ratio was evaluated by BCR-ABL mRNA RT-PCR on 14 days. Results. Colony formation of BMMNC from patients with CML-CP in combination with imatinib and IFN-alpha was more strongly suppressed than imatinib alone, and G-CSF did not abrogate the suppressive effect of the combination therapy. There were no significant differences in BCR-ABL mRNA of all colonies between imatinib plus G-CSF and IFN-alpha plus G-CSF. From these observations, it is concluded that combination therapy of imatinib and IFN-alpha with G-CSF can induce anti-leukemic effects effectively in BCR-ABL-expressing cells. Conclusion. Obtained data provide an evidence for the effective and safe combination therapy of imatinib and IFN-alpha with G-CSF against CML-CP patients. Further studies are needed to clarify whether combination therapy really increase normal cells or not, and when we should start the combined therapy for patients with CML-CP.

Abstract: 385 Poster: 292

MULTICENTER CLINICAL TRIAL OF THE TREATMENT WITH IMATINIB MESYLATE FOR THE PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA (CML) IN CHRONIC PAHSE (CP) IN NIIGATA PREFECTUER (JAPAN)

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(Aims) In order to establish an efficient treatment modality of imatinib, we explored the usefulness and side-effects of imatinib treatment in Japanese patients with CML-CP and tried to confirm the effects of the dose escalation of imatinib in respect of the cytogenetic state. (Method) During 36 months, 105 patients were enrolled in the study. Sixty-two patients, 32 of whom were newly diagnosed and 30 had been treated with INF and/or HU, were valuable in the study. Seventeen patients started to be treated within 6 months from the onset, in 24 patients the administration of imatinib was cancelled or continued in reduced dose because of severe side effects, and 2 developed BC during the treatment. Those 43 patients were excluded from the analysis of cytogenetic effects. Imatinib was administered at the starting dose of 400 mg a day. (Results) 86 percent of the patients, who were newly diagnosed and could be kept treated at the dose of 400 mg, reached CCR after 3 months. Percent bcr-abl positive neutrophils in the other 14% of the patients were also less than 5% after 3 months. 72 percent of the patients, who had been previously treated with the other agents and could be kept administered at the dose of 400 mg, got to the state of less than 20% bcr-abl positive neutrophils after 3 months and got to CCR thereafter by 6 months. Four of the other 7 patients, whose bcr-abl positive neutrophils was more than 20% after 3 months, were treated with an escalated dose of 600-800 mg imatinib and three patients could get to CCR thereafter by 12 months. The imatinib treated patients, who were identified to have entered into nearly CCR, were demonstrated to have 3-log reduction in bcr-abl positive cells by Q-PCR. Besides, by the continuous administration of imatinib for these patients, 4-5-log reduction of bcr-abl positive cells was obtained in most of the patients with one patient showing a negative nested PCR of bcr-abl RNA. Six out of 12 patients, who could not be treated with the standard dose of imatinib by the severe side effects, entered into nearly CCR even by the treatment with a diminished dose (100-200 mg) or an alternate administration of imatinib. One of the patients, who got to CCR after 3 months, developed lymphoblastic crisis after 6 months while the imatinib therapy at the dose of 400 mg. (Conclusion) Since it is proved in Japanese cases that entering into CCR in short duration of imatinib therapy brings a better prognosis, dose escalation of imatinib is recommended for the patients with CML-CP, who do not get to the hematological state of 20% or less bcr-abl positive neutrophils after 3 months of 400 mg imatinib therapy and are free from moderate to severe side effects. Considerable cytogenetic response could be observed in even the patients, who had been treated with a low dose or an alternate administration of imatinib, which implied that even a low dose administration is also recommended to the patients who do not have any donor for SCT.

Abstract: 386 Poster: 293

MOLECULAR RESPONSE IN CHRONIC MYELOID LEUKEMIA AT 12TH MOUNTH IS RELATED TO BODY WEIGHT IMATINIB DOSAGE

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Fixed dosage of 400 mg daily imatinib is the common accepted treatment for chronic myeloid leukemia in chronic phase (CP-CML). Higher dosage of 800 mg, if tolerated, promotes faster and deeper molecular responses. Less of 300 mg daily is considered inactive, so reduction dosage should be carefully considered. We evaluate the cytogenetic and molecular response (real time quantitative PCR with Roche LightCycler) after12 months of treatment in ten patients who received imatinib therapy for CP-CML. The group characteristics are described in Table 1. All patients begun treatment with fixed dose of 400 mg imatinib daily. Intolerable toxicity implied transient reduction dosage in 4 patients, 3 of them still supported some degree of reduction at 12h mounth therapy. Dose given for statistical calculation is fixed in basis of sum the daily dose really suministered divided for all days of 12 mounth period. Complete cytogenetic response (CCR) was achieved in 7 patients (all of them showed more than 2 log reduction in molecular quantification), major molecular response was achieved in 5 (more than 3 log reduction) and complete molecular response was achieved in 3 (sensibility of test is 5 x 10-5). All the seven patients with CCR received more than 5 mg/kg of body weight per day (average dose of 5.84) while the other 3 low responders received smaller dose (average dose of 3.9). We calculated the Pearson correlation coefficient between the degree of molecular response expressed as bcr-abl/g6pd ratio and the imatinib daily dose (total, body weight related and body surface area related). Statistical calculation was made with Prism 3.0 package software. Results are shown in table 2 We conclude that molecular response at 12 mounths in CP-CML imatinib therapy is related to body surface area and close related to body weight dosage, so adjusting the dose to body weight is recommended. More than 5 mg/kg daily, particularly in patients of more than 80 kgr of body weight, it would be the aim of therapy.

Abstract: 387 Poster: 294

BEHÇET`S DISEASE IN A PATIENT WITH CHRONIC MYELOGENOUS LEUKEMIA BEFORE HYDROXYUREA TREATMENT

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Chronic myeloid leukemia (CML) is a myeloproliferative disease due to abnormal stem cells. In literature, where the development of Behçet`s disease (BD) was observed during hydroxyurea or interferon maintenance in the chronic phase of CML. When the our case admitted to hematology department, the results of laboratory tests were determined hemoglobin level of 9.7g/dl, WBC count of 162 000/mm3, platelet count of 587 000 /mm3. With the peripheral smear and bone marrow findings, the patient had a diagnosis of CML. Then hydroxyurea treatment was started and the result of cytogenetic was expected. According to the patient`s history, there were sometimes oral aphtha and skin lesions like erytema nodosum prior to the CML. Under the hydroxyurea treatment in first mount, In patient was determined oral aphtha, genital ulceration, fever and erytema nodosum. At the result of skin biopsy, panniculitis was revealed. For the genital infection was started antibiotherapy. With this findings, a diagnosis of BS was thought and started colchicine and prednisolon. The lesions improved with the administration of prednisolon and colchicine. Glivec was started as result of philadel-phia-positive. Cases with symptoms of BD and CML have been reported after hydroxyurea treatment. Also a positive pathergy reaction was observed in patients with CML on interferon alpha (IFN-alpha) therapy. We discussed this case here, because there were BD symptoms prior to the CML diagnosis, co-existence of CML and BD have been reported without hydroxyurea and interferon treatment.

Abstract: 388 Poster: 295

CHRONIC MYELOID LEUKEMIA MAY PROGRESS TO ACUTE BLASTIC TRANSFORMATION WHILE BEING IMATINIB TREATMENT

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Background: Bcr-abl, a constitutively activated tyrosine kinase, is the product of the Philadelphia (Ph) chromosome. This enzyme is present in virtually all cases of chronic myeloid leukemia

(CML) throughout the course of the disease. STI571 (imatinib) is a potent and selective inhibitor of the tyrosine kinase activity of bcr-abl. Case 1: A 26-year-old female patient was diagnosed as CML chronic phase for 8 years, had been taking imatinib 400mg per day for the last 4 years. She was admitted with weakness 3 month ago. WBC count was 18.500/mm^3 with 35% blast. Hemoglobin was 8.4 g/dl and platelet count was 48.000/mm^3. A bone marrow specimen demonstrated lymphoblastic cell infiltration that was showed to be pre-ALL blast by flowcytometry. She was accepted as acute transformation of CML to acute lymphoblastic leukemia. Case 2: A 38-year-old male patient diagnosed as CML 1.5 years ago and hydroxyurea treatment was started. He quit the treatment as he felt better. He was administered with complaints of weakness and abdominal pain just one year after the diagnosis of the disease. In physical examination, his spleen was lying to the inguinal region in the middle line. WBC count was 154.000/mm^3. Hemoglobin was 6.5 g/dl and platelet count was 46.000/ mm^3. In bone marrow specimen, myelogranulocyte series was 85% and blastic cell was found 8-10%. He was accepted as accelerated phase of CML. Hydroxyurea treatment was started and later imatinib 400mg per day was given. After the treatment, WBC count was found 8.000/mm^3 and the spleen size was decreased. Imatinib was given continuously 400mg per day. After 3 monts, in physical examination of the patient, there was palpable servical lymphadenopathy and spleen was palpable in the middle line to the inguinal region, and WBC count was 73.000/mm^3. In bone marrow specimen, blastic cell infiltration of 95 percent was found. Bone marrow flowcytometry data was likely with AML-M1. He was accepted as acute transformation of CML to acute myeloblastic leukemia. Conclusion: These two cases have showed that CML may be to acute blastic transformation while being imatinib treatment.

Abstract: 389 Poster: 296

SPONTANEOUS INTRACRANIAL HYPOTENSION SYNDROME WITH ABDUCENS NERVE PARESIS IN A PATIENT WITH CHRONIC MYELOGENOUS LEUKEMIA

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Spontaneous intracranial hypotension is characterized by orthostatic headache, low cerebrospinal fluid pressure, and MRI findings of diffuse pachymeningeal gadolinium enhancement without previous history of head traume or lumbar puncture. A 46-year-old man with untreated philadelphia positive chronic myelogenous leukemia associated with spontaneous intracranial hypotension is described. This is the first time that such coexistance has been reported. He presented diplopia, vomiting and continuous nonpostural headache. Neurological evaluation revealed an abducens nerve paresis. Several image-guided lumbar puncture procedures were unsuccessful. Brain MRI showed diffuse pachymeningeal gadolinium enhancement. Other feature included subdural fluid collections without downward displacement of the brain. Imatinib (Gliveec(r), Novartis, Switzerland) at a dose of 400 mg per day orally was started. The patient had complet resolution of headache with conservative treatment. We concluded that myeloproliferative disorders may contribute to this condition. Although spontaneous intracranial hypotension is a relatively benign syndrome, it may be essential to control of underlying disease for prevention of unwanted fatal complications.

Abstract: 390 Poster: 297

MASSIVE HEMOTHORAX DUE TO THE INTRATHORACIC EXTRAMEDULLARY HEMATOPOIESIS IN A PATIENT WITH CHRONIC MYELOID LEUKEMIA

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Extramedullary hematopoiesis(EMH) is a compensatory process for some hematologic diseases, such as spherocytosis, myelofibrosis and thalassemia. Extramedullary hematopoiesis and/or leukemic transformation of EMH in the pleura is a rare occurrence and is usually asymptomatic. Pleural involvement is usually diagnosed on postmortem examination. Herein we describe a 46-year-old man with newly diagnosed chronic

myeloid leukemia who was evaluated for progressively worsening dyspnea, and unilateral pleural effusions. EMH involving the lungs and pleura was suspected. A sulfur colloid technetium 99m bone marrow scan was performed to detect that extramedullary hematopoiesis is positive. The diagnostic thoracentesis yielded bloody fluid that contained a large population of hemopoetic cells, including megakaryocytes and indicating pleural extramedullary hematopoiesis. Fine-needle aspiration and video-assisted thoracoscopy were considered, but deferred because of the potential risk of profuse bleeding. Subsequently Imatinib (Gliveec(r), Novartis, Switzerland) at a dose of 400 mg per day orally was started. The patient`s pleural effusion had completely resolved in two weeks. To our knowledge, this is the first case of chronic myeloid leukemia, which extramedullary hematopoiesis is described in the parietal pleura associated with massive pleural hemothorax and which was successfully treated with imatinib mesylate.

Abstract: 391 Poster: 298

TREATMENT RESULTS OF A SINGLE CENTER IN CHRONIC MYELOID LEUKEMIA: STEM CELL TRANSPLANTATION OR IMATINIB?

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Therapeutic decision making in CML is difficult. Although encouraging results have been reported with imatinib, allogeneic stem cell transplantation is still the only curative treatment. But it carries significant risk of morbidity and mortality. Besides, the probability of finding sibling match is only 30%. Aim of this retrospective study is to compare transplanted CML patients with those receiving imatinib in terms of cytogenetic response and leukaemia-free survival. A total of 70 chronic phase(CP) CML patients, 22 in transplant group (TG) and 48 in imatinib group (IG), were evaluated. Patient characteristics and study results are summarized in Table-1. Overall complete cytogenetic response (CCR) rate in IG was 41.7 % (CCR rates for patients receiving imatinib as first-line (n=29) and second-line (n=19) were 48 % and 31%, respectively). Mortality rate in the TG was 11% for patients transplanted in 1st CP (n=18) and 75% for those transplanted later. Due to short follow-up period in the IG (median 18 months) survival rate between two groups was compared at 2 years. Transplantation related early mortality was the most impotant reason for lower 2-year survival rate in TG (78 %) in comparison to IG (95 %). In conclusion, our CCR rate fist-line imatinib was inferior than those reported (60-70%). Since the mainstay of the cure in CML is the eradication of Ph+ clone, transplantation is still the best curative option with > 80 % durable CCR rate at 5 years in our patients. Extended follow-up is necessary to assess the long-term efficacy of imatinib.

