristoma (congenital corneal tumor) have been previously reported in patients with LS. Sarkozy A et al. reported myelodysplasia in a patient with LS and demonstrated Tyr279Cys mutation. More information about the patient has not been obtained. Previously, in patients with LEOPARD syndrome, leukemia have not been reported.This case report suggests that LEOPARD syndrome, especially with PTPN11 mutations, predispose for hematologic malignancies. Consecuently, after clinical diagnosis of LEOPARD syndrome, it is suggested that mutation studies and screening for hematologic malignancies must be done.

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TRIPLETS WITH AML

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Acute myelogenous leukemia (AML) is a group of hematological malignancies that contains about 15-25% of leukemia`s in childhood.(1,4) One of the most important predisposing factors in childhood acute leukemia especially AML, is genetic factor.(1,3,4) In this article we report a very rare presentation of AML development in three monozygotic triplets. Two were 10 months old, and the last one was 16 months old at presentation. Chemotherapeutic regimen was administered for all three sisters with success in maintaining remission induction and tolerates 3 years of treatment till now who are 6.5 years old.

Abstract: 246 Poster: 153

THE DIFFERENTIATION EFFECT OF 6ALPHA METHYLPREDNISOLONE ON HL60 CELLS

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BACKGROUND: In precent study, we observed the in vitro differentiation effect of 6.-methylprednisolone on HL-60 (human acute myeloblastic leukemia cell line) cells. HL-60 cells naturally express CD15 on its surface but not express CD11b and CD14 marker. For this purpose, granulocytic and monocytic (CD11b, CD14 and CD15) markers were analysed by flow cytometry. AIM: To evaluate the differentiation effect of 6.-methylpred-nisolone on HL-60 cells by analysing CD11b, CD14 and CD15 flow cytometric markers and to find efficient dose of 6.-methylprednisolone for these cells. METHODS: We treated HL-60 cells with 6.-methylprednisolone at a range of 10-6- 10-4 M concentrations and at a different set-up times of experiment. Before and after treating with 6.-methylprednisolone, the cells were stained CD11b, CD14 and CD15 monclonal antibody and analysed in flow cytometry. For morphological analysis, cells were cytospined and stained with wright dye. All analysis were set-up triplicate and results were evaluated as the mean plus or minus standart deviation (mean SD). Kolmo-grov-Smirnov test were used for the statistical analysis. RESULTS: We evaluated effect of 6.-methylprednisolone on HL-60 cells by flow cytometric data versus time zero of experiment. In 72th hours and at a 10-4 molar consantration, CD11 b cell surface marker slowly increase. In 96th hours, CD11b cell surface marker significantly increase on HL60 cells which treated with 6.-methyl-prednisolone at a concentration of 10-4 molar. We did not observe any significant effect of CD14 marker at the time and dose dependent experiment. CD15 cell surface marker slowly increased on HL-60 cells after treating with 6.-

methylprednisolone at a concentration of 10-6- 10-4 molar in 48-96 hours. When we tried to assay at 120th-168th hours, we observed the CD11b marker was significantly decrease since the cells go into apoptosis. CONCLUSION: As a result of CD11b increasing on HL-60 cells, confirm that 10-4 M concentration 6.-methylpred-nisolone differentiate the HL-60 cells to granulositic form. The wright stained cells also support the differentiation morphologically. Unfortunately, we did not show any significant monocytic differentiation effect on HL60 cells at a time/dose dependent of methylprednisolone. Analysis of CD15 marker, we observed %95 increase compared to untreated cells. Further time of experiment shows that apoptosis affect the differentiation of HL60 cells.

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ACUTE PEDIATRIC MEGA-KARYOBLASTIC LEUKEMIA IN GEORGIA

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Acute Pediatric Megakaryoblastic Leukemia in Georgia G. Meskhishvili, Ts. Chinchaladze, Georgian Fund of Pediatric Hematology, Children`s Central Clinic, Tbilisi, Georgia Background: First event of pediatric acute megakaryoblastic leukemia registered in Georgia 1997 year. Acute pediatric megakaryoblastic leukemia comprises 3-5% of all childhood AML and about 20% of infant leukemias. There were studied 6 cases of AML-FAB-M 7, 3 girl and 3 boy with age range from 12 month to 14 year /1997 -2004/. Methods: Cytomorphology: 2 patient`s peripheral blood and bone marrow smears except 4 patients what were difficult for diagnostical purpose showed giant blast cells of variable size, Some with two nucleus, blast cells were surrounded by numerous platelets shedding. Auer bodies were not recognized. Cytochemistry: Bone marrow smears showed POX and Sudan black negative blast cells, less blasts were PAS positive and negative to nonspecific esterase. Immunophenotyping: (Flow Cytometry, Becton -Dickinson Kits) performed in four patients except one, where diagnosis set only by cytomorphological and cytochemical features. Flow Cytometry confirmed right diagnosis in two doubtful cases. Immunophenotyping revealed cases with high expression of CD 61, CD 41 and CD 41a phenotypes, the markers for CD 33 were also positive. The most marked expression of following immunologic markers are presented in Table1. Karyotype: Chromosome analysis carried out using the G-banding technique /2patients/. The modal number of chromosomes was 46. This analysis showed polyploid metaphases with 90 - 100 chromosomes. Five patients were Treated by use AML BFM93 protocol except one 12 month old girl, who treated according her general condition C-Ara 100mg/m2 N5, Rbm 30mg /m2 N2. Only two pts achieved stable and complete remission, one boy died after intensification course due to Herpes viral infection, 2 patients relapsed follow short time of remission.

Abstract: 248 Poster: 155

ACUTE NONLYMPHOBLASTIC LEUKEMIA IN CHILDHOOD, CUKUROVA EXPERIENCES-I

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Acute myeloblastic leukemia (AML) constitutes 1525% of the leukemias of childhood. Radiation exposition in prenatal and postnatal periods, chemical substances and chemotherapy increase the incidence of AML. Anthracycline derivatives and cytarabine are used in the treatment. Stem-cell transplantation can also be applied in the patients during remission period. Although a lot of improvement has been performed in the last 30 years, survival rate without illness is below 50%. In this study, we would like to present the results of the patients with myeloid leukemia diagnosed in our clinic and treated by chemotherapy regimen with idarubicin. The participants of this study are 72 patients with ANLL treated between February 1998-May 2004. Of the cases; 38 were boys (52.8%) and 34 (47.2%) were girls. The average age was 83.5±48.4 months (3-168 months). The most common three complaints were weakness, fever and pallor respectively. 47 cases (65.3%) had complete remission after first induction therapy. Seven cases (9.7%) had partial remission, 6 cases (8.3%) had no remission, and 12 patients (16.7%) died during period of the first treatment. The complete remission and partial remission rates were detected as totally 75% (54 cases). When the survival times were examined, it was detected that overall survival was 62% for 12 months, 37%

for 36 months, and 37% for 60 months. Event-free survival was 54% for 12 months, 38% for 36 months, and 38% for 60 months. Disease-free survival was 50% for 12 months, 38% for 36 months, and 38% for 60 months. Finally, our results are lower than some of the studies whereas they are higher in others.

we can get better results by adding new chemotherapy drugs such as idarubicin, and improve nutrition which is important in patients with AML.

Abstract: 249 Poster: 156

IDARUBICIN BASED TREATMENT OF ACUTE MYELOID LEUKEMIA IN CHILDHOOD, CUKUROVA EXPE-RIENCES-II

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The results of acute myeloid leukemia (AML) treatment were not good in our previous experience, where we used idarubicin 12 mg/m2/day (3 days). Our in this study, aim was to reduce serious infections due to mucositis and myelotoxic affect which occurred secondary to chemotherapy. In regard to this, we decided to alter the therapy protocol. The first alteration was to begin total parenteral nutrition (TPN) (without lipid) to the patients whose oral intake was impaired and the second was to reduce idarubicin dose to 8 mg/m2. In this study, our aim was to compare the results of standard therapy (group I) of the newly diagnosed AML patients and the results of therapy that we altered (group II). The participants of this study were 68 patients with myeloid leukemia treated between February 1998-January 2005. There were 26 patients (38.2%) in group I and 42 patients (61.8%) in group II. After the first induction therapy of patients in group I, 20 patients (76.9%) had complete remission or partial remission. 6 cases (23.1%) are still being followed up at our clinic, 16 cases (61.5%) died, 4 cases (15.4%) are unknown. Of the patients in group II (42 cases); in 36 cases (85.7%) complete remission or partial remission have been determined after the first induction therapy. We have been following up 23 cases (54.8%) in our clinic out patient. Of these 23 patients; 11 (26.2%) are followed up without chemotherapy (2-24 months), 14 (33.3%) have died and 5 (11.9%) patients were unknown. The survival rates are shown in Table 1. We found OS and DFS rates statistically significant (Breslow, Tarone-Ware) but not EFS rates in group I and group II when compared. Finally, the survival in children with AML is still low. We suggest that

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HPF EOSINOPHILS COUNT ON BONE MARROW BIOPSY MIGHT PREDICT RESPONSE TO FIRST COURSE CHEMOTHERAPY IN SECONDARY ACUTE MYELOID LEUKEMIA PATIENTS

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Background Secondary leukemias are disease with poor prognosis. Nevertheless actually ther`is a lack of reliable prognostic factors allowing to discriminate patients with longer survival from those with shorter survival. Some evidence seems to show that eosinophilia could be a marker of good prognosis in de novo non secondary acute myeloid leukaemia, but this data are lacking in secondary acute leukaemia. Aims Aim of this study is to verify if ther`is a link between bone marrow eosinophil, monocyte, CD68+ cells, phagocyting histiocyte number and outcome of patients with secondary acute myeloid leukaemia, in terms of response to treatment and overall survival. Methods This is a single institution retrospective study on 17 patients (pat) affected by acute myeloid secondary leukemia (IIAML). Eosinophil (eos), monocyte and phagocyting histiocyte count was performed on paraffin included bone marrow biopsy, stained with hematoxylin-eosin. CD 68+ cells were identified by immunohistochemical stain. The count is the arithmetic mean of cell number observed in 10 cellulated different 40x fields at optical microscope. 12 months were identified as long-term and short-term survival cut-off, considering the short patient survival. The arithmetic mean of numbered cell was used as cutoff between patients with low and high cell count (18.7 for eosinophils, 62,5 for CD68+ cells, 17 for monocytes, 4 for phagocyting histiocytes). Two tail Fisher`s exact test was used to verify the correlation between patients with low and high cell count and long-term and short-

term survival. Results 17 pat (M/F:11/6), median age 65 years (R45-87), with IIAML were analyzed. 15 pat had an AML post myelodysplasia and 2 pat post chemotherapy with alkylating agents. 3 pat had the following karyotype abnormalities: t(8;21) with survival (SVV) of 12 months (mo), 5q-with SVV of 5 mo and complex with SVV of 18 mo. Median SVV was 6 mo (R1-27), 6 pat had a SVV ^{3}12 mo. 6 pat received low dose chemotherapy (CT), 9 standard dose and 2 supportive treatment. Among patients treated with low dose and support only 1 pat receiving low dose survived 12 months (pat with t 8;21). 9 pat achieved complete remission (CR) after I CT course. No correlation was found between CD68+ cells, macrophage, phagocyting histiocytes and SVV or response to PCT. Low eos count was related with CR achievement after I CT course (p 0.015). CR achievement after I PCT course was related with SVV ^{3}12 mo (p 0.009). No correlation between eos count and SVV was found (p 1). Summary/Conclusions Eos <18/ HPF might correlate, in secondary AML elderly patients, with a good response to I CT course, wich is related to a SVV ^{3}12 mo. This observation might be of clinical utility to define therapeutic approach in these patients. Nevertheless, the small number of patients do not allow an exact evaluation of the role of cytogenetic and type of treatment on prognosis.

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TREATMENT OF ACUTE MYELOID LEUKEMIA IN CHILDHOOD, CUKUROVA EXPERIENCES-III (CUKUROVA MODIFICATION)

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Relapse was seen in patients with acute myeloid leukemia (AML) which increased during maintenance chemotherapy after alteration of treatment of AML (to reduce idarubicin dose to 8 mg/m2 and to begin total parenteral nutrition)in our centre. In our previous studies, the relapse rate of group I (idarubicin used 12 mg/m2/day (3 days) in induction period) and group II (idarubicin used 8 mg/m2/ day (3 days) in induction period and parenteral nutrition) were 26.9% and 31.6% respectively. Idarubicin (6 mg/m2, one dose/every two month) appended to the maintenance chemotherapy to reduce relapse rate in group III (idarubicin used 8 mg/m2/day (3 days) in induction period, and parenteral nutrition, and 6 mg/m2, one dose/every two month in maintenance period (along 1 year). In this study, our aim was to compare the results of group I, group II and Group III. The participants of this study were 72 patients with AML treated between February 1998-May 2005. There were 26 patients (36.1%) in group I, 19 patients (26.4%) in group II, and 27 patients (37.5%) in group III. In group I; 6 cases (23.1%) are being followed in our clinic, 16 cases (61.5%) died and 4 cases (15.4%) are unknown. In group II (19 cases); 6 cases (31.6%) are being followed at our clinic, 10 cases (52.6%) died and 3 cases (15.8%) are unknown. In group III (27 cases): 20 cases (74.1%) are being followed at our clinic, 5 cases (18.5%) died and 2 cases (7.4%) are unknown. The survival rates are shown in Table 1. We found OS, EFS and DFS rates statistically significant ($p<0.05$) when compared in group I and group II, whereas did not in group III and group II ($p>0.05$). Finally, the survival rates improved in children with AML (group III) after we altered their chemotherapy.

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SYNCHRONOUS DOUBLE MALIGNANCIES OF RENAL CELL CARCINOMA AND ACUTE MYELOID LEUKEMIA: AN UNREPORTED COEXISTENCE

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Synchronous double malignancies of renal cell carcinoma and acute myeloid leukemia were found to be not reported before in PubMed search. In the present report, a case of renal cell carcinoma and acute myeloid leukemia is descibed. Right radical nephrectomy was performed because of renal mass in a 70 years old female patient. Pathology reported that the mass was renal cell carcinoma and except surgery no additional therapies such as radiotherapy or chemotherapy were given to the patient. After four months from surgery, complete blood count was obtained for the complaint of fatigue and the parameters were as follows: white blood cell 93 x10e9/l, platelet 12 x10e9/l and hemoglobin level 8,66 g/dl. Acute myeloid leukemia was diagnosed

and literature was reviewed because of this unreported coexistence.

Abstract: 253 Poster: 160

ALL-TRANS-RETINOIC ACID-INDUCED MYOSITIS IN A CHILD WITH ACUTE PROMYELOCYTIC LEUKEMIA

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Acute promyelocytic leukemia (APL) is characterized by typical morphologic findings, a balanced reciprocal translocation between the long arms of chromosome 17 and 15, and usually the presence of coagulopathy. Anthracyclin-based regimens and all-trans-retinoic acid (ATRA, tretinoin) as differentiating agent are commonly utilized for the treatment of APL. However, the use of ATRA may have some adverse effects including retinoic acid syndrome that is characterized by fever, dyspnea, weight gain, pleural or pericardial effusions and hypotension, and acute neutrophilic dermatosis (Sweet`s syndrome), hyperleukocytosis, and ATRA-induced myositis in rare cases of adults. Here in, we describe a 11-year-old girl with APL who developed ATRA-induced myositis during induction chemotherapy. The chemotherapy protocol (APL-93 protocol) that consisted of ATRA (45/mg/m2/d, days 1-28), cytosine arabinoside (200 mg/m2/d, days 1-7), and daunorubicine (60 mg/m2/d, days 1-3) was given. On day 5, the patient complained of pain in lower extremities. Physical examination revealed tenderness and firmness in the affected muscles and analgesics were given to relieve the pain. On day 11, she complained of severe pain in both arms with similar findings as seen in the lower extremities. Myositis was suspected. Creatine kinase, aldolase and LDH values were 40 U/L (normal=25-192 U/L), 5.30 U/L (normal=2.30-13.50 U/L) and 321 U/L (normal=240-480 U/L), respectively. Ultrasonography and magnetic resonance (MR) imaging of the affected region poped up myositis. Intravenous dexamethasone therapy (0.3 mg/kg/d) was administered for 3 days (days 16-18). ATRA was not discontinued during steroid therapy. On day 17, the patient`s fever resolved and in the ensuing days she felt less pain and tenderness in her muscles. On day 19, dexamethasone was discontinued. On day 20, she experienced high body temperature (39.70C) and abdominal pain. Examination showed abdominal tenderness. Therapy with intravenous dexamethasone (0.3 mg/kg/d) was reinstituted on day 21 and given for four days. Symptoms did not recur. A bone marrow aspirate obtained on day 28 confirmed a morphologic and cytogenetic complete remission. Muscular involvement associated with ATRA therapy in APL has only been described in four reports including 6 patients: in four cases associated with Sweet`s syndrome or acute febrile neutrophilic dermatosis and isolated myositis without cutaneos lesions in the two, as the case we report. Drug-induced myositis may also be seen during the use of ATRA in pediatric setting.