Abstract: 392 Poster: 299

ACUTE CORONARY SYNDROME IN ADVANCED CHRONIC MYELOGENOUS LEUKEMIA PATIENTS TREATED WITH IMATINIB MESYLATE: CASE REPORT

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Background: Imatinib mesylate, a selective inhibitor of the bcr-abl, c-kit and platelet-derived growth factor receptor tyrosine kinases, is a promising new form of targeted therapy for the treatment of patients with chronic myelogenous leukemia (CML) and, gastrointestinal stromal tumors. Former reports have indicated a variety of cardiac disorders, which is 1.6 -8.3% in advanced CML, with suspected casual relationship to imatinib mesylate. Aims: In this study we presented the clinical findings of 66 year-old male patient who developed acute coronary syndrome after treatment with imatinib in advanced CML. Case: A 66-year-old man was diagnosed with chronic phase CML with a huge splenomegaly in November 2002. He had type 2 Diabetes Mellitus. He didn`t possess any cardiac disease or complaint and he had no smoking story, with normal LDL cholesterol level. His ECG and echocardiography findings were all normal. He was hospitalized on October 2004 after his periodic control at hematology department for further investigation and treatment strategy. The patient was evaluated as blastic phase CML and Imatimib mesylate therapy 600 mg daily was initiated. Un-

fortunately, on the 20th day of Imatimib mesylate treatment, the patient admitted to emergency service with chest pain lasting more than an hour. Typically, he owned retrosternal pain and sensation of pressure at his chest. He was immediately taken to coronary care unit and diagnosed with acute coronary syndrome depending on cardiac enzymes and ECG findings. His echocardiography showed 60 % ejection fraction, normal systolic functions, but left ventricule diastolic dysfunction. Eventually, coronary angiography was carried on to the patient and left anterior descending coronary vessel was found %90 occluded. In the second process, coronary stent was implanted to LAD. Predispositions of cardiovascular diseases with related genetic mutations were detected to evaluate the risk of paitent; FV G1691A(Leiden), FV H1299R (R2), Prothrombin G20210A, MTHFR A1298C, Factor XIII V34L, GPIIIa L33P (HPA-1), HFE C282Y and Apo B R3500Q were normal and 4 heterozygote mutations (MTHFR C677T, beta-Fibrino-gen -455 G-A, PAI-1 4G/5G, Apo E2/E3/E4) were shown. Conclusion: This is the report, demonstrating an acute coronary syndrome in advanced chronic myelogenous leukemia patients after treatment of imatinib mesylate: In addition to cardiac events, a fluid-retention syndrome, involving pulmonary edema, ascites or hepatic dysfunction, which can have a negative influence on the cardiac function, has also been identified as a possible adverse effect of imatinib mesylate. In conclusion, a number of genetic and environmental risk factors should be evaluated for the risk of cardiovascular disease in imatinib treated patients. So, imatinib treated patients should be kept carefully and if cardiac complaints and findings reveal, further investigation including early coronary angiography should be intervented.

Abstract: 393 Poster: 300

THE RELATIONSHIP BETWEEN BONE MARROW FIBROSIS AND DISEASE PROGRESSION IN CML: DOES TREATMENT HAVE AN EFFECT ON MARROW FIBROSIS

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Introduction: Bone marrow fibrosis (MF) is a common finding in chronic myeloid leukaemia. It has been claimed that marrow fibrosis shortens survival and impairs quality of life in patients with CML. It has also been shown to be an indicator of therapy failure in Ph+ CML. Material and method: A retrospective clinicopathological study was performed in 67 CML patients to assess the impact of the presence of MF on progression free survival and to evaluate the efficacy of different therapeutic drugs (hydroxyurea, interferon and imatinib) in controlling MF. Results: The study group comprised 50 patients in first chronic, 9 in accelerated and 8 in blastic phase. Male to female ratio was 35/32. Median age was 44 years (range: 15-69) and median follow-up was 37 months (range: 6-205). Biopsy specimens of 56 (84%) patients showed MF of varying grades at the time of diagnosis whereas 11 (16%) patients had no fibrosis in bone marrow initially. Sixteen patients (24%) progressed to either accelerated or blastic phase during follow-up. No significant difference was found between fibrosis positive and negative groups in terms of progression free survival ($p > 0.05$). Mean Sokal score of the patients with progressive disease was high but not significantly different from those with stable disease. Pre-and post-treatment biopsy specimens were available in 39 hydroxyurea, 20 interferon and 47 imatinib episodes. The number of treatment episodes was higher than the number of patients, since most patients received more than one drug during their follow-up period. The impact of different treatment regimens on marrow fibrosis in CML was shown in Table-1. Contradictory to classical knowledge hydorxyurea doesn`t seem to decrease fibrosis in the marrow of our CML patients while imatinib caused marked regression in the MF. Conclusion: We could not demonstrate any relationship between marrow fibrosis and disease progression in patients with CML, but our data clearly and strongly implicate that imatinib reverses CML related marrow fibrosis.

Abstract: 394 Poster: 301

SEVERE FLUID RETENTION FOLLOWING IMATINIB MESYLATE (STI 571, GLIVEC) IN CHRONIC MYELOID LEUKAEMIA IN ACCELERATED PHASE: CASE REPORT

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Background: Imatinib Mesylate (STI 571, Glivec) is an inhibitor of the bcr-abl tyrosine kinase, the putative cause of chronic myelogenous leukaemia (CML). As a target therapy for CML, imatinib has been well tolerated in clinical trials. The most common side effects reported included nausea, rash, superficial edema, myelosupression, muscle cramps, and elevations in hepatic transaminases. According to the results of Novartis, clinical safety and epidemiology reports (ML. Hensley et. al Semin hematol 40: (suppl 2), 2003:21-25), less than 5% of the patients experienced excessive central fluid retention manifested as congestive heart failure, pleural effusion, ascites, pericardial effusion and pulmonary edema. Case report: a female patient, aged 47, with diagnosis of Philadelphia positive CML in the chronic phase in 2000. The patient was administered IFN-alpha 3 MU im on alternate days with Ara-C 10 mg s.c. daily and remained in hematological remission for three years. In July 2002 after bone marrow re-evaluation, which revealed 100% Ph(+) cells, trisomia 8, the results of analyses were as follows: FBC: WBC 33,700, Hb 12.2 g/dl, PLT 310,000/ul with no organomegaly and B symptoms. The suggested therapy was Glivec, but was refused by the patient, so IFN-alpha and Ara-C therapy was prolonged. In December 2003 the patient was admitted to our Clinic with presence of B symptoms, hepatosplenomegaly, WBC=99,000, Hb=8.3 g/dl, and PLT=323,000/ul. Bone marrow blast cells (CD=34+) were 4% and moderate degrees of fibrosis and displasia were observed in bone marrow biopsy. IFN-alpha and Ara-C were replaced by Glivec 400 mg/day. After 30 days of administering 400 mg/day of Glivec, her FBC showed: WBC=7,300, Hb=12.2 g/dl, and PLT=130,000/ul. However, the patient complained about weakness, fatigue, dry cough, dyspnea and superficial edema of both legs. Chest X-ray showed massive bilateral pleural effusion up to the third intercostal space. Ultrasound of upper abdomen showed the presence of a small quantity of ascites and slight splenomegaly. Echocardiography showed 100 ml of pericardial effusion. Intensive diuretic therapy was applied in the following order: day 1: Furosemide 60 mg iv with Aldactone 50 mg po; day 2: Furosemide 40 mg iv with Aldactone 50 mg po; day 3: Furosemide 20 mg iv with Aldactone 50 mg po; days 4 to 14: Furosemide 40 mg po with Aldactone 50 mg po. Concomitant therapy consisted of corticoids, cardiotonics and antibiotics while Glivec was discontinued. After 14 days of the therapy chest x-ray was normal, echocardiography showed less than 50 ml pericardial effusion and ultrasound findings showed no ascites. No other side effects were found. On the 15th day of diuretic therapy we started with Glivec again, 400 mg/day; the dosage of diuretics was decreased step by step and finally stopped after 30 days. The patient`s FBC was as follows: WBC=5,150, Hb=13.7 g/dl, and PLT=171,000/ul, no organomegaly, B symptoms and other side effects. Further experience in Imatinib administration will enable us to lower toxicity and identify common indicators of toxicities.

Abstract: 395 Poster: 302

BCR-ABL FUSION BINDING HLA MOLECULE FREQUENCIES AMONG PATIENTS WITH CML

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Background: The peptide binding HLA Class I and II molecules in CML for both bcr-abl and abl-bcr have been defined. The frequency of these alleles have been analyzed previously yielding inconsistent results. Aim: The aim of this retrospective analysis was to detect the frequency of these peptide binding HLA molecules, evaluate the role of DRB4 homozygosity, the role of age of onset. Material: In this study we will present the frequency of HLA Class I (AB) and II(DR) specificities among 192 CML patients (median age:38, M/F:117/76) diagnosed at our center all Philadelphia chromosome, bcr-abl positive in comparison to a control group of 333 healthy subjects (median age:47, M/F:163/170). Control subjects were chosen among our donor population, siblings were discarded to prevent haplotype accumulation. Care was taken to include patient/donor parents of all patients excluding CML patients. Methods: All patients and controls were typed by cytotoxicity assay and /or by DNA based typing for class I; Class II alleles were typed by PCR-SSP at low resolution. Results: In our study: Similar to the previous studies, the protective role of some antigens such as A3, B8, B14, DR1, DR3, DR4 that present CML peptides were appearent in combinations such as A3-DR4, B8-DR3, B14-DR1. On the contrary of other studies, homozigosity for HLA DRB4 were not detected as a risk factor and homozigosity for HLA DRB3 was not detected protective in our patient group. There was a significant difference on the frequencies of some antigens which had not been shown to have a link

with a CML transcript and/or peptide presentation; such as a decrease in B38 and B52 (respectively 13 vs 7.8%, 7.8 vs 3.9 %) and increase in B44 (18.2 vs 10.2%) frequencies. The delay in age of onset (equal or less than 38 vs older than 38) was significant in patients with certain antigens and haplotypes namely A3DR4, B8DR3, A3B44neg DR3, A3B44neg DR4.

Abstract: 396 Poster: 303

IMATINIB MESYLATE CAUSES PLATELET HYPOFUNCTION IN CHRONIC MYELOID LEUKEMIA PATIENTS

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Platelet dysfunction resulting from clonally derived megakaryocytopoiesis appears to contribute to thromboembolic complications and bleeding in patients with chronic myeloid leukemia (CML). Imatinib mesylate, one of the main chemotherapeutic agents currently used for the treatment of patientc with CML, may improve platelet dysfunction since specific supression of clonol megakaryocyte growth and recovery of polyclonal hematopoiesis can be achieved by this agent. A total of 23 newly diagnose CML patients were studied to investigate platelet function by optical aggregometry and whole blood platelet lumiaggregometry (WBPA), using four agonists (ADP, arachidonic acid, ristocetin and collagen). WBPA studies showed 9 patients had platelet hypofunction (impedance or release with one agonist below the reference range), 4 patients had platelet hyperfunction (impedance or release with one agonist above the reference range) whilst 10 had coexistence of hyper- and hypofunction. Optical agregometri demostrated that 11 patients had platelet hypofuction, 2 patients had platelet hyperfunction whilst 4 had co-existence of hyper- and hypofunction. 6 patients had normal results. Repeat platelet function studies were performed in 14 patients, following imatinib mesylate therapy (400 mg/d,12-30 months) 11 patients had hypoactive and 2 patients had mixed hypo- and hyperactive platelets by the luminesance method while 12 patients had mixed hypo- and hyperactive and 2 patients had hyperactive platelets by the optical method (Table 1). We also performed a direct comparison between the two methods we used and showed that luminesance method was more sensitive to detect an abnormality than optical method. 11 of 14 patients (79%) retested were found to have hypoactive platelets while platelet function of only 1 patient was normalized. We demonstrate that imatinib mesylate is not able to improve platelet functions in CML patients and make the suggestion that aspirin should be used only if otherwise clinically indicated in these patients since platelet hypofunction is the predominant abnormality over time, during imatinib mesylate therapy.

Abstract: 397 Poster: 304

SYSTEMIC MASTOCYTOSIS PRESENTING WITH A PROMINENT B LYMPHOCYTE PROLIFERATION IN THE BONE MARROW AND EXTENSIVE FIBROSIS OF THE SPLEEN

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BACKGROUND Systemic mastocytosis is a disease characterized by multifocal mast cell proliferation in the bone marrow or other extracutaneous organs. Because of loosely scattered and hypo-/agranular mast cells, the diagnosis is sometimes very difficult. In the bone marrow, mast cell infiltration may be associated with prominent lymphoid infiltration leading to a misdiagnosis of a low grade non-Hodgkin lymphoma. AIMS We present a case of systemic mastocytosis presenting with a prominent B lymphocyte proliferation in the bone marrow, extensive fibrosis of the spleen, and marginal zone hyperplasia in the mesenteric lymph node leading to difficulties in differential diagnosis. METHODS A 49-year-old woman presented with right arm and leg pain, psychiatric symptoms, and diarrhea for four years. Physical examination and laboratory investigation revealed hepatosplenomeg-aly, anemia, mild thrombocytosis, mild leucocytosis and lymphocytosis. Findings of magnetic resonance imaging and scintigraphy recommended a metabolic bone disease. In the bone marrow biopsy, there was a prominent B lymphocyte prolif-

eration reminiscent of a low grade non-Hodgkin lymphoma/leukemia in a hypercellular background and there were some spindle cells aggregates in paratrabecular location. These spindle-shaped cells had hypogranular or agranular large cytoplasms and round or lobulated nuclei. The consecutive bone marrow biopsies were similar to the first except the progressive decrease of the B lymphocyte population and an increase of spindle cell aggregates. The subsequent splenectomy specimen exhibited striking fibrosis consisting of fibroblasts and spindle cells resembling those in the bone marrow. In the lymph node sections, there was marginal zone hyperplasia. During follow-up of the patient, she complained of urticaria and pruritis. RESULTS Multifocal accumulations of mast cells with spindle cell morphology, persistent in all biopsies, were strongly positive with mast cell tryptase and CD117 (c-kit) on immunohistochemical staining, though no metachromasia was identified in Giemsa and Toluidine Blue stained aspirates and tissue sections, probably due to hypo-/agranulation of mast cells, rendering difficulties in diagnosis. CONCLUSIONS The case was presented to emphasize the importance of the antibody to mast cell tryptase as an immunohistochemical marker in the diagnosis of mastocytosis and to discuss problems of differential diagnosis due to the prominent lymphoid infiltration in the bone marrow and extensive fibrosis of the spleen obscuring the mast cell infiltration of systemic mastocytosis.

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THE DEVELOPMENT OF HEPATIC INVOLVEMENT IN A PATIENT WITH CHRONIC NEUTROPHILIC LEUKEMIA

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Chronic neutrophilic leukemia (CNL) is a rare myeloproliferative disorder characterized by a persistant increase of mature peripheral neutrophils, myeloid hyperplasia in bone marrow, hepatosplenomegaly, elevated neutrophil alkaline phosphatase and absence of philadelphia chromosome, with no evidence of infection or malignancy sufficient to mimic a leukaemoid reaction. A 61-year-old woman with CNL with hepatic involvement is described. This is the second decribing the development of liver injury in a patient with CNL. She presented in January 2004 for evaluation of leukocytosis of long term duration. In 2003, She had been diagnosed Philadelphia negative chronic myelogenous leukemia. She had a history of splenectomy for unknown cause. Several months before admission, she had recurrent upper gastrointestinal tract bleeding. On admission, physical examination was normal. The leukocyte count was 52X109/L with 70% segmented neutrophils an 15% band forms (neutrophilic leukocytosis). A bone marrow aspiration showed marked myeloid hyperplasia. Neutrophil alkaline phosphatase score, vitamin B12 levels were elevated. Cytogenetic analysis of the marrow aspirate showed normal karyotype, with no Philadelphia chromosome. A culture of the marrow aspirate in the media including no cytokine revealed spontaneous colony production, and an immunotyping analysis on flow cytometry confirmed that these colonies were myeloid origin. Blood chemistry showed a persistance elevation of gamma glutamyl transpeptidase with normal transaminase levels. A liver biopsy was performed which showed hepatitis with infiltration of neutrophils.A diagnosis of CNL with hepatic involvement was made, and interferon alpha (Roferon(r), Roche Diagnostics, Basel, Switzerland, at a dose of 3 MU/m2, 1-3 dose/wk, sc) was started. With decrease of total leukocyte count, levels of gamma glutamyl transpeptidase levels slightly decreased to near normal.