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ACUTE MYELOID LEUKEMIA AFTER SMALL DOSE OF IODINE-131 TREATMENT FOR THYROID CANCER

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Background: Radioiodine is widely used in the treatment of thyroid cancer. Radioiodine ablation of thyroid remnants has been defined as the destruction of residual, macroscopically normal thyroid tissue following surgical thyroidectomy. Acute side effects of 131I treatment are usually minimal and transient. Leukemia is an uncommon late complication of exposure to the ionizing radiation of radioactive iodine(131I). The risk of leukemia increases with higher cumulative activities, especially when associated with external radiation therapy. We report a case of AML occurring in a 40 year old woman 14 months after treatment with low dose 131I for papillary thyroid carcinoma. Case presentation: A 40 year old woman was admitted to the hospital in March 2005 because of fever, nonproductive cough and dispne. From her history it was learnt that she had papillary thyroid carcinoma diagnosis in September 2003 and a subtotal thyroidectomy was performed. After two months she was reoperated

with total thyroidectomy. She received a dose of 100 mCi 131I in January 2004. Physical examination did not reveal lymphadenopathy, splenomegaly, or hepatomegaly. Her hematologic findings were: hemoglobin, 8.6 g/dl; hematocrit, 25.8%; MCV: 84 fl; WBC, 14.8x109/l with 75 % blastic cells, 22 % bands, 3 % polymorphonuclear cells; and platelets 25x109/l. Bone marrow aspiration showed that 78 % of marrow cells were miyeloblast (FAB morphology AML-M2). Flow cytometric studies of the bone marrow cells revealed positivity for lineage markers for myeloid cells, but negativity for B, Tcells, natural killer cells, monocytic and megakaryocytic cells. The patient underwent remission induction chemotherapy and complete hematologic remission was obtained. She is now in the first complete remission without any complaints. The patient`s condition remained stable thereafter with a suppressive dose of thyroid hormone. Conclusion: Our patient received only a single dose of 100 mCi of 131I and was diagnosed to have AML after an interval of 14 months. The time interval reported in the literature is 5 to 11 years for the appearence of CML, whereas this is shorter in cases of acute leukemias (1-3 years). Patients who are given 131I should be followed-up more closely due to the risk of acute leukemia development.

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HEMOPHAGOCYTIC SENDROME WITH ERYTHROCYTE PHAGOCYTOSIS IN THE MYELOID PRECURSORS IN A PATIENT WITH AML-M2

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Hemophagocytic syndrome (HPS) is an unusual acute syndrome presenting with fever, hepatosplenomegalia and cytopenias. Histologic finding is phagocytosis of hematopoietic cells in the activated macrophages. In adults, most cases are secondary to infection or malignancy. We described seventy-six years old man admitted to hospital because of left hypocondrial pain and epistaksis. Physical examination revealed fever, pallor and hepatosplenomegalia. A complete blood cell count indicated the following values: Hemoglobin:9 g/dL, WBC count: 14000 cells/mm3 and platelet count: 97000 cells/ mm3 Acute myeloblastic leukemia-M2 with Hemophagocytic Syndrome is diagnosed. Bone marrow examination demonstrated that not only macrophages but also myeloid precursors have hemophagocytosis.

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ACUTE PROMYELOCYTIC LEUKEMIA IN A PATIENT WITH BEHCET`S DISEASE UNDER LONG-TERM COLCHICINE THERAPY

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Behçet`s disease (BD) is characterized by a continuous T-lymphocyte mediated inflammatory reaction in small arteriols resulting with gradual destruction. In patients with BD, the efficiency of colchicine has been reported in literature. Acute leukemias have seldomly been associated with BD according to the knowledge in literature. As already known, acute promyelocytic leukemia (APL) is a particular subtype of leukemias typically seen together with special cytogenetic abnormalities. We report a male case of APL in a patient with BD under the long-term treatment of colchicine. This coexistence is reported as the first time in literature and it may suggest that the long-term colchicine therapy may be important in the pathogenesis of APL. The patient has been followed by the treatment with retinoic acid and idarubicine (ATRA-IDA protocol) with a good clinical remission.

Abstract: 257 Poster: 164

REPORT OF A RARE CASE OF ACUTE MEGAKARYOCYTIC LEUKEMIA

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Acute megakaryocytic leukemia (AMegL) is a rare subtype of acute myeloid leukemia (AML) developing from primitive magakaryoblasts. The disease was first described by Von Boros and colleagues in 1931. It is also referred to as FAB M7. The aim of this study is to present the clinical and laboratory data, and the evolution of a rare finding of M7, seen in our department. A 41 year old female was admitted to the hospital because of weakness, fatigue, epygastrial pain and vomiting, and a very high platelet level on routine laboratory investigations. There was absence of overt hepatosplenomegaly and lymph node enlargement on presentation. The hemoglobin level was 9.8g/dL, WBC 18 x10(6)/L and the platelet count was 1045 x 10(9)/L.On peripherial blood smear 40% blast cells were seen. The diagnosis of AMegL was established mainly on morphological grounds. The bone marrow aspirate showed a leukemic blast cell population of about 40% of the myeloid marrow. The majority of these cells were undifferentiated by the routine cytohemical methods but some were identified as early or dysplastyc megakaryocytic precursors. Immunocythochemistry stain on bone marrow biopsy showed positivity on PECAM-1 in about 30% of cell population. Cytogenetic studies were also carried out on peripherial blood and bone marrow blasts.Peripherial blood karyotype showed sigh of gene amplification in a form of double minute chromosomes in 6 out of 17 analyzed methaphases. Bone marrow karyotype showed hyperdiploidia with more than 60 chromosomes in 3 out of 25 analyzed methaphases. In one mathaphase, deletion of short arm of two chromosomes from group B was detected together with polyploidy, witch suggested presence of a clone. The patient was treated according to DAE protocol (Idarubicin, Ara-C and Etoposide) but after two cycles of chemotherapy, no remission was achieved. Then, chemotherapy according to Flag-IDA protocol (Fludarabine, Ara-C, Idarubocin and G-CSF) was started and now, after completing two cycles, she is in a stable partial remission. We plan to continue the treatment with alogenic transplantation if HLA-identical donor (her brother) is confirmed. This is the first case of AMegL seen in our department in the last 10 years. Reports in literature about this disease have been sporadic because of both the rarity of the disease and the lack of well-established diagnostic criteria. Initially treatments with cytarabine and an anthracycline (daunorubicin or idarubicin) or a nonanthracycline DNA intercalator (amsacrine or mitoxantrone) are reported but also, a variety of treatment including low-dose cytarabine, etoposide and bone marrow transplantation. The median survival reported is about 4-10 months.

Abstract: 258 Poster: 165

ANTIPHOSPHOLIPID ANTIBODY POSITIVINESS IN AN AML PATIENT: A CASE REPORT

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Acut leukemia is a disease resulting from hematopoetik progenitor cell`s neoplastic proliferasyon and clonal proliferasyon. And it is characterised by increase immature cell in bone marrow. Antiphospholipid anitibody (APA) is a clinic syndrome caused by repeating arterial and venous thrombosis, recurrent fetal loss, positiviness of lupus anticoagulant or/and anticardiolipin antibody. It is called primary if no underlying disease has been detected. In literature APA positiveness is %30 for AML patients. We here discuss a patient 51 years old woman with AML and APA together. She referred to rheumatology clinic because of right arm and down extremities pain. She first diagnosed APA syndrome and she was admitted to our hospital. But she had neutropenia and sedimentation rate increase. After that bone marrow sample was taken. It confirmed diagnosis of AML M2. On laboratory findings PT:13,7sec, APTT: 90sec, mixing APTT: 56 sec, D- Dimer: 1458 ng/ml, Anti .2 glikoprotein IgM, anti-cardiolipin antibody and antiphospholipid antibody was positive. ANA, anti DNA, anti ds DNA was negative. The patient was admitted remission phase after chemoterapy, and while her consolidation therapy was going on her arthralgia have disappeared. After the management control APA levels will be monitored.

Abstract: 259 Poster: 166

AN UNCOMMON PRESENTATION OF ACUTE MYELOID LEUKEMIA: ORBITAL INFILTRATION

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Orbital infiltration of acute leukemia is a rare condition. A 58-year-old man with acute myeloid leukemia presented with left orbital leukemic

infiltration presenting as tense proptosis and exopthalmus (Figure 1). During the examination his leukocyte count was found to be 450x103, hemoglobin was 9.4x106 g/dl and platelets were 27x103.His peripheral blood smear revealed 95% blasts. Acute myeloid leukemia M4 was diagnosed by flow cytometric examination. Leukopheresis was performed for four times and his leukocyte count dropped to 85x103 /ul. Orbital CT showed thickening of left medial rectus muscle and left orbital proptosis, exopthalmus with anterior subcutaneous thickening. Cranial CT examination revealed bilateral anterior frontal hypodense area, a possible leukemic infiltration. We conclude that orbital lesions could be the first manifestation of acute leukemias.

Figure 1. Left Orbital Infiltration

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LONG-TERM EVALUATION OF ADULT ONSET STILL`S DISEASE IN A HEMATOLOGY DEPARTMENT

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Adult onset Still`s disease (AOSD) is a rare disorder. We report a rare case of a 21 - year old male with AOSD. He was admitted to hospital with 31.000 WBC/mm3, fever and muscle weakness. He was referred to our clinic with a preliminary diagnosis of acute leukemia. Peripheral blood examination and bone marrow aspiration biopsies were performed. A detailed investigation was performed and finally he was diagnosed to be AOSD. Corticosteroid therapy of 60 mg total divided in two doses was started, his fever dropped to normal levels in the very first day, his leucocytosis and clinical symptoms resumed within a week. AOSD should be kept in mind in a hyperleucocytosis case with fever which cannot be explained otherwise.

Abstract: 261 Poster: 168

BETA THALASSEMIA PREVENTION PROGRAM IN DENIZLI-TURKEY: RESULTS OF TEN YEARS EXPERIENCE

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Background: Thalassemia is an important public health problem in many areas of the world including Turkey. As effective management of thalassemia major is too expensive and an economic and social burden for most countries; disease prevention is a critical part of management. Therefore, premarital screening program aimed at the identification of carrier couples, genetic counselling and offering of prenatal diagnosis was initiated in Denizli province of Turkey by the provincial office of the Ministry of Health in 1995. Aims: To evaluate ten years results of beta thalassemia prevention program. Methods: Prospective couples were screened for beta thalassemia trait by automatic red cell indices and Hb A2 determination. Couples at risk were informed about their risk and options before they have children. Results: In a ten year period, 65501 subjects were screened and 2004 of them were defined as high Hb A2 type beta thalassemia trait. The overall carrier frequency of beta thalassemia was found to be 3.2 %. In 62 prospective couples, both partners had carriers of the beta thalassemia. After counselling, only 3 couples decided to separate, remainder proceeded to marriage. Seventeen couples refrained from having a child. Two couples could not have a child due to other medical problems. 23 couples (26 pregnancies) conceived a child and applied for prenatal diagnosis. Prenatal screening revealed 6 foetuses to be normal, 18 had thalassemia minor and 2 had thalassemia major. Both of pregnancies with affected foetuses were voluntarily terminated. Two pregnancies ended in spontaneous abortion before prenatal diagnosis. Five couples had a child without prenatal diagnosis. Follow up of 10 couples was lost due to leaving from Denizli. Conclusions: Our results suggest that premarital thalassemia screening program is highly effective in reducing the number of newborns with severe thalassemia.

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HEMATOLOGICAL FINDINGS IN CHILDREN WITH METABOLIC DISEASES

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Background and Aims: Metabolic diseases are not rare in Turkey. Because consanguinity marriage is common in our country (20-25% in different nationwide surveys). This study aimed to evaluate the frequency and type of hematological findings in children with various inherited metabolic diseases. Material-Methods and Results: Between January 2004 and June 2005, 28 children with metabolic diseases were admitted to Hacettepe University Faculty of Medicine Pediatric Hematology Unit for evaluation of hematological findings. The mean age of the children with metabolic diseases was 55,0±69,7 months (range: 2 months-18 years, median: 14.5 months) and of these 28 children, 9 (32 %) were female and 19 (68 %) were male. The most common hematological findings in our series was anemia (n:26, 92.8%). Fourteen of them (53.8%) had anemia of chronic disease (CD), 6 (23%) had megaloblastic anemia due to vitamin B12 (Vit B12) deficiency, 5 (19.2%) had IDA, and 1 (3.8%) had IDA and Vit B12 deficiency anemia. In addition to the anemia, bicytopenia was found in 3 children (anemia and thrombocytopenia in 2 children and anemia and neutropenia in 1 children) and pancytopenia in 3 children. Of 11 children with methylmalonic acidemia, 4 had anemia of CD, 1 had anemia of CD and pancytopenia, 1 had anemia of CD and thrombocytopenia, 2 had iron deficiency anemia (IDA), 1 had Vit B12 deficiency, 1 had deep venous thrombosis (DVT) and 1 had no hematological abnormalities. Among 5 children with propionic acidemia, 3 had anemia of CD, 1 had anemia of CD and thrombocytopenia and 1 had anemia of CD and pancytopenia. Among 3 children with phenylketonuria, 1 had IDA, 1 had otoimmun hemolytic anemia (OIHA) and Vit B12 deficiency, and 1 had OIHA. One children with cystinosis had IDA and the other had anemia of CD. One children with MSUD had IDA and the other had anemia of CD. One children with isovaleric acidemia had Vit B12 deficiency and hemolytic anemia. One children with argininosuccinic acidemia had anemia of CD and pancytopenia. One children with homocystinuria had Vit B12 deficiency and cranial infarct. One children with citrullinemia had Vit B12 deficiency and neutropenia. One children with erthropoietic protoporphyria had Vit B12 and IDA. Conclusion: Anemias including IDA, megaloblastic anemia and anemia of chronic diseases; various cytopenias including bicytopenia and pancytopenia may complicate metabolic diseases because of the restricted diets and arrested maturation of hematopoietic precursors by toxic metabolites. We suggest that evaluation and treatment of hematological abnormalities in such patients may improve the outcome and life quality of these children with metabolic diseases.

Abstract: 263 Poster: 170

COMPLETE BLOOD COUNT PARAMETERS FOR HEALTHY, SMALL-FOR-GESTATIONAL-AGE, FULLTERM NEWBORNS

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Background: In newborns, complete blood count (CBC) is an important test for diagnosing diseases like infections. In literature, we could find a few studies concerning some of the CBC parameters in small-for-gesta-tional age (SGA) newborns. Aim: In this study, we aimed to investigate differences of CBC and peripheral smear parameters between healthy-term SGA and appropriate for gestational age (AGA) neonates, and to establish reference values for term SGA newborns. Method: One-hundred-thirty-two term newborns were included in the present study. Of them, 73 were healthy-term SGA (SGA group), and 59 were healthy-term AGA (AGA group) neonates. On postnatal day 1 and day 7, 1 ml venous blood sample was obtained for CBC and peripheral smear. We compared data including CBC parameters and peripheral smear findings between groups. Results: Absolute normoblast count, hemoglobin, hematocrit and red blood cell counts on day 1 were higher in SGA group than AGA group. However, there was no difference between two groups on day 7. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) on both days were higher in SGA than those in AGA neonates. Platelet and absolute neutrophil counts were lower in SGA than those in AGA group. However, there was no difference in platelet and absolute neutrophil counts between SGA neonates born to hypertensive or normotensive mothers. Absolute metamyelocyte count was higher in SGA newborns than those in AGA group. Consequently, immature:total neutrophil (I:T) ratio was higher in SGA group than AGA neonates. Abso-

lute lymphocyte counts were similar between two groups on day 1, but lower in AGA newborns on day 7. Conclusion: CBC parameters of healthy term SGA newborns are different from AGA neonates. This is the first study mentioning differences in MCV, MCH, MCHC, metamyelocyte count, I:T ratio, and lymphocyte counts in SGA babies. Therefore, we suggest use of our results as reference values in healthy-term SGA newborns.