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RETROSPECTIVE ANALYSIS OF NON-CML CHRONIC MYELOPROLIFERATIVE DISEASE: SINGLE CENTER EXPERIENCE

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Background and aim: Essential Thrombocythemia (ET), myelofibrosis with agnogenic myeloid metaplasia and polycythemia vera (PV) are classified under the title of chronic myeloproliferative diseases (CMPD). Strict discrimination of these diseases from each other is mostly difficult and the diagnosis and treatment is mostly based on the clinical features and prominent laboratory findings. There have not been consistent cytogenetical anomalies in these disorders. There are

several guidelines for the diagnosis and treatment of these diseases. We hereby, aimed to evaluate retrospectively the patients with CMPD treated in outpatient unit of Ege University Hospital Hematology Department. Material and methods: 101 patients (54 essential thrombocythemia, 42 polycythemia vera and 5 myelofibrosis) monitored with diagnosis of CMPD were analysed retrospectively between October 1992 and December 2004. Mean age of patients was 55.3 (18-78) years, 59.2(23-80) years and 64.4(53-73) years for ET, PV and myelofibrosis respectively. Results and conclusion: The main complaints for ET patients were as follows; neurological symptoms (headache, stroke, tinnitus etc), gastrointestinal symptoms (Budd-Chiari, ulcer and pancreatitis etc). Treatment was started with hydroxiurea (HU) (81 %), busulfan (18.2) and anegralide HCl (1.8%). After obtaining the reduction in platelet count, maintenance was given by interferon (IFN), anegralide or the same drug with the dose reduction. Anegralide has been the first option for the newly diagnosed patients. One patient was transformed to acute leukemia after 7 years of diagnosis. The main complaints for PV patients were as follows; headache, epistaxis and pruritis and cardiac problems. Phlebotomy was used successfully and mean 5.3 units per patient (2-17) were performed. HU and IFN were also used as a first line treatment or for maintenance. Patients with myelofibrosis were admitted due to weakness and abdominal pain. HU or busulfan was given as the first line treatment. Cardiac and gastrointestinal events are among the most clinical newly onset events detected after starting the treatment.

Abstract: 400 Poster: 307

THROMBOEMBOLIC EVENTS IN MYELOPROLIFERATIVE DISORDERS

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Background: In myeloproliferative disorders (MPD), thromboembolic events (TE) are one of the most common causes of mortality and morbidity. Aim: To investigate the prevalence of TE in MPD. Methods: Previously or newly diagnosed 56 patients (27 male, 29 female, mean age 60 years) with MPD were enrolled to this study in-between November 1999 and February 2005. Twenty-six patients had essential thrombocythemia (ET), 13 chronic/acceler-ated phases chronic myeloid leukemia (CML), 8 agnogenic myeloid metaplasia (AMM) and 9 polycythemia vera (PV). The mean duration of diseases was 44±43 months. Results: During this follow-up period, TE were detected in 21 patients (37.5%) with MPD. The prevalence of TE was 67% in PV, 37.5% in AMM, 35% in ET, and 23% in CML, respectively. There was not statistically significance difference for TE in-between four groups ($p>0.05$). TE were seen in the arteries of cerebrovascular (16%), coronary (11%), pulmonary (2%), and the veins of lower extremities (4%), portal (5%), and cavernous sinus (2%). Splenic infarction and missed abortion were detected in 7% and 2% of the patients, respectively. 10 patients (18%) died from cerebrovascular thrombosis, chronic renal failure, cerebral hemorrhage due to thrombocytopenia, and leukemic transformation in this period. The patients received the drugs including hydroxyurea (77%), acetylsalicylic acid (73%), interferon-a (39%), and anagrelide (38%). There was thrombocytosis in %73 of the patients Thrombocytosis was detected in 71% of 21 patients with TE. Hypertension was detected in 39% of patients. There were no differences for hemoglobin level, platelet and white blood cell counts, hypertension in-between patients with TE and without TE ($p>0.05$). Mean and median survivals were not different in patients with TE and without TE, respectively. Hemorrhages including intracerebral, gastrointestinal (23%), gingival and skin were found in 32% patients with MPD. There was no difference for hemorrhages in-between subgroups ($p>0.05$). Conclusion: TE often occurs in the patients with MPD. Although TE have no effect on survival, anticoagulant and/or anti-platelet drugs may be useful in the prevention of TE.

Abstract: 401 Poster: 308

EFFECTS OF SPLENECTOMY ON THE GENERAL STATE AND PROGNOSIS OF PATIENTS WITH HEMATOLOGICAL PROLIFERATIVE DISORDERS

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SUMMARY Splenectomy is an auxiliary treatment method applied in many disorders of hematopoietic system including also myeloproliferative syndromes. Splenectomy was performed on 50 pa-

tients with various hematological proliferative disorders. Main indications were: compression symptoms, hipersplenism and diagnostic aims (Table 1). Post-splenectomy observation period ranged from 6 to 82 months (mean time 26 months). Improvement lasting not less than 6 months was achieved in 52% of patients. The best prognosis group included patients with primary splenic marginal zone lymphoma; 5 long lasting clinical remissions and 3 deaths due to basic disease progression were reported (altogether 15 cases). The worst prognosis group included patients with primary myelofibrosis -no clinical remission was reported and 6 deaths occurred due to disease progression (out of 9 cases). Splenectomy due to hipersplenism resulted in platelet increase over 100 x 10e9/l (89% of patients) and white blood cell increases over 3 x 10e9/l (all patients). The post-splenectomy mean increase of red blood cell count in patients with hipersplenism was not large (0.07 x 10e12/l), in two patients however it reached 0.7 and 1.7 x 10e12/l, so blood transfusion could be avoided. In 22% of patients serious post splenectomy clinical complications appeared including one death due to cerebral hemorrhage. In 50% of patients thrombocytemia was observed and in 28% patients occurred asymptomatic thrombosis of splenic vein. The best prognosis in patients with proliferative hematological disorders was found in the group with primary splenic marginal zone lymphoma, while respectively the worst prognosis concerned patients with primary myelofibrosis.

Abstract: 402 Poster: 309

MYELOFIBROSIS AND EXTRAMEDULLARY HEMATOPOIETIC TUMOUR CAUSING SPINAL CORD COMPRESSION

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Myeloproliferative disorders may be associated with extramedullary hematopoiesis usually in chronic myeloid leukemia (CML), rarely in myelofibrosis (MF) essential thrombocythemia (ET) and policythemia vera (PV). It can differentiate to solid tumour, chloroma, and inflammatory lesions, so in these cases, it is necessary to make a biopsy sample for the histological diagnosis. The authors review a case-report of 48 year-old woman, who was diagnosed with essential thrombocythemia in 1997. From August 2000 she suffered from progressive anaemia, thrombocytopenia and spleen enlargement, which caused abdominal compression symptoms. The repeated bone marrow biopsy showed myelofibrosis Grade II. In January of 2003, the patient underwent splenectomy, after that her peripheral blood picture normalized and no transfusion was necessary. A year later, the patient complained of severe pain in back and paresis of legs. The neurological examination and MRI showed tumour mass in the level of thoracic III-XII. vertebrae with spinal cord compression. Biopsy was made, and histology showed massive erythropoietic infiltration with polimorph megakaryocytosis. The patient achieved complete remission after the irradiation therapy. In myeloproliferative disorders we can sometimes see the appearance of myeloid metaplasia and extramedullary hematopoiesis. Histology and biopsy sample are essential for precise diagnosis and for determining the specific treatment procedure.

Abstract: 403 Poster: 310

DENTAL FILLING: CAUSE OF EOSINOPHILIA

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BACKGROUND: Eosinophilia is defined as an increase in peripheral blood eosinophilic leukocytes. Eosinophilia occurs in a wide range of conditions. It can also occur in relation to common allergic, neoplastic, idiopathic, autoimmune, skin diseases, medicine reactions, and parasitic infections. Eosinophilia secondary to cobalt dental fillings is not published in the world literature. We report a case of eosinophilia in relation to cobalt dental fillings. CASE REPORT: A 44-years old man was admitted to hospital because of nonproductive cough, shortness of breath, night sweats, and weight loss of 4 kilogram over 2 months. The night sweats had been persisting for several months. There were no drug and alcohol abuse, environmental exposures in his medical history. He had no a history of allergy, asthma and other atopic disease or history of tuberculosis, had no HIV infection and its risk factors. On

physical examination, there were two dental fillings in the oral cavity. His hematological laboratuary findings: a white blood cell count was 18.2 x 109/L consisting of 80% eosinophil. On the periferal blood smear 80% increase in eosinophil leukocytes were seen. The bone marrow showed 70% increase in eosinophil cells and its precursors. The biochemical profile, antinuclear factor, and amebic indirect hemagglutination test were within normal range. A tuberculin skin test was negative. Fecal examinations for intestinal parasite on three occasions were normal. The serological studies for hepatitis B and C viruses, Epstein-Barr virus, Human immunodeficiency virus were negative. The thorax and abdomen CT showed normal. Skin prick tests were normal. Patch tests with 1% cobalt chloride showed positive reactions. After the patient was started on removal of cobalt dental fillings, his symptoms diminished rapidly, with resolution of his eosinophilia. During two years of follow up, although he continued to smoke he did not show relapse of respiratory symptoms. DISCUSSION: The term eosinophilia refers to conditions in which abnormally high amounts of eosinophils are found in the blood. There are the striking associations between peripheral blood eosinophilia and various human diseases. Eosinophils kill helminths through their ability to generate potent oxidants and through their content of cationic proteins, which likely achieve high concentrations at points of granule deposition. Eosinophils also participate in inflammation in human disease especially asthma, skin diseases, and heart disease. The eosinophils may cause injury to the respiratory epithelium. Major basic protein, eosinophil peroxidase and hydrogen peroxide which could mediate this injury, release from its granules. Because of these, his symptoms were seen. After allergen as cobalt fillings removed from his teeth, improvement was appeared in all clinical symptoms. To our knowledge, this is the first case of eosinophilia secondary to cobalt dental fillings.

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CUSHING SYNDROME PRESENTING AS ABSOLUTE ERYTHROCYTOSIS

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The polycythemias can be classified as relative and absolute. Relative polycythemia is a disorder in which a modest elevation of the hematocrit without an elevated red cell mass possibly due to deplesion of the plasma volume. Absolute erythrocytosis is associated with an actual increase in the circulating red cell mass. Primary polycythemias are characterized by increased sensitivity of the erythroid progenitors to regulatory growth factors. However, secondary polycythemias are characterized by an increase in erythropoietin (EPO) and normal responsiveness of their erythroid progenitors to hematopoietic growth factors. Secondary polycythemias can be due to hypoxia, autonomous EPO production, administration of EPO or other factors which stimulate erythpoiesis. Some studies have mentioned the occurrence of polycythemia in patients with endocrine disorders (Cushing Syndrome, primary aldosteronism, long-term androgen replacement therapy). These conditions can frequently be distinguished by in vitro assays of erythroid progenitor cells and serum EPO levels. Here we are presented a case who is followed with a diagnosis of absolute polycythemia for one year, then he developed clinical findings of Cushing syndrome. 39 year old man with suffering from headache and hypertension was admitted to our hospital. The patient had a one year history of polycythemia vera (PV) and only flebotomy was recommended in another center. His blood pressure was 150/90 mmHg and pulse 85 beats per minute and regular. At the examination, mild facial plethora was evident. There were dorsocervical and supraclavicular fat pads. There was no bruising of the soft tissue. The thyroid gland was not enlarged. Examination of abdomen showed truncal obesity without striae. His blood tests revealed white-blood cell count 7900/mm3, hematocrit 51%, hemoglobin 16.9 g/dl and platelets 236.000/mm3, Epo levels 2 mU/ml (below normal limits), red cell mass 58 ml/kg (elevated) at that time. Abdominal ultrasonography was revealed minimal hepatosteatosis and no splenomegaly. Bone marrow aspiration and biyopsy were nondiagnostic. Bone marrow cytogenetic result was 46,XY. His basal cortisol level was 31.4 microgram/dl (elevated), 24 hour urine cortisol level was also elevated and computed tomograpy of abdomen showed bilateraly nodular hyperplasia. 8 mg overnight dexametazone test revealed cortisol supression. Cushing syndrome was diagnosed and then magnetic resonance imaging of hypophisis showed that adenom and surgery was recommended.

Abstract: 405 Poster: 312

RAPID PRENATAL TESTING OF BETA THALASSEMIA MUTATIONS BY REAL TIME FLUORESCENT PCR AND MELTING CURVE ANALYSIS

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Beta thalassemia is the most common genetic disorder in Turkey with 5000 affected and 1.500.000 carrier patients. Rapid, cost effective and reliable tests for prenatal, carrier and preimplantation genetic diagnosis is of great importance for effective fight against this public health problem. Tests based on reverse dot blot hybridization or ARMS-PCR strategies do not fulfill all of these criteria. Real time fluorescent PCR technology is widespread, sensitive and has low running cost. We have developed multiplex real time fluorescent PCR melting curve analysis protocols and optimised for studying in single cells. Beta thalassemia mutations: IVS I:110, IVS II:745, Codon 15, IVS I:6, CD39, CD8/9, CD44, IVS I:5, IVS II:1. IVS I:1 have been detected in as low as 1 single villus from CVS; 200 microliter amniotic fluid or blood, or from single blastomers. The turnover time for testing was 90 minutes including DNA isolation. Combined with early CVS, rapid detection of mutations allow same day reporting of the test results. The protocols provide economic and fast detection of thalassemia mutations.