Abstract: 264 Poster: 171

RESPONSE TO ANTITHYMOCYTE GLOBULIN THERAPY IN SEVERE APLASTIC ANEMIA: A SINGLE CENTER STUDY FROM INDIA

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Background: Aplastic anemia(AA) is considered to be an immune mediated bone marrow disease,characterized by bone marrow aplasia and peripheral blood cytopenia. Most patients can be successfully treated with either haematopoeitic stem cell transplantation(HSCT) or immunosuppressive therapy(IST).In developing countries,HSCT facilities are limited,and even IST is unaffordable for many patients.The response to IST using lower doses merits evaluation in a country like india. Aim:The aim of this study was to evaluate the response to Anti-Thymocyte Globulin (ATG) thrapy in Aplastic Anemia. Method:This study was conducted from September 2003- May 2005 in the Department of Hematology,AIIMS, New Delhi,India.The diagnosis and classification of AA was established as per standard criteria.Three preparations of ATG were used:ATGAM(Pfizer);ALG(Aventis)and Thymogam(Bharat Serum,India).Those who could not afford ATGAM or ALG were offered the indian preparation Thymogam,as it was less expensive.ATG was infused in a dose of 15mg/kg/d for 5 days.Cyclosporin 3-5mg/kg/d was started after 2 weeks of ATG,with majority getting the lower dose of cyclosporin.Response criteria:Patients were assessed for hematologic response after 3 months of ATG treatment.A complete response(CR) was defined as blood counts normal for age.Partial response(PR) was defined by transfusion independence and no longer meeting the critera for severe aplastic disease.None responder were still severe aplastic anemia(SAA).All remission had to be confirmed by at least 2 blood counts at least 4 week apart. Results:A total of 61 courses of ATG were given to 60 patients with a median age of 26 year(8-75 year);Male-39;Female-21.Diagnosis of SAA in 50(Very SAA -12)and moderate AA in 10 patients. Base line median hemoglobin was 5.4g/dl(2.3-11.3g/dl);median absolute neutrophil counts were 400/mm3 and median platelets counts were 19,000/mm3(1,000-50,000/mm3). Pre ATG treatment:Androgen-37 patients,Cyclosporin-35 patients.Median time from diagnosis to receive ATG was 3 months(1week-84 months).Preparation used:ATGAM in 48 patients;Thymogam in 11 patients and ALG in 2 patients.55 patients were evaluable(minimum 3 months of post ATG).Median blood component support during ATG therapy:Packed RBCs-2 U (0-20 U);RDP-2U(0-20U)and SDP-2U(1-7U).Allergic reaction to ATG was observed in 11 patients and managed with antihistminics and hydrocortisone.Median period of hospitalization during ATG therapy was 11 days(5-42 days).Serum sickness was observed in 10 patients. Response:Overall response of 49% was observed.Details of response to diffrent preparations are given in Table.Eight patients died:4-nonresponder,1 developed acute myeloid leukemia 2 months post ATG, 1 developed disseminated aspergilosis, 1 developed renal mucormycosis and 1 developed HIV infection post transfusion. Conclusion: About 49% patients of aplastic anemia showed response to lower doses of ATG and cyclosporin, and an indian preparation is promising.

Abstract: 265 Poster: 172

PLASMA NITRIC OXIDE AND FREE HEMOGLOBIN CONCENTRATIONS IN SICKLE CELL ANEMIA PATIENTS WHO UNDERGO APHAERESIS

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Among the most important of the multiple biologicaI actions of nitric oxide (NO) in the cardiovascular system are the stimulation of vasodilatation and the inhibition of vascular cell adhesion and aggregation. There is evidence that in sickle cell disease (SCD) vascular production of NO is elevated to maintain vasodilatation. It was reported that restore NO bioactivity by supplying

exogenous NO, reducing NO scavenging by superoxide, or inducing NO synthase expression or activity may prove beneficial for patients with sickle cell disease. Meanwhile, cell-free hemoglobin may limit NO bioavailibility, and consume a part of the NO in SCD. Thus, therapies that inactivate or remove plasma free hemoglobin (PFH) can restore NO concentration and bioavailibility. Automated red cell-exchange (ARCE) is a procedure by which about 60 % of a patient`s red cells are exchanged for those of a donor by using an automated process called aphaeresis; the aim of this procedure is to prevent the complications of SCD. We aimed to investigate the effects of ARCE on the NO, and PFH concentration in the blood of patients with SCD. Stable NO product (nitrate, and nitrite) and PFH concentration in human plasma were measured by using spectrophotometric methods. Thirty red cell-exchange procedures were performed on 16 male and 11 female patients aged 18-48 years, with the diagnosis of sickle cell anemia and with different indications for therapy. Mean NO values were 21.19 ±8.95 µmol/L before ARCE and 19.75± 10.39 µmol/L after ARCE. Mean PFH concentrations were 28.34±34.09 mg/dL before ARCE and 19.82±27.23 mg/dL after ARCE. There were no statistically significant differences in regard to NO level before and after ARCE (P > 0.05). When comparing before procedure PFH concentration to after procedure PFH concentration, significant decreases were observed after the procedures (P < 0.05). A statistical significant difference was noticed in arterial oxygen saturation before and after the procedure (pO2 = 93 vs. 97) (P = 0.003). This study is the first to assess the NO and PFH concentration in patients undergoing ARCE in SCD. Data obtained have supported the theory that ARCE procedures might have positive effects on NO bioavailibility by lowering PFH concentration in the blood

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THE RETICULOCYTE PARAMETERS IN IRON DEFICIENCY, B12 VITAMIN DEFICIENCY AND THALASSEMIA TRAIT PATIENTS

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Aim: The new reticulocyte parameters are currently used in determinating of iron deficiency in hemodialysis patients and children. Since there are a few study in this area, we aimed to evaluate the value of these new parameters in the differential diagnosis of microcytic anemias and macrocytic anemia due to B12 deficiency. Method: In this study, the reticulocyte parameters of 41 iron-deficient (ID), 22 B12 deficient (B12), 34 thalassemia trait (TT) patients and 34 healthy control (C) were studied by ADVIA 120 autoanalyser, and the parameters of % reticulocyte (R), absolute reticulocyte count (ARC), MCVr, CHr, CHCMr are examined. Results: The significantly increased R parameters was found in TT group as compared with controls(C:0.94 ±0.37 vs TT:1.48 ±1.35 p=0.029) and also, increased ARC parameters was found in TT group (C:46.0 ±7.0 vs TT:75.9 ±46.3p=0.001). Altough MCVr was found significantly decreased in ID and TT, it was found significantly increased in B12 group when compared with controls(C:97.7 ±7.5, ID:92.9 ±7.5, B12:117.4 ±17.7,TT:86.7 ±8.8, pvalue 0.008, <0.001, <0.001 respectively). Though CHr was found significantly decreased in ID and TT, there was a significant increase in B12 group as compared with controls (C: 28.2 ±1.7, ID: 21.8 ±3.3, B12 32.0 ±5.7, TT:21.0 ±2.9, p value<0.001,=0 .002,<0.001 respectively). Significantly decreased CHCMr was found in ID and TT groups compare with control group (C:29.1 ±2.1, ID: 24.2 ±3.4, TT: 24.4 ±2.4, p<0.001, <0.001 respectively). MCVr significantly decreased in TT when compared with ID group (86.6 ±8.7, 92.9 ±7.5,p=0.001). CHr and CHCMr parameters were not different in ID and TT groups, but they were significantly differnt in B12 group when compare withthese groups (for CHr ID: 21.8 ±3.3, TT: 21.0 ±2.9 vs B12 32.1 ±5.7 p<0.001, for CHCMr ID:24.2 ±3.4, TT: 24.4 ±2.4 vs B12: 27.8 ±3.8 p<0.001)The ratio of CHr/CH was significantly increased in ID and TT groups as compasre with control (C: 0.92, 0.98,0.97, p<0.001,=0.001 respectively). And there was a difference between controls and TT group`s CHCMr/CHCM. According to the results of control group, R and ARC parameters showed statistically significantly increase only in TT group as a different than ID group. Conclusion: These parameters can be clinically useful in differential diagnosis of microcytic anemias and macrocytic anemia due to B12 deficiency. Additionaly, we can take advantage of these parameters for differentiation of ID and TT.