Abstract: 406 Poster: 313

A NOVEL &BETA; -THALASSEMIA MUTATION, IVS-II-2 (T-A), IN A FAMILY FROM TURKEY

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Inherited hemoglobinopathies are a vast group of disorders, which include the thalassemias and abnormal hemoglobins. Thalassemias are the most common single gene disorders, caused by defective and imbalanced a/b globin chain production. B-thalassemias are caused by mutations that reduce or abolish the expression of the B-globin genes. B-thalassemia major patients frequently present with severe anemia, requiring regular blood transfusions to sustain life. The disease poses a major public health concern, especially in regions of the world, previously endemic for malaria. More than 200 mutations have been described thus far on the B-globin gene. The thumb rule is that 6-8 mutations account for about 90% of the B-thalassemia alleles in a given population. In contrast to most Mediterranean countries, Turkey is very heterogeneous regarding B-thalassemia mutations and more than 32 mutations have been described thus far in the Turkish population. The availability of accurate knowledge on the spectrum of mutations in a population group represents an invaluable tool to study human population dynamics to implement cost-effective programs for B-thalassemia based on prenatal diagnosis by DNA analysis. Here we report the first description of a novel mutation in a Turkish family originating from the Black Sea region. The 11-year-old proband and her mother are typical B-thalassemia carriers, with elevated Hb A2 levels. The father of the proband has a borderline Hb A2 value, and a possible heterozygosity could not be excluded. Initially this familly was referred to us since a second pregnancy was planned and the b-thalassemia carrier status of the father was ambigious. However, further hematological investigations excluded his heterozygosity, thus only the proband and her mother from whom she had inherited the disease, were due to DNA analysis. Since the B-Globin StripAssay (Vienna Lab), which detects 20 B-thalassemia mutations and two abnormal hemoglobins, was not informative, the proband and her mother, were subjected to genomic sequencing. The results revealed the presence of a novel mutation at IVS-II-2 (T-A) in the proband, which was later confirmed in her mother. The invariant GT at the donor splice site of the second intron of the b-globin gene, which ensures proper mRNA splicing, is converted into a GA, in our family. This is expected to lead to a severe B0-like phenotype, when it is combined with another severe B-thalassemia mutation. However since our patients are heterozygotes, they exhibit a mild and symptomless hypochromic, microcytic anemia. To the best of our knowledge, thus far, two mutations have been reported at the IVS-II-2 position of the B-globin gene; one of these mutations [IVS-II-2/3, +11bp -2 bp] was detected in three chromosomes from Iran, in a father and his two children, also in heterozygous form. The other one, [IVS-II-2 (-T)], was observed in a Chinese patient in combination with a 4 bp-deletion at codon 41/42.

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RAPID HLA MATCH TESTING BY REAL TIME FLUORESCENT PCR AND MELTING CURVE ANALYSIS FROM BUCCAL CHEEK SWABS MAY INCREASE THE NUMBER OF AVAILABLE DONORS

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Rapid, simple and reliable HLA match strategies would be valuable for screening of larger number of candidate transplantation donors. The costs and long turnover times of the HLA SSP typing tests used currently by many clinical laboratories limits the size of the screened samples. Number of available donors are further limited by the unfeasibility of blood sampling and transport from relatives at rural hometown areas. Here we describe a rapid HLA match test procedure that uses real time fluorescent PCR and melting curve analysis for common HLAA, HLA-B and HLA-DRB1 alleles in DNA obtained from buccal cheek swabs. Real time fluorescent PCR technology is widespread, provides fast and economic analysis of single nucleotide polymorphisms. Buccal cheek swabs can easily be used without the help of a health professional and they can be transported at room temperature over several weeks thus enabling the accessibility to distant relatives, friends and hometown individuals. We have optimised the test for single cells so that the HLA match testing could be done on a few cells from cheek swabs. Peripheral blood lymphocytes or buccal cheek swabs have been prepared by centrifugation. Initially, HLA-A, HLA-B and HLA-DRB1 typing of the parents and the affected child has been performed. The first round multiplex PCR has been carried out by using common PCR primers for HLA-A, HLA-B and HLA-DRB1 followed by nested PCR using sequence specific primers, anchor probes and labelled hybridization probes for Lightcycler. The turnover time for HLA-A, HLA-B and DRB1 match test of 32 donors was 60 min in Lightcycler instrument. The matching donors have been further studied by high resolution genotyping of the HLA alleles. Rapid HLA match test by real time fluorescent PCR and melting curve analysis of buccal cheek swabs minimize the test costs and turnover time while increasing the feasibility of access to higher number of potential donors.

Abstract: 408 Poster: 315

SIGNIFICANCE OF M-FISH APPLICATIONS IN HAEMATOLOGICAL MALIGNANCIES

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Multicolor Fluorosescence In Situ Hybridization (MFISH) is recently developed molecular cytogenetic tech-nique for evaluation of the chromosomal abnormalities especially in cancer cells. This technique allows identification of derivative and marker chromosomes and complex chromosomal rearrangements. In this study, M-FISH technique was applied in conduction with conventional cytogenetics in 22 cases (13 AML, 5 ALL, 2 MDS, 1 CML and 1 NHL) with hematological malignancy to resolve the nature of the marker/derivative chromosomes and complex chromosomal rearrangements (19 cases) or karyotype refinement (3 cases). We identified variant translocations in three cases. These variant translocations were as follows: t(8;16;21) (q22.1;q13;q22.1) in an AML patient, der(7) t(1;7;22) (p31;p21;q13.2) in an ALL patient and t(1;6;9;22) (p36.1;p21.3;q34;q11) in a CML patient. Five cryptic translocations were also detected in three cases. These cryptic translocations were as follows: der (6)t(6;14)(p15.3;q32.3) and der(15)t(5;15)(q35;q26.3) in a patient with ALL, der (5)t(5;21) (q35;q22.3), t(12;21) (q24.3;q22.3) in a patient with NHL and der(2)t(2;3) (q36;q27) in a patient with MDS. In our study, the chromosomal abnormalities of chromosomes 3, 7, 11 and 20 are frequently observed in AML patients who have complex karyotype. We also observed that, t(9;22) translocation and abnormalities of the chromosome 1 are most frequently observed chromosomal aberrations in ALL cases with complex karyotype. In addition to well known leukemia associated breakpoints, other breakpoints are also observed in the complex chromosomal aberrations. Among these rare breakpoints, 12q11 is observed in two adulthood AML patients. Our results show that, M-FISH is a powerfull technique which allows detection of cytogenetically undetermined hidden translocations in leukemia cells.

Abstract: 409 Poster: 316

FLT3-ACTIVATING GENE MUTATIONS IN ACUTE PROMYELOCYTIC LEUKEMIA -THE SINGAPORE GENERAL HOSPITAL EXPERIENCE

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Mutations activating FMS-like tyrosine kinase (FLT3) gene are present in some patients with acute leukemia. The presence of the FLT3 internal tandem duplication (FLT3-ITD) and Asp835 point mutations was determined in a chohort of patients with acute promyelocytic leukemia (APL). Detection of mutations was performed by polymerase chain reaction (PCR) followed by nucleotide sequencing for aberrant PCR products. Clinico-hemato-logic features of 41 APL cases together with molecular analysis for PML-RAR alpha fusion transcripts by reverse transcription PCR was also studied. Sixteen patients (39%) possessed the FLT3 mutations - 7 with FLT3-ITD, 8 had Asp 835 while 1 had both. The FLT3-ITDs were all in-frame. No statistically significant association was demonstrated for the different PML-RAR alpha transcripts in relation to both mutations although there appeared to be a trend towards ITD postivity in patients with bcr2 (1/3) and bcr3 (5/15) fusion transcripts compared to bcr1 (2/23) PML-RAR alpha fusion transcript. No statistically significant correlation iwth age or gender was found. A positive correlation with high presenting white cell count (>10,000/ µl was demonstrated in ITD positive patients only (p=0.015, Mann Whitney test). In summary, FLT3 mutations - both ITD (20%) and Asp835 (22%) are common in APL. The clinico-pathogenetic impact of the presence of these mutations in patients with APL remains to be elucidated.

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INTERNAL TANDEM DUPLICATION OF THE FLT3 GENE IN BULGARIAN ACUTE MYELOID LEUKEMIA PATIENTS - A PILOT STUDY

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BACKGROUND: Fms-like tyrosine kinase 3 (FLT3) is a receptor-type tyrosine kinase involved in the proliferation and differentiation of hematopoietic stem cells. Internal tandem duplications of the juxtamembrane domain of FLT3 (FLT3-ITDs) are considered as the most frequent molecular abnormalities in acute myeloid leukemia (AML) patients, which predict unfavorable outcome. However, the data concerning the incidence and association with patients` characteristics vary in different studies. AIMS: To determine the incidence of FLT3-ITDs in a cohort of 59 Bulgarian AML patients and to correlate FLT3-ITD-positive [FLT3ITD(+)] status with clinical and biological features of patients. PATIENS AND METHODS: Bone marrow and/or peripheral blood from 59 patients (28 males; 31 females; with a median age of 45 years, range from 3 to 80 years) with de novo AML (n=55) or secondary AML (n=4) were screened for presence of FLT3-ITDs using reverse transcription polymerase chain reaction (RT-PCR) and gel electrophoresis. Subclassification of the patients with de novo AML according to French-American-British (FAB) criteria was as follows: M1 (n=5), M2 (n=14), M3 (n=13), M4 (n=17), and M5 (n=6). RESULTS: FLT3-ITDs were detected in 16/59 (27%) of the patients, including 14/55 (25%) of the patients with de novo AML and 2/4 (50%) of the patients with secondary AML. The frequency of FLT3-ITDs positivity was higher in the FAB M3 (5/13; 38%), M4 (6/17; 35%) and M5 (2/6; 33%) subtypes as compared with M1/M2 (1/5; 20%) and M2 (0/14; 0%). There were significant differences in the mean white blood cell count (WBC) between FLT3-ITDs(+) and FLT3-ITDs(-) patients - mean 69.6 [+/-94.6x10(9)/l] versus 28.6 [+/-44.3 x10(9)/l], respectively (p=0.027). There was no correlation between patients` FLT3-ITD status and gender and age. CONCLUSIONS: In this pilot study we demonstrate that the FLT3/ITDs is a frequent molecular lesion in Bulgarian AML patients, and the presence of FLT3ITDs are associated with M3/M4/M5 FAB morphology and with high white blood cell count. The incidence of FLT3-ITDs observed in our study (27%) is similar to that reported before.

Abstract: 411 Poster: 318

HEMATOPOIETIC CHIMERISM ANALYSIS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IN PEDIATRIC CASES

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Allogeneic hematopoietic stem cell transplantation represents the treatment of choice for the patients with malignant and non-malignant hematologic diseases. Monitoring of hematopoietic chimerism could be achieved by fluorescent in situ hybridization (FISH) of X/Y probes, only in sex mismatched donor and recipient cases. In this study, PCR based analysis of polymorphic short tandem repeats (STR) was applied in a total of 24 children and CEP X/Y probe FISH analysis was performed in 34 children after bone marrow transplantation due to different diagnosis including beta-thalassemia, immunodeficiency, adrenoleukodystrophy, juvenile myelomonocytic leukemia, aplastic anemia, Fanconi aplastic anemia, osteopetrosis, acute myeloid leukemia, acute lymphoblastic leukemia and non-hodgkin lymphoma. Analyses were performed at different time points after transplantation, with a total of 196 samples studied. For STR analysis, fifteen tetra-nucleotide repeat loci (CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX, vWA) and the Amelogenin gender determining marker in a single PCR amplification were amplified by using AmpFlSTR(r) Identifier TM PCR Amplification Kit. STR polymorphisms were analyzed on ABI PRISM(r) 310 Genetic Analyzer system. Calculations were based on relative areas of donor and recipient peaks. Patients were evaluated with regard to the cytogenetic, molecular cytogenetic and molecular results and clinical findings. Of the 58 patients, complete chimerism in 29 cases (50%), partial chimerism in 24 cases (41.4%) and no chimerism in 5 cases (8.6%) was observed in an average follow up time of 13.8 months. Our conclusion is that after stem cell transplantation, STR based analysis of chimerism and X/Y probe FISH analysis, are important tools in post-transplant hematopoietic chimerism quantification, leading to early detection of graft failure, relapse, donor cell derived disease as far as minimal residual disease.

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A POLYMORPHISM (LYS751GLN) OF THE DNA REPAIR GENE XPD CORRELATES WITH RISK OF HEMATOLOGICAL MALIGNANCIES IN TURKISH POPULATION

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Aim: The xeroderma pigmentosum group D (XPD) gene encodes a DNA helicase that functions in nucleotide excision repair (NER) of chemotherapy-induced DNA damage, the efficiency of which is predicted to be affected by a lysine to glutamine variant at codon 751 (A›C, Lys›Gln). We aimed to investigate whether the XPD 751 polymorphism is involved in the susceptibility to different hematological malignancies. Methods: Lysine›Glutamine substation in codon 751 of exon 23 in XPD gene polymorphism was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in patients who were hospitalized between the period of 1 July 2004 and 1 June 2005; 24 patients were diagnosed as acute leukemia (7 ALL, 17 AML), 8 patients had multiple myeloma and 18 patients had non-Hodgkin lymphoma. Fifty healthy subjects formed control group. The frequencies of genotypes AA, AC and CC in the patients and control groups were compared Results: Median age of the patients with hematological malignancies and control group were 45 years (16-73) and 38 (16-52), respectively. The distribution of CC genotype (codon 751 polymorphism) and C allele frequency in XPD gene were significantly lower in the acute leukemia group compared to the control group (p= 0.047 and 0.022 respectively). But, there were no significant difference in multiple myeloma/ NHL patients compared to the control groups. Conclusion: This preliminary data suggested that XPD variant allele C/C may be associated with reduced DNA repair capacity and increased leukomogenic risk. At the same time, a protective effect of the CC genotype in XPD gene was confirmed in this study. However, due to the small sample size, further studies are needed to evaluate these associations within acute leukemia and in other populations.

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CYTOGENETIC FINDINGS OF ADULT ALL CASES

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The results of cytogenetic examinations of 62 bone marrow and 15 peripheral blood specimens of ALL cases that were referred to the cytogenetic laboratory of Medical Biology Department, Cerrahpaşa, a Medical Faculty, İstanbul University; by the Division of Hematology, Department of Internal Medicine, Cerrahpaşa, a Medical Faculty, İstanbul University, are presented. 45% of the patients were female and 55% were male. The average age of the patients was 31.61. Cytogenetic results were obtained from 42 of the bone marrow and 13 of the blood specimens. Only normal karyotypes were observed in 9 of marrows and in 6 of blood samples. 33 of marrows and 7 of blood samples showed clonal and nonclonal numerical and structural chromosome abnormalities. The clonal findings that were observed in the bone marrow samples are seen in the Table 1. Cytogenetic, molecular cytogenetic and molecular genetic analyses are important in identifying prognostic markers in ALL. Furthermore t(9;22) is almost exclusively found in B-precursor ALL t(9;22)(q34;q11)(Philadelphia (Ph1) chromosome), which is known as the most frequently seen cytogenetic abnormality in adult ALL, was observed in bone marrow samples of 4 cases (two cases-KML&61614;ALL, one caseALL&61614;KML,one case ALL), and in blood sample of one case (common B ALL) in this study. The translocation t(4;11)(q21;q23), with the corresponding fusion gene ALL1-AF4 is found %5 of all adult ALL patients. In this study, one case demonstrated t(4;11). All other nonrandom translocations in adult ALL show an incidence of less than 5-10%. The translocation t(1;19)(q23;p13) was observed in bone marrow of only one case in this study that is also commonly observed in ALL cases. -20 is one of the most frequently seen abnormalities in this study. As indicated in the previous studies, the most frequent deletions and point mutations in ALL involve gene regions with a functional role as tumor suppressor genes. However, in this study, a lot of additional chromosome aberrations are observed and the results suggest that these regions involve oncogenes. All results obtained in this study are compared with literature and discussed in detail in the poster.

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A PRELIMINARY STUDY OF TELOMERASE ACTIVITY EVALUATION IN CHILDHOOD AND ADULT-ONSET HEMATOLOGICAL MALIGNANCIES

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The ends of eukaryotic chromosomes are termed as telomere. Human telomeric DNA consists of a tandemly repeated sequence (TTAGGG)n, and shortens in each cell division. It is synthesized and repaired by an enzyme called telomerase, particularly in germ cells and stem cells. Telomerase activity has also been reported to be associated with the hematological malignancies, and may be used in the diagnosis and monitoring those diseases. Many studies have been established to reveal the importance of telomerase activity in adult onset malignancies, however no study has been reported evaluating the hTERT mRNA ratios comparing the hTERT mRNA changes regarding the age in hematological malignancies. We aimed to evaluate the hTERT mRNA ratios in the patients with leukemia and myelodysplastic syndrome from different ages, and to compare those ratios with the prognostic factors described previously. We also investigated hTERT mRNA ratios in adults with multiple myeloma (MM). The online real-time reverse transcriptase PCR was used for the quantification of hTERT mRNA in peripheral blood and bone marrow in 15 cases (Childhood: 13, Adult: 2) with acute lymphoblastic leukemia (ALL), 7 cases (Childhood: 2, Adult: 5) with myelodysplastic syndrome (MDS), 5 cases (Childhood: 2, Adult: 3) with acute myeloblastic leukemia (AML), 4 cases with MM. Cytogenetic analyses were available in 15 patients. In all cases with acute leukemia and in MDS, peripheral blood (PB) hTERT mRNA levels correlated significantly with bone marrow (BM) hTERT mRNA levels. Higher hTERT mRNA levels were observed in ALL patients followed by MDS and AML patients. Average of BM and PB hTERT mRNA 3.55 in 6.65 and 5.00 191.05 in ALL, 7.33 175.62 and 128.20 ratios were 162.84 18.06 in MDS. The average bone marrow hTERT mRNA was 22.02 and 20.20 AML, 29.86 2.07 in MM patients. No correlation was observed between the hTERT

mRNA 1.60 levels and age. The known prognostic factors were not significantly correlated with the telomerase activity in all malignancy groups. The preliminary results of this study has three important conclusions, first there is a correlation between the hTERT mRNA levels of BM and hTERT mRNA levels of PB in hematological malignancies; secondly hTERT mRNA levels were not influenced by the increasing age in all hematological malignancies; thirdly known prognostic factors described previously in ALL are not correlated with the telomerase activity. This is a preliminary study and the final results of this study are expected to contribute to the literature regarding the association between hTERT mRNA ratios and the prognosis of acute leukemia, MDS and MM in different ages, subgroups of acute leukemia and cytogenetic abnormalities.

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COMPARISON OF FISH-DIAGNOSED RESULTS OF MDS CASES WITH CYTOGENETIC RESULTS

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Cytogenetic findings are important parameters in the diagnosis of myelodysplastic syndrome and its prognosis. In respect to karyotypes, the MDS patients can be classified as patients with good, moderate or severe prognosis. Loss of 5q or 20q or Y chromosome alone is related with good prognosis whereas chromosome 7 aberrations and complex karyotypic abnormalities are related with the severe prognosis. Other chromosome abnormalities that are not in these two groups are grouped as aberrations related with moderate prognosis. Of 30 cases, 18 were RA, 1 was RARS, 7 were RAFB, 2 were KMML, 2 were RAFB-t. All cases were primary MDS. Classical cytogenetic (GTG bands) and molecular cytogenetic analyses were performed in all cases. In the molecular cytogenetic analyses, LSI EGR1/D5S23, D5S721 Dual Color Probe; LSI D7S486(7q31)/CEP7 Probe, CEP8 Probe, LSI MLL Dual Color Probe and LSI D20S108 (20q12) probe were used. Of 30 MDS cases, no cytogenetic analyses could be performed in 8 cases (26.67%) whereas normal karyotypes were revealed in 13 patients (59.06%), single chromosome aberration was seen in 7 (31.82%), double abnormalities were detected in one case and one case had a complex karyotype. In the FISH analyses, deletions of 5q31 region, 7q31 region, 7q31 and CEP 7 regions, MLL gene region and 20q were seen in 4(13%), 2(6%), 1(3%), 3(10%) and 3(10%) cases, respectively. In three cases, trisomy 8 was detected. Based on chromosome constitutions of the cases, 14 could be classifed as the cases having the disease with good prognosis because 13 had normal karyotypes and one had only Y chromosome deletion. Of the cases, one was classified in to the poor prognostic group because of his complex karyotype. The remainings were in the intermediate prognostic group since trisomy 8 (2 cases), chromosomes 3 (2 cases) and 19 (2 cases) abnormalities were revealed. The classification of the cases in respect to prognosis of the disease were changed because of their molecular cytogenetic results. In the FISH analysis, two cases had 5q deletions and they were evaluated as the cases having the disease with cytogenetically determined Y chromosome deletion, additional abnormalities including chromosomes 5q and 7q deletions were determined and therefore the prognostic group of this case was changed from the good prognosis to poor prognosis. Of the cases without karyotypic analysis and therefore prognostic evaluation, two were classified into poor prognostic group because of their abnormalities detected by FISH analysis: one case had 5q deletion and monosomy 7 whereas the other showed both 7q and 20q deletions. In two cases, the trisomy 8 diagnosis was supported by the FISH and they were still in the intermediate prognosis group but in one of them, additional abnormality including loss of 11q23 was seen. Two cases were in the good prognostic group because of their normal karyotypes, but chromosome 20q deletion was determined in these cases and therefore their prognostic group was changed from good prognostic group into intermediate one. Molecular markers, that are important in evaluation of prognosis of the disease, should be analysed by the FISH. Molecular cytogenetic analyses have ability to show cryptic deletions and chromosomal rearrangements not detected by the conventional karyotype analysis. We concluded that prognostic evaluation of the disease with high accurate rate can only be performed by using the combination of cytogenetic and FISH analyses.

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MINOR ANOMALIES IN CHILDREN WITH HEMATOLOGICAL MALIGNANCIES

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The association of childhood malignancy with major genetic anomalies (e.g., Down Syndrome, Beckwith-Wiedemann syndrome, WAGR syndrome) has been well described. Recent advances in molecular genetics have clarified the common genetic background in an increasing number of such associations. At the same time, a possible correlation between childhood malignancies and non-syndromic congenital anomalies has been investigated in several studies. Thus, we aimed to determine the prevalence of minor malformations in children with hematological malignant diseases. Between January 2000 to December 2004 109 children with leukemia and 109 healthy age- and sex-matched children as a control group were included in this study. The prevalence of 62 well-defined minor malformations was determined in both 109 patients and controls. Pearson correlation test and x2 tests were used for statistical analysis. The demographic data and the results of minor malformations observed in both patients and controls are shown in Table 1. Concerning the cumulative data, 64.22% of patients and 26.6% of the controls had at least one minor malformation ($p<0.05$). Pigmented nevi (15.31%), café-au-lait spots (18.01%), confluent eyebrows (8.1%), simian crease (5.4%) clinodactyly (4.5%) were the most common malformations observed in our patients. However, only pigmented nevi and café-au-lait spots were statistically more frequent in patients compared with controls ($p<0.05$). Our findings contribute to the understanding of the role of genetic factors in children with hematological disorders. This study suggests the need for prospective study in children with hematological disorders, which focuses on cutaneous lesions, because this may provide direction for future studies of genetic influences on hematological disorder risk.

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PROGNOSTIC CYTOGENETIC MARKERS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA: CASES FROM MANSOURA, EGYPT

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Objective. To evaluate children with acute lymphoblastic leukemia (ALL) showing resistance to immediate induction chemotherapy in relation to conventional and advanced cytogenetic analysis. Subjects and methods. This work was conducted on 63 ALL children (40 males and 23 females) with age range 4.5 months -16 years (mean = 7.76 years). They included 37 cases attained true remission and 26 complicated by failure of remission, early relapse or death. They were subjected to history, clinical examination and investigations including CBC, BM examination, karyotyping, FISH for translocations and flowcytometry for immunophenotyping and minimal residual disease diagnosis. Results. Cases aged < 5 years; male sex with organomegaly had better remission although statistically insignificant. Initially low HB < 8 gm/dl, high WBCs and platelet counts > 50.000/mm3 also showed better but non-significant remission rates. Most of our cases were L2 with better remission compared to other immunophenotypes. Forty informative karyotypes were subdivided into 15 hypodiploid, 10 pseudodiploid, 8 normal diploid and 7 hyperdiploid cases; the best remission rates were noticed among the most frequent ploidy patterns. Chromosomes 9, 11 and 22 were the most frequently involved by structural aberrations followed by chromosomes 5, 12 and 17. Resistance was noted with aberrations not encountered among remission group; deletions involving chromosomes 2p, 3q, 10p and 12q; translocations involving chromosome 5; trisomies of chromosomes 16 and 21; monosomies of 5 and X and inversions of 5 and 11. Conclusions. Some cytogenetic and molecular characterizations of childhood ALL could add prognostic criteria for proper therapy allocation.

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MONOSOMY X AS THE SOLE CYTOGENETIC ABNORMALITY IN A CHILD WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Specific clonal abnormalities are well recognized in hematologic malignancies. The nonrandom gains and loses of autosomes have been reported in association with acute myeloid and lymphoblastic leukemia. This finding is one of the factors that determines the classification, treatment and prognosis. Loss of an X chromosome as the sole acquired cytogenetic abnormality is rare and usually found in myeloid hematological malignancies. Only six cases of acute lymphoblastic leukemia (ALL) with this abnormality at the initially diagnosis have been reported previously. There was no apparent association between monosomy X and spesific hematological disorder. We identified a new pediatric patient diagnosis with ALL and monosomy X as the sole cytogenetic abnormality. A 3-year old girl was admitted to our hospital with weakness, and spontaneous ecchymosis. She had no significant past medical history. Peripheral blood revealed white blood cell (WBC) of 10x109/l, hemoglobin 10.6 g/dl, and platelets 53x109/l. A WBC differential count showed 72% blast and 28% lymphocytes. Bone marrow examination showed predominantly FAB L1 type lymphoblast infiltration. Immunophenotyping with flow cytometric analysis displayed CD10, CD19, TdT, and cIgM positivity. A diagnosis of B-precursor ALL was established. No metaphases could be obtained in cytogenetic study. Fluorescence in-situ hybridisation (FISH) using a centromeric probe for X-chromosome found only one signal in 166 out of 200 interphase nuclei examined. ALL BFM 95 chemotherapy protocol was initiated, achieving complete remission after 15 days. No central nervous system disease was demonstrated. The patient is currently alive and in complete remission and still receiving chemotherapy. Isolated monosomy X was generally reported in older patients with myeloid leukemia and lymphoid malignancies with this cytogenetic abnormality is relatively rare. These patients who reported before were generally young and had low or normal white cell count at the diagnosis similarly as our patient. To our knowledge, there are only 4 pediatric cases with ALL and monosomy X. Our case might add to current literature. Loss of all or part of a chromosome may contribute to the neoplastic process by loss of the site of a tumor suppresor gene.

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RAPID TRANSFORMATION OF ATYPICAL MYELOPROLIFERATIVE DISORDER WITH CONSISTENT T(8;13) TO ACUTE LYMPHOBLASTIC LEUKEMIA: A CASE REPORT

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There are several reported cases of an atypical myeloproliferative disorder in which the atypical cells have a consistent t(8;13) translocation. There have been different breakpoints reported in different patients, but the most frequently seen is the t(8;13)(p11;q12). Main characteristics of this disorder can be described as indolent course and rapid transformation to malignant hematological diseases mostly to acute lymphoblastic leukemia and non-Hodgkin lymphoma. Since there have not been enough data to establish appropriate treatment, therapy after transformation remains to be clarified. But allogenic bone marrow transplantation has been offered as the only curative therapeutic option. We hereby present a case admitted to hospital because of weakness and fatigue. No organomegaly was detected and laboratory analysis showed leukocytosis with prominent myelocytes and metamyelocytes, anemia and normal platelet count. Leukocyte alkaline phosphatase score was found to be 2%. Cytogenetical analysis showed no Philadelphia chromosome and t(8;13)(p12;q12) in 2 of 16 metaphases. Patient was diagnosed as atypical chronic myeloproliferative disease with t(8;13). Supportive treatment with erythrocyte suspension was started and hydroxiurea was given to decrease the leukocyte count. Bone marrow biopsy revealed existence of 15 to 20% of lymphoblastic cells. Patient was hospitalized due to pallor, weakness and presence of blastic cells in peripheral blood smear at 3rd months after first complaints with the diagnosis of acute lymphoblastic leukemia. Hoelzer`s protocol was given and after first cycle, remission was obtained. The patient has now been under treatment and aoutologous peripheral hematopoietic stem cell transplantation would be treatment option for this patient since she has no full-match sibling donor.

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MILESTONES IN MOLECULAR HEMATOLOGY: THE SAUDI EXPERIENCE

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Over the last decade molecular diagnostics technology has developed dramatically from the most laborious, time consuming Southern Blot Methology through the revolution of Polymerase Chain Reaction (PCR) technology to the most reliable, fast and contamination free molecular analyzer, the Real-Time Quantitative RT-PCR. The Section of Hematology, Department of Pathology and Laboratory Medicine at King Faisal Specialist Hospital and Research Center has shared this experience during the last 10 years with more than 8038 samples submitted for the analysis of different gene rearrangements, fusion gene transcripts and gene mutations including Ig heavy chain gene rearrangement for B-cell malignancies, T-cell receptor gamma chain gene rearrangement for T-cell malignancies, BCR/ABL-P210 and P190 fusion gene transcripts for Chronic Myeloid Leukemia (CML) and Philadelphia Positive Acute Lymphoblastic Leukemia (ALL) respectively, PML/RARA fusion gene for Promyelocytic Leukemia (PML), AML1/ETO for AML (M2) with t(8;21), CBFB/MYH11 for AML (M4E0) with inv (16), BCL-2 for follicular lymphoma and BCL-1 for mantle cell lymphoma. Hence, most molecular assays are qualitative in nature, quantitative assays are deem necessary in the monitoring and follow-up of Minimal Residual Disease (MRD) in Leukemia and Lymphoma, and proved in our experience to serve as an essential tool to confirm complete remission (CR) post-chemotherapy and Bone Marrow Transplantation (BMT), and to detect signs of early relapse for proper clinical intervention. In this presentation, we share our experience and guidelines in Molecular Hematology and concentrates on Molecular monitoring of CML patients on Gleevec.