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CLINICAL USEFULNESS OF ERYTHROCYTE CREATINE AS A

SENSITIVE AND RELIABLE MARKER OF INTRAVASCULAR HEMOLYSIS IN PATIENTS WITH CARDIAC VALVE PROSTHESIS

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Study objectives: To evaluate clinical usefulness of erythrocyte creatine (EC) as a marker of intravascular hemolysis, we compared EC with other hemolytic markers and hemodynamic parameters in patients with cardiac valve prosthesis. Patients and Methods: Erythrocyte creatine was enzymatically assayed in 33 patients with prosthetic valve including 15 patients with aortic valve replacement, 13 patients with mitral valve replacement and 5 patients with double (aortic and mitral) valve replacement and 33 controls. Blood flow velocity and valvular regurgitation were determined by Dop-pler-echocardiography. Other hemolytic markers (lactate dehydrogenase, reticulocyte count and haptoglobin) and cardiac muscle markers (myoglobin and myosin light chain 1) were also measured. Results: Erythrocyte creatine and lactate dehydrogenase were significantly higher ($p<0.0001$) and haptoglobin was lower ($p<0.0001$) in patients with prosthetic valve as compared with controls. However, there were no significant differences in these makers between those with (n=17) and without (n=16) regurgitation. Patients with high erythrocyte creatine (>1.8 micro mol/g hemoglobin) exhibited significantly higher total peak flow velocity (sum of peak flow velocities at mitral and aortic valves) than those with normal erythrocyte creatine ($p=0.006$). Erythrocyte creatine had a significant correlation with total peak flow velocity ($r=0.64$, $p<0.0001$), but lactate dehydrogenase and haptoglobin had no significant correlation with total peak flow velocity. Patients with high lactate dehydrogenase (>460 IU/L) showed significantly higher myoglobin ($p=0.008$) and myosin light chain 1 ($p=0.02$) than those with normal lactate dehydrogenase, whereas erythrocyte creatine was not related to cardiac muscle markers. Reticulocyte count is also a conventional marker, used as an erythropoietic or hemolytic marker, but it did not have a sufficient sensitivity to detect mild hemolysis. Conclusions: Erythrocyte creatine is a reliable and quantitative maker to estimate the severity of intravascular hemolysis in patients with cardiac valve prosthesis. Mild and subclinical hemolysis due to abnormal destruction of erythrocytes caused by prosthetic valve is ascribable to an increase in valvular flow velocity rather than valvular regurgitation.

Abstract: 268 Poster: 175

A NEW FORMULA FOR DIFFRENTIATION OF IRON DEFICIENCY ANEMIA (IDA) AND THALASSEMIA TRAIT (TT)

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Introduction: The most commonly encountered disorders with mild microcytic anemia are iron deficiency anemia and thalassemia trait. Sensitivity and specificity of many discrimination indices have been reported using red blood cell indices. Youden`s index provides an appropriate measure of validity of a particular technique or question by taking into account both sensitivity and specificity. We compare the Youden`s index for these indices as well. Methods: We studied 284 individuals with microcytic anemia aged between 6 month and 75 years. There were 188 females and 96 males involved in our study with mean age equal to 24.23(SD, 15.44). Ferritin, HbA2, and Complete Blood Cell (CBC), in which RBC, Hemogluboline(Hb), Hematocrite(Hct), Mean Corpuscular Volume(MCV), Mean Corpuscular hemoglobin(MCH), and Mean Corpuscular Hemoglobin Concentration(MCHC), were measured for all the participants. We diagnosed individuals with HbA2>3.4% as patients with Beta Thalassemia (BTT) and those who has a serum ferritin <12ng/ml or respond to administered Iron and anemic situation in their blood subsides as patient suffering from Iron deficiency anemia (IDA). England Index, Mentzer Index, Srivastava Index, Kawakami Index, have been calculated for all formulas as well as OUR INDEX (Ehsani Index) = MCV-(10*RBC) Blood Counts were obtained by H1 Technicon Cell Counter System while ferritin was measured and HbA2 value determined by electrophoresis. Sensitivity, specificity, Positive IDA Predictive Value (PPV), Negative IDA (BTT) Predictive Value (NPV), and Youden Index (YI) was calculated. Results: Considering the above criteria we diagnosed 130 patients with BTT and 154 patients with IDA. Sensitivity and specificity for England Index was 99.2 and 69.5, Mentzer Index 94.6 and 95.5, Srivastava Index 88.5 and 85.7, Kawakami Index 86.2 and 98.1, and Ehsani`s Index

90.0 and 95.5. Conclussions: The most frequently encountered diseases with microcytic anemia are TT and IDA. Screening for TT is of great importance in order to address the patient to a genetic counselor. Iron should not be administered to patients with TT as an attempt to normalize MCV so differentiating TT from IDA has a great importance. Decreased levels of SI, TS and ferritin with increased levels of SIBC are the main diagnostic criteria for IDA. The diagnosis of BTT is established by the presence of characteristic red blood cell microcytosis and elevated levels of HBA2. However in some mutations of BTT and in heterozygous alpha thalassemia, HBA2 is not elevated. The use of Ehsani Index is so easy and anybody can subtract the ten fold RBC from MCV in mind with no need for calculator.

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NUTRITIONAL INFANTILE VITAMIN B12 DEFICIENCY DUE TO MATERNAL VEGAN DIETS

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In this report we present thirteen infants (3-24 month-old) with nutritional B12 deficiency who are exclusively breast-fed and born from mothers with inadequate animal protein consumption and no vitamin supplementation during pregnancy and lactation. Although none of the mothers were aware of veganism they consumed almost no animal products due to poor economic status. Infants presented with lemon color paleness, weakness, refusal to wean, neuromotor retardation, tremor, abnormal movements mimicking convulsion, apathy and hypotonia. They had severe anemia (median 6.2 g/dl, range 3.1-10.6) and some had also neutropenia (9/13) and thrombocytopenia (6/13). Bone marrow showed megaloblastic changes. None of them had proteinuria or family history of megaloblastic anemia. Serum B12 levels were low (median 88 pg/ml, range 44.1-233) whereas folic acid were normal (median 15.1 ng/ml, range 5.1-34.6). None of the mothers had macrocytic anemia although some had low serum B12 levels. Infants had packed red cell transfusion as initial therapy. Multiple doses of B12 vitamin is introduced subcutaneously in the first month. B12 is also introduced to mothers. Serum ferritin levels were high at presentation but oral iron medication was started in the second week. Their neurologic improvements were evident even in the first few days. Weaning could be started at the end of the first week. Thrombocytopenia and neutropenia recovered at the end of the second week. In the one-year follow-up period the patients had no hematologic or neurologic abnormality. Symptoms of megaloblastic anemia in exclusively breast-fed infants due to maternal B12 deficiency may begin early, even in the third month. Pediatricians must be aware of neurologic symptoms of infants with B12 deficiency. Immediate treatment is mandatory. Mothers with inadequate animal product consumption must be encouraged for vitamin supplementation during pregnancy and lactation.