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CARRIER DIAGNOSIS OF HEMOPHILIA A BY THE ARMS METHOD

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The human factor VIII gene, with 186 Kb length composes about %0.1 of the X-chromosome. This gene with 26 exones after splicing its 25 introns, produces mRNA with 9 Kb length after translation it produce protein with 265 KD comprising 2351 amino acids. The disorder of factor VIII gene includes point mutation, deletion, duplication and inversion which lead to a bleeding disorder. Hemophilia-A are studied. A blood sample was drawn from affected individuals and after DNA purification by proteins-K and boiling methods with ARMS Technique (one of PCR techniques), the situation of factor VIII gene for number 18 and 24 exones were studied. For this reason specific primers designed and synthesized by DNA synthesizer. DNA derived from samples of patients were treated with specific primers then amplified by a specific method with 25 cycle thermocycle instrument. After electrophoresis through agarose gel, the product of PCR was analysed by UV transluminator. In this study the affected individuals because of mutation in 1941 situation of number 18 and 2209 of number 24 exones were deter-mind. After determination of mutation, carrier detection were performed which this procedure was successful and used first for carrier detection in Iran.

Abstract: 422 Poster: 329

IL-10 AND ADIPONECTIN LEVELS IN PATIENTS WITH LYMPHOMA AND CLL

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Background: There is increasing data that adipose tissue and immunologic events are closely related. Adiponectin is the abundant protein within adipocytes. It has been suggested that adiponectin had a negative regulator role in hematopoiesis, and inhibited predominantly the proliferation of myelomonocytic lineage cells. In addition, it is known that adiponectin has strong immunosuppressive effects, and induces antiinflammatory cytokines like IL-10. It was reported that IL-10 level increased in lymphoma and CLL patients; and this was associated with poor prognosis. Aims: In our study, we evaluated adiponectin and IL-10 levels in lymphoma and CLL patients; and determined their relationship with the patients`

clinical features. Methods: We included newly diagnosed or relapsed 43 lymphoma and 23 CLL patients into the study. The control group composed of 17 healthy subjects within the same age range. Of all lymphoma patients, 18 had Hodgkin lymphoma (HL), 25 had nonHodgkin lymphoma (NHL). Patients with documented infection within the last 2 weeks; those with febrile neutropenia, sepsis, any organ failure; patients with hypertension or diabetes were excluded. The patients` clinical and laboratory data, body-mass indexes (BMI) were recorded down from the hospital files. Serum adiponectin and IL-10 levels were determined by ELISA. Results: The groups were similar in age, sex, lipid profiles and BMI. Hemoglobin level in the control group ($p<0.01$), and leucocyte count in CLL group ($p<0.001$) were higher than in other groups. CRP level in lymphoma patients was higher than in controls ($p=0.03$). The groups did not differ in their adiponectin level ($p>0.05$). IL-10 level in lymphoma group was significantly higher than in CLL and control groups (p values=0.006 and 0.005). The clinical and laboratory parameters; IL-10 and adiponectin levels of the groups are seen in the Table 1. When HL and NHL patients were compared: it was seen that HL patients were younger (44.3±19 vs. 63.2±13.7, $p=0.001$), IL-10 levels were similar; and adiponectin level was significantly higher in the NHL group (15.6±7.1 vs. 9.9±7.4, $p=0.02$). The adiponectin level in the lymphoma group had a positive correlation with age ($r=0.35$, $p=0.02$), and a negative correlation with the platelet count ($r=-0.42$, $p=0.006$). Conclusions: IL-10 level was increased in lymphoma patients; and adiponectin level was increased in NHL patients when compared to HL patients associated with age. We might suggest that IL-10 and the adipocyte-origin adiponectin play possible roles especially in immunoregulation and hematopoiesis in lymphomas.

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DRUG RESISTANCE INHIBITION IN LEUKEMIC CELLS BY NANOPARTICLES

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Background: Acute myeloblastic leukemia (AML) is the most common leukemia in adults. Although the clinical outcome of acute leukemia (AL) has been proved by recent progress in chemotherapy, it is still a difficult disease to treat. One major problem is the emergence of leukemic blast cells that are resistant to anticancer drugs. This phenomenon is named multidrug resistance (MDR). A representative cause of MDR is the expression of the MDR1 gene and its product, P-glycoprotein (Pgp) on the cell surface membrane. Expression of Pgp is associated with its resistance to several types of antineoplastic agents such as Anthracyclines, Taxanes, Epipodophyllotoxines and vinca alkaloides. Aim: In this study we tried to reverse MDR phenotype in leukemic cells by antisense in complex to nanoparticle against MDR1 gene. Materials & Methods: In the present study, the Pgp expressing cell line was established from parental K562 (Erythroleukemia) cell line with increasing concentrations of Doxorubicin starting with 5 ng/ml. The Pgp expressing cell line was obtained in 20 ng/ml and named KDI/20. In order to reverse the MDR Phenotype due to Pgp, expression, four different sequences of sense, antisense and one random sequence with phosphorothioate (PTO) modification (PS-ODN) against MDR1/mRNA was synthesized. They were treated on the KDI/20 in combination with two non-viral vectors: 1) Fugene 6 transfection reagent (cationic lipid) and 2) polyethylenimine (PEI, cationic polymer). The effect of PS-ODN was assessed at the cellular level by flowcytometry (for Pgp detection), Rhodamin 123 assay (for functional assessment of Pgp), RT-PCR at the molecular level (for MDR1/mRNA detection) and MTT assay (in order to assess the sensitivity of cells to Doxorubicin). Results: The results showed a decrease in the percentage of Pgp protein and MDR1/mRNA exprestion and an increase in the accumulation of Rh123 and drug sensitivity of cells to Doxorubicin by antisense I and III. Summary: The results showed that antisense can reverse MDR Phenotype at transcription level and the PEI vector is more efficient than cationic lipid.

Abstract: 424 Poster: 331

BETA-GLUCAN REDUCES METHOTREXATE-INDUCED INTESTINAL DAMAGE IN RATS

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Background: Methotrexate (MTX), a folic acid antagonist, is widely used as a cytotoxic chemotherapeutic agent for leukemia and other malignancies. Enteritis following MTX administration poses an important problem in limiting the therapy`s effectiveness and negatively effecting the patients` quality of life. Beta-glucan is a polysaccharide which is presumed to have antiinfective and antioxidant properties and immunoregulatory effects. This investigation elucidates the role of free radicals in MTX-induced enteritis and the putative protective effect of beta-glu-can. Materials and methods: Following a single dose of methotrexate (20 mg/kg in saline), both sexes of Wistar albino rats were administered either saline or beta-glucan (50 mg/kg) for 5 days. In other rats, physiological saline (control group) or beta-glucan (50 mg/kg) was injected for 5 days, following a single dose of saline injection. Each group consists of 8 rats. Rats were decapitated and ileal segments were fixed for light microscope examination. To evaluate the oxidative damage in the tissue samples, the levels of malondialdehyde (MDA), the end product of lipid peroxidation and glutathione (GSH), a key antioxidant were also determined. Myeloperoxidase activity in the tissue samples was measured as an indicator of neutrophil infiltration in the damaged tissue. The occurrence of fibrosis in the tissue was determined by measuring the collagen levels in the tissue sections by histologic methods. Results: Our results showed that in the saline-treated MTX group, the ileal MDA and collagen levels were increased significantly ($p<0.001$), while the GSH levels were decreased. The significant increase ($p<0.001$) in the MPO activity supports the role of neutrophils in the damage. These changes induced by MTX were reversed in beta-glucan-treated group ($p<0.05$-0.01). Conclusion: The present study demonstrates that beta-glucan administration is cabaple of reversing MTX-induced intestinal damage and thus may be beneficial in ameliorating the symptoms of chemotherapy-induced enteritis, while increasing the effectiveness of therapy through its immunomodulator effects.

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ENHANCED EXPRESSION OF THE LOCAL HEMATOPOIETIC BONE MARROW RENIN-ANGIOTENSIN SYSTEM IN POLYCYTHEMIA RUBRA VERA

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Local bone marrow (BM) renin-angiotensin system (RAS) affects physiological and pathological haematopoiesis, including erythropoiesis. The RAS was initially postulated to influence erythropoiesis since the demonstration of the hematopoietic side effects of RAS blockers. Elucidation of the activity of the local autocrine/ paracrine RAS-mediated regulation of the clonal erythropoiesis of polycythemia rubra vera (PV) is both (patho)biologically and clinically important, since the angiotensin peptides represent a molecular target in pathological red blood cell production. Bone marrow aspirate samples were obtained from three patients with PV (1 male, 2 females) and six patients with other nonmalignant hematological disorder (2 males, 4 females). The quantitative expression of the mRNAs of the major RAS components, namely angiotensin converting enzyme (ACE, CD143), renin, and angiotensinogen in bone marrow samples were measured by quantitative RT-PCR to search the activity of local bone marrow RAS in polycythemia rubra vera (PV) in comparison to normal erythropoiesis. The presence of CD143 was also investigated by flow cytometry at the same bone marrow samples (Figure 1). The characteristics flow cytometric analysis and relative gene expression of quantitative real-time PCR is depicted in. Increased local syntheses of the major RAS components have been identified via demonstrating their corresponding mRNAs in the bone marrow of the patients with PV. Similar flow cytometric analysis (CD 143+, CD 34+, CD 143+/CD 34+) for PV and control

patients was observed. Our findings indicate the up-regulation of the local bone marrow RAS together with the down-regulation of the cell surface ACE receptors in the autonomous neoplastic clonal erythropoiesis of PV. Further experimental and clinical studies should focus on the elucidation of the critical erythropoietic mechanisms in relation to the molecular and cellular basis of the local bone marrow RAS for the better management of the patients with PV.

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OVER-EXPRESSION OF ANGIOTENSIN CONVERTING ENZYME (CD143) ON LEUKEMIC BLASTS AS A CLUE FOR THE ACTIVATED LOCAL BONE MARROW RAS IN AML

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Local bone marrow renin-angiotensin system (RAS) is an autocrine-paracrine system affecting hematopoiesis. Angiotensin II type 1a (AT1a) receptors are present on the CD34+ hematopoietic stem cells. Angiotensin II stimulates the proliferation of bone marrow umbilical cord blood hematopoietic progenitors. There are preliminary data that local RAS might also be involved in leukemogenesis. ACE hyperfunction may lead to the acceleration of negative hematopoietic regulator peptide, AcSDKP, metabolism, which in turn lowers its level in the bone marrow microenvironment, finally removing the anti-pro-liferative effect of AcSDKP on the hematopoietic cells and blasts. Renin expression could have a role on the leukemia development and angiotensin may act as an autocrine growth factor for acute myeloid leukemia (AML) cells. The aim of this study is to search ACE (CD 143) surface antigen by flow-cytometric analyses on the leukemic blast cells taken from the bone marrow of the patients with AML. Bone marrow aspiration materials and peripheral blood samples were obtained from 11 patients with AML (8 males, 3 females; aged 46 (range 26-67) years) and 6 patients with non-malignant hematological disorders (4 males, 2 females; aged 56 (range 22-71) years). ACE (CD 143), surface antigen was shown to be over-expressed in leukemic myeloid blast cells. ACE is positively correlated with bone marrow blast count. Elucidation of the pathological activity of the local RAS-mediated regulation of the leukemogenesis is both pathobiologically and clinically important, since the angiotensin peptides represent a molecular target in the disease management.

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CYCLIN D1 EXPRESSION IN ACUTE LEUKEMIA

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Back ground Disorders of the cell cycle regulatory machinery play a key role in the pathogenesis of cancer. Over expression of cyclin D1 protein has been reported in several solid tumors and certain lymphoid malignancies, but little is known about the involvement of cyclin D1 in acute leukemia. Patientsl and methods In this study, we analyzed the expression of cyclin D1 at protein level in, 40 AML, 10 ALL, and 11 normal controls using flow cytometry. Results The expression of cyclin D1 was not significantly different in AML group as compared to normal controls. On the other hand, over expression of cyclin D1 was evident in ALL group (4/10) as compared to that in healthy control. The ALL cases with cyclin D1 over expression were significantly correlated to blast cell counts in the peripheral blood and bone marrow but not with hemoglobin level, WBCs, and platelets count. The ALL group with lymphadenopathy and organomegaly express significantly higher cyclin D1 over expression as compared to those without. Conclusion: the biological value of cyclin D1 over expression might be different in AML and ALL

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THE CYTOTOXIC AND APOPTOTIC EFFECT OF 6ALPHA METHYL-PREDNISOLONE ON HL-60 CELLS

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BACKGROUND: 6.-methylprednisolone is a steroid compound which effects the leukemic blast to differentiate and to cell death in patients with acute myeloblastic leukemia (AML). In the recent study, we tested in-vitro apoptosis in HL-60 (human acute myeloblastic leukemia cell line) cells which is best model for leukemogenesis experiments. For this purpose, annexin-V FITC staining cells were analysed by flow cytometry. AIM: To evaluate the effects of methylprednisolone on human acute myeloblastic leukemia cell line HL-60 and to find the in-vitro apoptotic dose of methylprednisolone for these cells. METHODS: HL-60 cells were cultured with 10-4-10-3 M consantration 6.-methylprednisolone for 12-48 hours. Cytotoxic effects of methylprednisolone in these cells were analysed either tyripan blue dye exclusion method or annexin-V FITC by flow cytometric method. Early and late apototic effects of the drug were tested with Annexin-V FITC by flow cytometrically. RESULTS: We observed the cytotoxic effects of 6.-methylprednisolone on HL-60 cells at a concentration of 10-3 M in 48th hours by evaluating tyripan blue dye exclusion assay and annexin-V FITC staning analysis. In HL-60 cells, the treatment of 6.-methylprednisolone for annexin-V FITC flow cytometric test, we showed that the early (primer) apoptosis was at a concentration of 10-3 M 6.-methylprednisolone in 12th hours and the late (secondary) apoptosis was at a concentration of 10-3 M 6.-methylprednisolone in 24th hours. Statistic analysis showed that high dose (10-3 M) 6.-methylprednisolone significantly affect of apoptotic patway on human acute myloblastic leukemia cells. CONCLUSION: In the present study, we evaluated the in-vitro cytotoxic and apoptotic dose of 6.-methylprednisolone on HL-60 cell line. Our observations confirm that the high dose (10-3 M) 6.-methylprednisolone treated HL-60 cells go to apoptosis in 24 hours (in the early hours) and go to necrosis in 48 hours (in the late hours) compared to HL 60 cells without drug.

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RECOMBINANT HUMAN GRANULO CYTE COLONYSTIMULATING FACTOR (rhG-CSF) PROMOTES IN VITRO PLATELET AGGREGATION

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Backgrounds RhG-CSF is increasingly used for stimulation of granulopoiesis and also in healthy donors for allogeneic transplantation. However, possible association between thrombosis and rhG-CSF administration has been reported. Aim To clarify this situation, in this study, we investigated the effect of rhG-CSF on platelet aggregation in whole blood of 10 healthy volunteers. Method Three concentrations of rhG-CSF solution were prepared. Each concentration of rhG-CSF solution and the diluent not including rhG-CSF for control were incubated with whole blood. Incubation with rhG-CSF solution would result in blood levels of 0.1, 1.0 and 10 ng rhG-CSF/ml. After incubation, aggregation responses were evaluated with ADP (5 and 10 µM) and collagen (2 and 5 µg/ml) in whole blood. Results When compared to control, preincubation with all dilutions of rhG-CSF augmented aggregation of platelets induced by ADP and collagen in a statistically significant manner. And also, there was a relationship between rhG-CSF concentration and platelet aggregation response. Conclusion rhG-CSF administration may lead to possible hypercoagulable state related to stimulation of platelets.