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PARATHYROID FUNCTIONS IN THALASSEMIA MAJOR

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The aim of this study was to evaluate parathyroid functions in children with thalassemia major. The study consists of totally 90 patients, 55 males (61.1%) and 35 females (38.9%), followed at Dicle University, Department of Pediatric Hematology between July 2004 and April 2005. The mean age of patients was 7.17.± 3.78 years (1-18 years). 36 patients (40%) had erythrocyte transfusion once in 3 to 4 weeks interval and 54(60%) in 1 to 3 months intervals. Serum ferritin levels were ranged between 430 and 9872 ng/mL and in only 7 patients (8%) was below 1000 ng/mL. Splenectomy had been performed in 19 patients (21.3%). 55 patients (61.1%) were taken DFO treatment. 84 patients (93.3%) were taken supportive therapy (zinc, folic acid, vitamin C or vitamin E). Blood samples were obtained from all patients before transfusion or at least 15 days after transfusion. Serum total Ca, P, ALP, PTH levels were measured to evaluate parathyroid function of children with thalassemia major. 79 patients (68%) had normal Ca level but 11 patients (12%) had low total Ca level. 84 patients (93.4%) had normal, three patients (3.3%) had high P level and three (3.3%) had low P level. Serum PTH levels were normal in 67 patients (74.5%), high in 23 patients (25.5%). While hypoparathyroidism was not determined, secondary hyperparathyroidism was found in the 25.3 percent of patients. This was considered as a result of iron loading in the liver so that decreasing 25 hydroxylation of vitamin D3 and ultimately lack of 25(OH)2D3. When the parameters related to para-

thyroid function were taken in to account significant relation were found between serum PTH and ferritin levels. Our study shows that in thalassemia major altered endocrine organ functions such as secondary hyperparathyroidism might occur even in the first decade. We also showed that there were relation between endocrine dysfunction and transfusion frequency and ferritin level. For this reason patients with thalassemia major should be followed up regularly for parathyroid function.

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OBSERVATIONS ON ETIOLOGY AND TREATMENT OF IRON DEFICIENCY ANEMIA IN INFANCY AND IN PRE-SCHOOL PERIOD

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Iron deficiency anemia (IDA) is the commonest type of anemia in infancy and childhood. The status of iron deficiency and IDA in our country was assessed by the UNICEF survey "Multi-indicator group research with micronutritive components". According to this survey that was made in year 2000 and comprised 1079 children aged from 6 months to 5 years, the prevalence of IDA was moderate -26% (range from 15,0-39,9%). Purpose of the study: To evaluate the etiology and treatment modalities of IDA in infancy and in preschool period. Materials and methods: 284 children with IDA aged from 1 month to 6 years diagnosed during a 5-year period at the Hematology-Oncology Department -Pediatric Clinic in Skopje. Results: Most of the patients -186 (65,5%) are 1-3 years old. Male to female ratio was 63%:37%. The level of hemoglobin (Hb) was usually between 50-80 gr/l -206 children (72,5%). The most common etiological factors were: 1. Poor dietary practices - 206 patients (72,5%), 2. Recurring infections -45 patients (15,8%), 3. GIT disease -30 patients (10,5%), 4. Prematurity and low birth weight -20 patients (7%), and 5. Blood loss -10 patients (3,5%). The treatment of the patients was with oral administration of iron, only 13 children (4,5%) required transfusion of eritrocites. Conclusion: In the evaluated group of 284 patients aged from 1 month to 6 years dominates the risk aged group between 1-3 years with the level of Hb<70gr/l. The commonest causes of IDA in this age group are: faulty dietary habits with recurrent infections. Oral iron therapy is the treatment of choice, as it is cheap, safe, effective and well tolerated -daily increase of Hb is approximately 1 gr/l.

Abstract: 272 Poster: 179

HEMATOLOGICAL PRESENTATION OF SYSTEMIC LUPUS ERYTHEMATOSUS AT THE TIME OF REFERRAL AND THEIR RELATIONSHIP WITH DISEASE ACTIVITY AND OTHER ORGAN INVOLVEMENT

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Abnormalities of hematological system are very common in systemic lupus erythematosus (SLE). The aim of the study is to evaluate hematological findings in patients with SLE at the time of referral and their relationship with disease activity and organ involvement. The study was carried consecutive 115 patients, including 20 males and 85 females. Most of our cases had anemia at the time of presentation due to various etiologies as well. The rate anemia of chronic disease was 46,09% and it was the most common encountered picture. The rate of hemolytic anemia was 27,83%, and Coombs` positivity rate was 21,74% among our patients. Leukopenia (<4,0 x10e9/l), neutropenia (<1,8 x10e9/l), and lymphopenia (<1,5 x10e9/l) rates were 57,39%, 20,00%, and 81,74%, respectively. Thrombocytopenia was noted 40,00% for <150 x10e9/l, 26,09% for <100 x10e9/l, and 7,83% for 50 x10e9/l. Of our patients, 9,56% had antiphospholipid syndrome (APS) at the time of diagnosis. Increased fibrinogen levels were observed in 34,78% without relating to disease activity. The rates of C3 and C4 hypocomplementemia were 86,09%, and 64,35%, respectively, and both are closely correlated with the disease activity. Moreover, C3 hypocomplementemia was more prominent in cases with renal or serosal involvement. Leukopenia and hyperfibrinogenemia were more common in patients with skin/mucosal involvements. C3 hypocomplementemia, APS, and elevated IgG levels were more common in our patients with renal involvement. SLE should be kept in mind while evaluating patients with mentioned hematological findings, especially in those with accompanying organ involvements.

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HOLOTRANSCOBALAMIN (HOLO TCII) AS THE EARLY MARKER OF NEGATIVE VITAMIN B12 EQUILIBRIUM IN PATIENTS WITH MEGALOBLASTIC ANEMIA DUE TO VITAMIN B12 DEFICIENCY AND IN RISK GROUPS (ISCHEMIC CEREBROVASCULAR DISEASE)

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Neurological deficitis may develop in vitamin B12 deficiency before anemia occurs of without having anemia. The neurological deficitis are progressive, and can be irreversible and even fatal if diagnosis is made at a late stage. The conventional tests for the diagnosis of vitamin B12 deficiency lack specificity, and are also expensive and impractical for clinic use. Plasma homocysteine level increases in vitamin B12 deficiency, and the elevated homocysteine level is an independent risk factor for development of the ischemic cerebrovascular, and cardiovascular diseases. Decreasing the plasma homocysteine level by treatment with cyanocobalamin can prevent recurrence of cerebrovascular disease. A decreased concentration of plasma halotranscobalamin II (holoTCII) is an early marker of vitamin B12 deficiency. In our study holoTCII levels measured by utilizing a radioimmunoassay (RIA) test in 65 patients; 20 with megaloblastic anemia due to vitamin B12 deficiency and 45 with ischemic cerebrovascular disease. The plasma holoTC II levels were determined in all patients prior to and on the 10th day of the cyanocobalamin treatment. The plasma holo TC II levels prior to the treatment were found to be decreased in majority of the patients with megaloblastic anemia and ischemic cerebrovascular disease (19/20 and 35/45,resceptively), and the levels significantly improved on the 10th day of the treatment in the both groups. According to these findings, holo TC II can be used as an early diagnostic marker for patients with clinical or subclinical vitamin B12 deficiency.

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THE EARLY CHANGES IN THE NEW ERYTHROCYTE AND RETICULOCYTE PARAMETERS AND RESPONSE TO ORAL IRON THERAPY IN THE IRON DEFICIENT PATIENTS

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Aim: In the iron deficiency (ID) anemia, it is observed that with the oral iron treatment a reticulocyte response is seen approximately in the seventh and tenth day. In the studies, it has been found that content hemoglobin (CHr) from the reticulocyte parameters increased after the second and fourth days of the parenteral treatment. As there is no information about when and how the oral iron treatment causes a change on this parameters, it has been aimed to search the early response to the treatment in this research. Method: In the 2nd and 7th day of the oral iron treatment and before the treatment of 41 patient who are ID and who have not done blood transfusion, absolute reticulocyte number (ARN), % reticulocyte (%R), mean corpuscular volume reticulocyte (MCVr), MCV, content hemoglobin reticulocyte (CHr), CH, mean hemoglobin concentration of reticulocytes (CHCMr), CHCM, parameters were studied by Advia 120 (Bayer Diagnostics, Tarrytown, NY) autoanalyzer. The rates of MCVr/MCV, CHr/CH, CHCMr/CHCM were compared. In addition, the values were compared with 34 healthy control (C). Results: ARN and %R increased significantly in the 2nd day (p=0.00). This increase continued in the 7th day (p=0.03). Hb was not increased in the 2nd day, but in the 7th day there was a significant increase. MCV was not changed in the 2nd and 7th day. The increase of MCVr was insignificant in the 2nd day, but in the 7th day it was significantly increased (p=0.00). Basal MCVr/MCV was higher from the control, and it increased in the 2nd and 7th days (C:1.17±0.1, day0:1.26±0.09, day2:1.29±0.12, day7:1.38±0.16), but, only in the 7th day, the difference was significant. The basal CH was lower from the control, and it was not changed in the 2nd day. Although it was increased in the 7th day, it was still lower from the normal. The basal CHr was lower from the C (21.87±3.34) and it showed a significantly increase in the 7th day (p=0.04). The basal value of CHr/CH was higher from the C, and occured a significant increase in the 2nd and the 7th days (C:0.92, day0:0.98, day2:1.04,