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THROMBOPOIETIN

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Thrombopoietin (TPO) also known as c-Mpl ligand is a primary regulator of the proliferation and maturation of Megakaryocytes as well as of platelet production. The primary target cell population for TPO in bone marrow comprises Megakaryocyte progenitors at the late stage of differentiation, such as colony-forming unit-megakaryocyte (CFU-MK) expressing GpIIb/IIIa. The entire human TPO gene was mapped to chromosome 3q26-q28. Originally shown to span 6.2 kb and contain six exons and five introns. However, an additional exon was subsequently detected upstream of exon 1 produced in hepatocytes. TPO receptor (c-Mpl) binds the NH2-terminal, two disulfide bonds in the NH2-terminal domain are essential for the biological activity in human. Glycosylated COOH-terminal domain is thought to

be necessary for survival of TPO in the circulation. In addition, the sugar chains have recently been shown to be important for the secretion of TPO from cells. TPO is produced in hepatocytes, and to a lesser extent are found in the kidney, brain and testes. TPO secreted at sites of production enters the bloodstream and is transported to bone marrow, where it acts directly on Megakaryocyte progenitors to influence platelet production. Serum concentration of TPO, which ranges from 0.33 to 1.72 fmol/ml. Potential clinical uses of Thrombopoietin Human Thrombopoietin studies in a wide range of areas have only recently been started using both the full-length, glycosylated Thrombopoietin and the truncated, PEG-Thrombopoietin. None of these studies is sufficiently mature to have provided data for interpretation yet. so Potential clinical uses of Thrombopoietin may include; Chemotherapy of solid tumors, Chemotherapy of acute Leukemias, Radiation therapy, Bone marrow transplantation, Aplastic anemia, Bone marrow failure states, Myelodysplastic syndromes, HIV thrombocytopenia, Immune thrombocytopenic purpura, Intraaor-tic balloon counter-pulsation, Cardiac surgery, Platelet apheresis. While Potential clinical risk of Thrombopoietin administration expected will be Thrombocytosis. Other side effects may include; Thrombosis Marrow fibrosis Venoocclusive disease Interaction with other growth factors Stimulate growth of Leukemic blasts Since TPO was first cloned, several recombinant TPOs have been developed for clinical evaluation. There are two of these preparations, rhTPO and PEG-rHuMGDF

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QT DISPERSION AFTER ADMINISTRATION OF RAPID INTRAVENOUS VARIOUS ANTIEMETICS IN CHILDREN PRIOR TO CHEMOTHERAPY

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Children with acute leukemia are at an increased risk of cardiac arrhythmias, from their cardiac infiltrations and cardiotoxic treatments. There are many reasons why the children with acute leukemia is at increased risk of potentially life-threatening cardiac arrhythmias. The autonomic response to chemotherapy and radiation therapy (nausea, retching and vomiting) or their biochemical ejects (vomiting-induced electrolyte disturbance) can have important implications. It is, therefore, important to ensure that any medications to-administered to the children, do not further increase the risk of cardiac complications, particularly arrhythmias. Nausea and vomiting are considered to be the most distressing and debilitating side effects of therapy, and can profoundly affect patients` quality of life. The aim of this study was to determine the effects of the rapid administration of intravenous tropisetron, granisetron and ondansetron on measures of cardiac depolarization in children receiving chemotherapy for acute leukemia, by comparing twelve-lead ECGs before (baseline) and after 2nd and 24th hours after the drug administration. The study was performed in total 75 children with acute leukemia (25 children for each antiemetic). QT dispersion was calculated as the difference between the maximum and minimum QTc in twelve-lead surface electrocardiogram lead. It was concluded that no clinically important cardiovascular side effects are associated with the administration of tropisetron, granisetron and ondansetron following first 24 hour. There are no dysrhythmic or hemodynamic changes in all patient groups.

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THE PREPARATION FOR PROMOTION ANTICANCER TREATMENT

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Introduction. Anticancer treatment can be cause of the toxic alterations. The severe toxic complications are myelosuppression and immunosuppression. The new preparation blasten decreasing toxity of chemotherapy and stimulating hemopoiesis and immunity has been developed. Methods. The preparation was obtained from a spesial strain Lactobacillus Delbrueckii by enzimatic and chemical hydrolysis. Blasten was tested preclinical studies and clinical studies in brest cancer patients. Results. It was established that preparation produced a stimulating effect on the proliferation and differentiation of cells precursors of granulomonocytopoiesis. Small doses of preparation stimulate cellular and humoral immunity. The include blasten in combined treatment brest cancer patients normalized leukocytes and erythro-

cytes in patients with cytopenia arising in the chemo- and radiotherapy treatment. The use preparation increased survival brest cancer patients, augmented the therapeutic efficacy of anticancer drugs and decrease the toxic effects of current treatment. Conclusion. The preparation blasten can be use in complex treatment cancer patients for to increase antitumoral properties drugs and to stimulate hemopoiesis and immunity.

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VASCULAR ENDOTHELIAL GROWTH FACTOR, HEPATOCYTE GROWTH FACTOR AND UROKINASE PLASMINOGEN ACTIVATOR: PREDICTIVE MARKERS FOR PROGRESS OF CHRONIC LIVER DISEASE AND DIAGNOSIS OF HCC IN HCV INFECTED PATIENTS

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Background: Human hepatocellular carcinoma (HCC) is associated with high rate of intrahepatic invasion, which requires tightly controlled extracellular matrix degradation by proteolytic enzymes including metalloproteinase, plasmin and urokinase plasminogen activator (u-PA). The tissue expression of these enzymes is induced by angiogenic factors including vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF). Aims: To assess the angiogenic factors which may mediate fibrinolysis in hepatitis C chronic liver disease and HCC; and to evaluate the potential implementation of VEGF, HGF and u-PA as possible biological markers to predict invasiveness of HCC in hepatitis C virus (HCV) infected patients. Methods: Forty-six individuals were the subjects of this study; 12 patients with chronic active hepatitis C, 12 patients with liver cirrhosis, 12 patients with HCC and 10 healthy subjects. Blood levels and hepatic expression of VEGF, HGF and u-PA were measured using enzyme-linked immunosorbent assays and immunohistochemistry using biotin-streptavidin amplified system and VEGF, HGF and u-PA antibodies. Results: Serum level of VEGF was low in patients with liver cirrhosis compared to those with chronic active hepatitis C while VEGF hepatic expression was comparable in both groups. In contrast, circulating levels and degree of hepatic expression of HGF ($p<0.05$) and u-PA ($p<0.01$) were significantly elevated in cirrhotics compared to patients with chronic active hepatitis C. Patients with HCC had marked elevation of VEGF, HGF and u-PA circulating levels and higher degree of hepatic expression compared to other groups ($p<0.01$). A positive correlation($p<0.01$) was detected between the circulating levels of VEGF, HGF and u-PA and the degree of hepatic immunoreactivity of the same factor in patients with HCC. Conclusion: VEGF, HGF and u-PA have direct relationship with angiogenesis in HCC and can be used as satisfactory markers for early diagnosis of HCC. VEGF, HGF and u-PA play an important role in the activation of the fibrinolytic system and in the pathogenesis of HCV infection.

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MESENCHYMAL STEM CELL DIFFERENTIATION DEFECT IN MALIGNANT INFANTILE OSTEOPETROSIS

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. Background: Acidification defects associated with vacuolar proton pump or chloride channel in human have been described as intrinsic defects of osteoclasts leading to defective bone resorption in patients with malignant infantile osteopetrosis (MIOP). However, extrinsic defects interfering with osteoclast function that may be attributed to mesenchymal stem cells (MSC) as a major component of bone marrow microenvironment have not been described. The growth kinetics, phenotype, differentiation capacity of bone marrow-derived MSCs were examined in three patients with MIOP two of whom underwent allogeneic hematopoietic stem cell transplantation (HSCT). In addition, the chemokine, stromal derived factor-1 (SDF-1) con-

centrations, as a component of bone marrow microenvironment, were determined in MSC culture supernatants. Methods: Bone marrow MSCs, obtained from bone marrow aspirates of healthy marrow donors and 3 patients with MIOP were expanded in culture in DMEM containing 10% fetal calf serum of selected batches. The cells were identified by morphology, adhesive characteristics, and by flow cytometry (CD105,CD29, CD166, CD73, CD44 positivity and CD45, CD14, CD34, CD3, and CD 11b negativity). The differentiation potential of MSCs towards osteocytic, chondrocytic, and adipocytic lineages were determined by defined stainings, and previously described protocols were used for induction of differentiation. SDF-1 concentration was determined by ELISA in culture supernatants. Results: MSCs from patients with MIOP grown in culture showed differentiation defects when compared to the control samples. None of the patient samples showed adipocytic differentiation after induction of adipogenesis when compared with nearly 100 % adipocyte differentiation in those from healthy marrow donors. In addition, defects in chondrocytic and delays in osteocytic differentiation were demonstrated in patient samples. The adipocytic differentiation defect was partially improved following HSCT in one patient examined who demonstrated 10-15 % adipocyte differentiation. The SDF-1 concentration in culture supernatants of one patient before and after HSCT revealed; 3039 pg/ml concentration at passage 1, and 3254 pg/ml at passage 2; which decreased to 1379 pg/ml and 729 pg/ml respectively at passages 1 and 2 after transplant. Conclusion: To our knowledge mesenchymal stem cell defects have not been described in MIOP patients or in animal models Figure 1. Liver section from a case of hepatocellular carcinoma showing positive intracytoplasmic of osteopetrosis. The novel finding of defective differentia-brownish granules in the hepatocytes for VEGF (Immunostain, DAB, ×400) tion in particularly adipogenic lineage in those patients needs further exploration that may reveal new mutations. Only partial correction of the defect after HSCT suggests that HSCT alone may not provide optimum therapeutic benefit to patients with particularly extrinsic defects. Additional cell therapy with MSC may completely correct the microenvironmental defect in patients with MIOP and may contribute to neurodevelopmental improvement.

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THE IMMUNOMODULATORY EFFECTS OF HUMAN MESENCHYMAL STEM CELLS ON CORD BLOOD T CELLS

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Mesenchymal stem cells (MSC) derived from adult BM or form several mesenchymal tissues after appropriate stimulation. Reports indicate that MSC have unique immunologic properties, making them ideal for cellular therapy. MSC are not immunogenic, they do not stimulate alloreactivity, and they escape lysis by cytotoxic T-cells and natural killer (NK)-cells. Thus, MSC may be transplantable between HLA-mismatched individuals without the need for host immunosuppression. MSCs were plated in 96-well plates (2,000/well), and cocultured for 3 days with T cells isolated from cord blood. Cord blood T cells non-cocultured with MSC acted as control group. After cord blood T cells stimulated by PHA for 60 hours, T cell proliferation was assessed by MTT assay. Expression of immunoregulatory molecules on MSC was analyzed by flow cytometry. MSC express major histocompatibility complex (MHC) class I and lymphocyte function-associ-ated antigen (LFA)-3 antigens constitutively and MHC class II and intercellular adhesion molecule (ICAM)-1 antigens upon gamma-interferon treatment but do not express CD80, CD86, or CD40 costimulatory molecules. The results showed that cord blood T cell proliferation was suppressed when 2,000 MSCs were plated each well. MSC actively inhibit T-cell proliferation, suggesting that allogeneic MSC transplantation might be accomplished without the need for significant host immunosuppres-sion.MSCs of any person may be use for modulation of immune system in hyper reactive and autoimmune diseases.

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CO-CULTURE EXPANSION OF NON ENRICHED CORD BLOOD STEM/PROGENITOR CELLS ON MSCS INDUCES MARKED IN INCREASE OF CXCR4 EXPRESSIONS

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Introduction: A number of potential cell adhesion molecules, which mediate essential cell-to-cell or cell-to-matrix interactions, are expressed on the surface of Cd34+ HPCs, such as integrins, CD44 and CXCR4. These molecules are essential to the homing process. We investigated the expression of CD44 and CXCR4 on expanded CD34+ hematopoietic progenitor cells. Material and Methods: Cord blood Cd34+ cells was expanded by using of bone marrow mesenchymal stem cells and cytokins(TPO,SCF,FLt-3,IL-6 and IL-3), then expression of CD44 and CXCR4 were evaluated on CD34+ cells by immunoflurescence analysis. Result: After 2 week of suspension culture, expression of CXCR4 was decreased on CD34+ cells that expanded in serum free media($P<0.05$). In contrast, expression of CXCR4 on CD34+ cells that expanded on hMSCs was increased.($P<0.05$).The expression of CD44 on expanded CD34+ cells was without significant changes. Conclusion: the results indicate that co-culture of cord blood stem cells on hMSCs significantly increases CXCR4 expression on cord blood CD34+ cells.

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REACTIVATION OF FETAL HEMOGLOBIN IN PERIPHERAL BLOOD CD133+ CELLS

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Background: Switching of Hb F in the adult hematopoiesis was controlled with mechanisms that are unclear. Understanding of mechanisms underlying this process can be useful for treatment of beta globin disorders. Aims: The study of cytokine-mediated enhancement in gamma globin expression in adult human hematopoietic progenitor cells. Methods: The PB (Peripheral Blood) was collected from normal person, which previously had received G-CSF. Mononuclear cells were isolated by density gradient, and CD133+ cells were isolated using magnetic beads. Isolated cells have cultured for two weeks in IMDM with 20% FBS supplemented with EPO (Erythropoietin), SCF (Stem Cell Factor) and TGF-beta. Then, RT-PCR and flow cytometry were done for detection of gamma globin and Hb F respectively. Also, the Colony assay was accomplished. Results: Flow cytometry analysis showed occurrence of 83% Hb F positive cells in differentiated populations. This result was confirmed by increase of gamma globin expression detected by RT-PCR in comparison with control. The hematopoietic colony forming assay showed that hematopoietic progenitor cells have ability to forming colony the same as untreated cells. Summary/Conclusion: In conclusion, the cytokines used in this study, can be a suitable candidate for treatment and investigation purposes instead of conventional drugs that can increase the Hb F.

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DOES MOUSE SPLEEN NICHE AFFECT ON THE EXPRESSION OF HUMAN GLOBINS GENES?