day7:1.16, p=0.00, p=0.01, p=0.00, respectively). Pretreatment CHCM that was lower from the C was observed to be the same level in the 2nd and the 7th days. CHCMr value was lower from the C, it remained the same in the 2nd day, and increased in the 7th day, but still remained lower from the C. For CHCMr/CHCM, C and the basal value were the same, they were did not changed in the 2nd day and they increased significantly in the 7th day (C:0.79, day0:0.79, day2:0.79, day7:0.84, p=0.01). Conclusion: ARN, %R and CHr/CH were found as the earliest parameters (in the 2nd day) that increased with the oral iron treatment. Hb, MCVr, MCVr/MCV, CHCMr/CHCM showed significant increases in the 7th day. Following of these parameters can be useful especially for the evaluation of the early response to the treatment in patients with diagnostical problem.

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MOLECULAR HETEROGENEITY IN RECOMBINANT ERYTHROPOIETIN USING SURFACE ENHANCED LASER INDUCED DESORPTION IONIZATION (SELDI). CLINICAL IMPLICATIONS

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Several erythropoietin preparations (EPO) are used globally for anemia management. Repeated EPO use has been reportedly associated with thrombotic complications. Molecular profiles of these recombinant proteins vary widely, impacting their clinical profile. This may be due to cellular/receptor interactions and other modulatory properties. There are WHO and FDA regulatory guidelines regarding molecular profile; data on protein profiling is usually not provided by the manufacturers. Purpose of this investigation was to compare various clinically-used recombinant erythropoietin products Epogen (Amgen), Vintor (Emcure), Eprex (Janssen-CILAG) and Neo Recormon (Roche) utilizing protein chip array technology (Ciphergen, Fremont, CA). All products were analyzed using anionic SAX2 chips and molecular profile was obtained in the range of 0-150 KDa. Epogen and Vintor exhibited a main component at 32.4 KDa range with minor peaks at 13.5, 21.7 and 43.2 KDa. Intensity of the main component varied widely even within the 3 batches of Epogen. The Epogen preparations and Vintor brand contained albumin in different quantities. One batch of Epogen contained 4x higher amount of this material with a heterogeneous distribution. Epex and Neo Recormon did not have any albumin. One of the Epogen product also contained a component at 110.0 KDa. Glycosylation profiles of each of these preparations also differ. These data suggest that molecular heterogeneity exists within different recombinant erythropoietin preparations. Molecular heterogeneity among these preparations may be partly responsible for differential interactions within erythropoietin receptors on cells and anti-epo antibody generation. Protein chip array technology may be helpful in examining the protein components within different recombinant erythropoietins and may be helpful in establishing molecular profile guideline to minimize pharmacodynamic variance in erythropoietin preparations. Furthermore, molecular profiling of these recombinant proteins may be helpful in establishing the purity and homogeneity of these drugs.

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BIRTH MCV & MCH ARE VERY RELIABLE PREDICTORS OF THE PRESENCE OR ABSENCE OF ALPHA THALASSAEMIA IN THE NEONATE

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BACKGROUND: In parts of the world where thalassemia is common there could be diagnostic problems if patients with the trait present with microcytosis later in life, when diagnosis will be difficult to resolve, especially in the presence of iron deficiency anemia.Tha-lassemia minor is known to present with microcytosis at birth. However, it is not the only condition that does so, and there is alpha and beta AIM: We did a study to see whether the level of MCV and MCH as such can be reliable predictors of one of the two thalassemias and to differentiate between them at birth. METHODS: 202 consecutive births with an MCV of 95 fl or less were tested for Hb separation, basically to look for the presence of Hb Barts 100 controls were neonates with an MCV higher than

95. Coulter counter was used for routine blood counts on cord blood samples or a venous sample of day 1 of life. HPLC was used for Hb separation (Variant II, BIO-RAD). RESULTS: Correlation of the MCV and MCH with the presence of Hb Barts, and hence the diagnosis of alpha thalassemia minor, is shown in the table. Hb Barts Positive: Total patients MCV<90 MCV<90 MCV>90 MCV>90 129 106(82%) 2(1.7%) 9(6.4% 12(9.9%) Hb Barts Negative 73 2(2.7% 2(2.7%) 1(1.4%) 68(93%) * All 100 controls were negative for Hb Barts It was possible to date to follow up 23.3% of the cases for 6 months or more.Diagnosis was confirmed in the Barts positive cases either by the presence of low HbA2, low red cell indices without anemia, high red cell count, HbS lower than 30% in cases of mixed sickle and alpha thalassemia traits or a combination of those findings. CONCLUSION: The positive predictive value for alpha thalassemia of having MCV < 90, together with an MCH < 30 is 0.82. If cases with only one of the values below those level are added then the positive predictive value is 0.91. On the other hand, negative predictive value for alpha thalassemia of having MCV> 90 and MCH> 30 is 0.93

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SERUM AND SALIVARY IRON, TOTAL IRON BINDING CAPACITY AND FERRITIN LEVELS IN THALASSEMIA AND IRON DEFICIENCY ANEMIA

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Background: The thalassemias are a group of autosomal recessive blood diseases of varying degrees of underlying genetic defects that include total or partial deletion of globin chains and nucleotide substitutions, deletions or insertions. Current therapy includes regular blood transfusions and iron chelation. Cronically increased iron load is due to excessive hemolysis, increased intestinal iron absorption and frequent blood transfusions that causes organ damage and dysfunction. Iron deficiency anemia is the most common type of childhood anemia. Especially in childhood period, serum iron level measurement methods are tecnically invasive. Difficulty of the current methods used to evaluate the iron accumulation in organs suggests the importance of saliva usage for diagnosis. In this study, it has been supposed that salivary iron amount could be a marker of total body iron storage in patients with thalassemia and iron deficiency anemia. Material and methods: 34 healthy children as control group were compared with 30 iron deficiency anemia, 71 thalassemia major, 10 thalassemia intermedia and 15 thalassemia trait. Salivary and serum iron and ferritin levels were measured in all groups. Results:there was no statistically significant difference between the control group and other gorups by means of age and gender ($p > 0.05$). There was a correlation between serum and salivary iron levels in thalassemia major, intermedia and trait groups ($p = 0.000$, $r = 0.972$, $r = 0.720$, $r = 0.955$). Also, this correlation was present in control and iron deficieny anemia groups ($p = 0.000$, $r = 0.885$, $r = 0.368$). As conclusion, salivary iron and ferritin levels increases as well as seum levels. This increament in salivary iron amount may be an indicator of total iron accumulation. Therefore non invasive, salive samples for measurement of iron and ferritin may prefer instead of blood samples in patients with thalassemia and iron deficiency anemia. For this reason, we think that more extensive and controlled studies are needed to use the saliva as a routine diagnostic material.

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EFFECT OF ANTIOXIDANTS ON OXIDATIVE STRESS IN PATIENTS WITH THALASSEMIA MAJOR: IN VITRO STUDY

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Excess iron that plays role in occuring hydroxyl radicals is main reason of oxidative stress in patients with beta thalassemia major. Long term oxidative stress impair activity of immune cells and increase possibility many disease such as leukemia and AIDS. Antioxidants, like vitamin C, vitamin E and selenium may decrease the oxidative stress. In this study; firstly, plasma levels MDA, protein thiol groups and free iron were measured in 26 patients with beta thalasemia major and 10 healthy volunteers. Then natural killer (NK) activity and mitogenic lymphocyte transformation were investigated by colorimetric MTT test. After lymphocytes were incubated with vitamin E (150,50,15 mg/ml), vitamin C (200,100,20 mg/ml) and selenium (10-5, 10-6,10-7 M), NK