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Background: The Embryonic Stem Cells (ESCs) are interesting cells in study of hematopoiesis. Although the in vitro study of stem cells-derived hematopoietic cells is valuable in hematopoiesis but the effect of niche cannot be studied with. Aims: With doing of this work, we want to understand that niche can affect on expression of globins genes in transplanted hematopoietic cells derived from embryonic stem cells. Methods: The human ESC line, Royan H1, was differentiated into hematopoietic cells in feeder free culture supplemented with hematopoietic growth factors. The expression of globins genes were detected by RT-PCR. The mice were lethally irradiated and transplanted intravenously by the globins expressing hematopoietic cells derived from human ESCs. Two weeks after transplantation, the spleens were removed and the expression of globins genes and ESC specific genes were analyzed by RT-PCR. Results: The RT-PCR result in ESC-derived hematopoietic cells showed the expression of alpha, gamma, epsilon and zeta globin genes. The appearance of transplanted mice`s spleens was nodular and the size of it was bigger than the normal ones. The expression detection of globins and ESC specific genes in spleen lysates of transplanted mice by RT-PCR revealed that human globins and human ESC specific genes had switched off whereas the expression of internal

standard (human beta-actin) had remained in the high level. Sum-mary/Conclusion: In conclusion, the mouse spleen niche can affect on gene expression profile of transplanted cells. The expression of human internal standard confirmed locating of the transplanted cells in spleen. This investigation suggests that in future cell therapy strategies, the target tissue niche must be considered as a critical factor in the successful transplantation.

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ULTRASTRUCTURE EVALUATION OF IN VITRO DIFFERENTIATION OF CARDIOMYOCYTES FROM HUMAN BONE MARROW DERIVED MESENCHYMAL STEM CELLS

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Abstract text: Objective- resent studies have shown that bone marrow cotains cells capable of differentiating into cardiomyocyte in vivo.In this report, we investigated the ultrasturacture analysis of cardiomyocyte during differentiation and maturation from BM-drived mesenchymal stem cells(MSCs) under physiological culture condition. Methods- BM samples from 12 healthy donors were used as a source of MSCs.To indiuce cardiomyocyte differentiation MSCs were culture in the presence of 5% FCS, PDGF-AB, bFGF and 5-Azacytidin.Differenti-ated cardiomyocytes evaluated by transmission electron microscopy(TEM) during different developmental stages. Results- The ultrastructure of differentiating cardiomyocytes showed gradually development from scattered filaments at early stage to sarcomeric structure at the end stage. Conclusion- The ultrastructure of cardiomyocytes differentiated from BM-derived MSCs are similar to normal cardiomyocyte.

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UNCHANGED GLOBAL FIBRINOLYTIC CAPACITY (GFC) DURING THE COURSE OF HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Hemostatic alterations due to vascular endothelial damage have been detected during the complicated course of the hematopoietic stem cell transplantation (HSCT). Fibrinolytic response to the ongoing hemostatic cascade activation in HSCT still remains to be elucidated. Global fibrinolytic capacity (GFC) is a unique novel method, which is reflected by the amount of generated D-dimer when the fibrinolysis of a freeze-dried fibrin clot is stopped by introducing aprotinin. GFC is sensitive to all the factors involved in the process of fibrinolysis. The aim of this study is to serially assess GFC at the critical points (days -1, +7, +14, +21 following transplantation) during the course of HSCT.Nineteen patients with hematological malignancies [14 females, 5 males; aged 36±10 years], in whom HSCT had been performed, comprised the study group. Thirty healthy adults [21 females, 9 males; aged 31± 7 years] were served as controls. Serial peripheral blood samples at days -1, +7, +14, +21 following transplantation had been taken for the determination of GFC and other essential coagulation parameters. Global fibrinolytic response, as reflected by GFC, has been unchanged as an inappropriate response to ongoing hemostatic activation, as indicated by D-dimer, and microvascular damage of HSCT. GFC remained stable despite the development of thrombocytopenia associated with HSCT procedure before the platelet engraftment. Our results indicate that global fibrinolytic response was not evident as a compensatory response to the enigmatic prethrombotic state of the HSCT. Hemostatic abnormalities following HSCT is not only an academically significant issue but also their better understanding may lead to better management of patients with HSCT

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OCCURENCE OF THE SPONTANEOUS AGGREGATION IN THE PREPARATIONS OF THE CRYO-

PRESERVED MONONUCLEAR CELLS OBTAINED FROM UMBILICAL CORD BLOOD:

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Background: The phenomenon of the nonspecific cell aggregation (cell clumping) appears after thawing in the nucleated cell preparations obtained from the Bone Marrow, Peripheral Blood and the Umbilical Cord Blood (UCB). Such preparations, containing both the population of the mature cells as well as the immature haematopoietic progenitors are obtained by processing of the whole cord blood and freezing. Different techniques may be applied for thawing of such preparations. Aims: To evaluate the cell clumping phenomenon, the influence on it was examined of the increasing density of the nucleated cell suspension extracted from the UCB and freezed. The two selected techniques of thawing were also evaluated on their influence on cell clumping. Methods: The fraction of the nucleated cells from the UCB was obtained by sedimentation. The probes containing the suspensions (5; 10; 20; 50 mln/ml) of these cells were cryopreserved (cryopreserved solution: 70% FCS, 7% DMSO and 23% IMDM medium) The cells were kept in liquid nitrogen at -196 C (MESSER system) for six months. To evaluate the productivity of recuperation of cryopreserved hematopoietic cells we used two selected methods: classic and according to Rubinstein method. After thawing, the cells were resuspended either in IMDM medium supplemented 10% FCS or in the medium prepared according to Rubinstein method. The cell count and viability were determined in cell suspension. The in vitro cultures of the colony forming cells (CFC) were performed before freezing and after thawing of the cells. Results: The intensity of the cell clumping increased simultaneously with the growing density of the cell suspension. It rised from 15±3% in the 5 and 10 mln/ml groups to 43 ± 7% in the 20 and 50 mln/ml groups, when thawed accordingly to the "classic" technique. If the solution containing dextran (Rubinstein`s technique) was applied post thaw to dilute the cell suspension, the clumping phenomenon was markedly inhibited. It didn`t exceed 16 ± 4% in any density group. Independently to the intensity of the aggregation process, the number of the CFC among the whole pool that remained suspended after thawing (per 100 000 cells), maintained on a quite stable level of 70 ± 9% per raport to the prefreezing value. Conclusions: The intensity of the cell clumping phenomenon is directly influenced by the probability of the cell contact. Neverthless, the substances like dextran may markedly inhibit this process. This phenomenon remains to be not selective, it affects as well the early haematopoietic cells (CFC) as the mature cells, independently to the initial density of the freezed suspension. It seems, that for the protection of the thawed nucleated cell suspensions from clumping, in consequence from looses, the future labours will have to concentrate on the composition of the adequate freezing mediums containing clumping inhibitors.

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MULTI HOUR STORAGE OF FRESHLY PBSC AUTOLOGOUS APHERESED HARVESTS -THE INFLUENCE ON VIABILITY AND ENGRAFTMENT POTENTIAL

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Introduction: A major challenge in harvesting PBSC from previously mobilized patients with hematological malignancies is to collect a maximum amount of viable peripheral progenitor cells. Recent studies revealed the possible problem of DNA gel from dead cells during the multi hour or overnight storage of fresh PBPC harvests. The aim of the study was to determine the incidence and influence on viability of multi hour or overnight storage of fresh PBPC harvest at 4oC temperature, as well as the engraftment potential during the autologous setting for patients with hematological malignancies. Material and methods: We evaluated PBSC harvests from 40 mobilized patients with hematological malignancies (HD 12., ALL 2., AML 11., NHL 9., MM 5., CLL 1) with chemotherapy or G-CSF in 2-5 (median 3) apheresis procedures using the Baxter CS 3000 cell separator. PBSC grafts from consecutive patients were cryopreserved in solutions containing 5% and 10% DMSO by controlled rate freezing procedure (Nicole plus PC Espace 330) and stored at -196&61616;C. Trypan Blue viability was assessed for 78 PBPC harvests within 1 hour after apheresis procedure before cryopreservation and repeated on a 0,5ml aliquot stored overnight on 4oC tem-

perature. Results: The viability of the fresh harvests before storage vas median 97% (range 68, 5-99, 9%). The poorer viability was associated with harvest cell count. Bellow 300x10(9)/L the median viability was 98% and only 2/40 cases had <85% viable cells. Harvests count above 300x10(9)/L the median viability was 78% (67,8%-99%) and 8/10 (p<0,001) had <85% viable cells. The viability of harvests after overnight storage was below 25%-30% from the starting viability point, or median 78% for harvests bellow 300x10(9)/ L and median 54% for harvests above 300x10(9)/L (p<0,001). Conclusions: In our study fresh PBPC harvests below 80% viability gave concern for continuing further autologous transplant procedure, especially in the group stored overnight with viability bellow 65%. We conclude that harvest assessment before autologous setting is very important in the terms of defining the amount of viable progenitor cells as well as determine engraftment potential.

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CHARACTERIZATION OF BONE MARROW-DERIVED MESENCHYMAL STEM CELLS FROM HEALTHY MARROW DONORS OF PEDIATRIC AGE

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Background: Mesenchymal stem cells (MSCs) has gained a lot of attention recently as potential vehicles for cell and gene therapy. Bone marrow-derived MSCs as a widely used subgroup, has been characterized by several investigators. However, due to the heterogeneity in culture protocols including cell numbers, different medium conditions, differentiation protocols and the number of passages studied the expanded cells show different characteristics. Older age-related decreases in osteogenic potential have been shown in elderly humans. Here, MSC characteristics were studied in the pediatric age. Materials and Methods: Bone marrow cells were isolated from posterior iliac crest or tibia of healthy marrow donors including infants. The characteristics of MSCs were studied in 10 donor samples of ages ranging from 4mo to14 years. Bone marrow nucleated cells were plated and primary cultures were grown in DMEM containing 10% fetal calf serum of selected batches for 10-14 days until confluency, then trypsinized and expanded in further passages. The adhesive characteristics, fibroblastic morphology and surface antibody profile by flow cytometry were used for phenotypic characterization of MSCs. Multi-lineage differentiation potential towards adipocytic, osteocytic and chondrocytic lineage was assessed by previously described differentiation and staining assays. The cells were pelleted into aggregates and cultured in a medium containing transforming growth factor-beta3 for chondrocytic differentiation. Results: The volume of the initial marrow aspirate ranged from 3 to 10 ml. The cells were grown up to 12 passages in culture without losing their phenotypic characteristics consisting of CD29, CD44, CD105, CD166, CD73, CD44, CD26 positivity (ranging from 71.5 % to 97.2 %) and CD45, CD14, CD34, CD3, and CD 11b negativity, their adhesive properties or fibroblastic morphology. A rounder phenotype was evident in some late cultures. Trilineage differentiation potential towards adipocytic, osteocytic and chondrocytic lineages as shown by oil red O, alizarin red and toluidine blue stainings respectively, was preserved in the MSCs of infant donors. Conclusion: Bone marrow-derived MSCs obtained from young children including infants are easily expandable in culture and display similar phenotypic characteristics as MSCs obtained from different sources and of adult ages. Although adipogenic, chondrogenic and osteogenic differentiation potential of infant donors has been shown in this study, multilineage differentiation capacity needs further exploration.

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A NOVEL METHOD FOR DIFFERENTIATION OF EMBRYONIC STEM CELLS INTO HEMATOPOIETIC CELLS

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Background: Embryonic stem (ES) cells can differentiate into multiple cell lineages, such as hematopoietic cells. For this reason ES cells are unique model for tracing of processes that take place in

early hematopoiesis. Aims: In the study of hematopoiesis with using of embryonic stem cells, it is critical to minimize the interfering agents such as feeder cells and condition media. In spite of conventional method for differentiation of ES cells that using feeder for hematopoietic differentiation, in this work the mouse embryonic ones were differentiated to hematopoietic cells in a feeder free condition. Methods: The mouse ES cell line, Royan B1, was differentiated to hematopoietic precursor cells in IMDM with 15% fetal bovine serum (FBS) supplemented with hematopoietic growth factors. Expression of globins genes evaluated by Reverse Transcription-Polymerase Chain Reaction (RT-PCR).Also flow cytometry and colony assay for detect of ES cell-derived hematopoietic progenitor cells were performed. Results: Flow cytometry studies were showed that CD34 (+) cells are present in differentiated population. The embryonic globins (alpha and beta like globin) were detected by RT-PCR. The colony assay`s result showed that ES cells were differentiated to erythroid, myeloid colonies. Summary/Conclusion: In conclusion, the feeder free system can provide a suitable condition for differentiation of embryonic stem cells to hematopoietic ones; because we omitted the interfering agents (feeder cells) from differentiation medium. In this new method, differentiation can be considered more reliable in the study of hematopoiesis.

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PREVALENCE OF LEUKOCYTE ADHESION DEFICIENCY TYPE I IN IRANIAN PATIENTS WITH IMMUNODEFICIENCY FEATURES DURING TWO YEARS

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AN Background: Leukocyte Adhesion Molecule Deficiency type I (LAD1) is an autosomal recessive disorder due to absence of CD18 molecule on the surface of leukocytes. Defect in CD18/CD11 complex leads to abnormal leukocyte adhesion to vascular endothelium and subsequent migration to infected sites. These patients characterized by immunodeficiency features. These patients succumb to infection, commonly when they are younger than 2 years. Bone marrow or stem cell transplantation is the choice treatment and has success rate. Aims: In this study we investigated prevalence of LAD1 in patients with above features who reffered to Iranian Blood Transfusion Organization (IBTO) during two years. Methods: In this descriptive study we evaluated CD18 and CD11a,CD11b,CD11c on leukocytes gate.Whole blood lysing technique and Epics-xl Flowcytometry were applied.The results were analyzed by chi-square test. Results: The analysis revealed that 12.4% (14 out of 113) of patients had no CD18 expression on the surface of leukocytes. They were known as LAD1.All of the LAD1 patients were under 14 years and this phenotype showed significant difference in age groups ($p<0.05$).10 out of 14 LAD1 patients had recurrent and severe bacterial infection, 2 had delayed umbilical cord separation, one had aseptic meningitis and one with failure to thrive.3 out of 14 (21.4%) LAD1 patients had none of CD11a, CD11b and CD11c molecules on the surface of their leukocytes. Summary/conclusion: In spite of rare incidence of LAD1 throughout the world(less than 200 in US) our results showed high prevalence of LAD1 in patients with immunodeficiency features (6.3% to 18.5% with CI=95%). Therefore investigation of adhesion molecule expression is recommended in the work up of these patients, especially in pediatric groups.

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THE GENOTYPE FREQUENCIES OF HLA CLASS-I AND CLASS-II IN PATIENTS WITH DIFFERENT HEMATOLOGICAL MALIGNANCIES

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The major histocompatibility complex (MHC) superficial antigens play an important role in the immune response and in the predisposition mechanisms for different disease, including malignancy. The aim of this study is to define important HLA antigens in patients with different hematological malignancies in the Turkish population. From 2000 to 2003, we observed 176 pediatric and adult patients suffering from hematological malignancies such as ALL, AML, CML, MDS and NHL. Fifty of these patients were alive and 76 of 176 were dead. We have typed for HLA-A*, B*; DRB1* alleles in all patients in Central Anatolia of Turkey by PCR-SSP (sequence specific primers) low resolution DNA technique. The genotype frequencies in dead group were as follows: A*02

42 (28.38%), A*24 24 (16.21%), B*51 30 (20.54%), B*35 22 (15.06%), DRB1*04 38 (26.76%), DRB*13 26(18.30%). The genotype frequencies in survivors were as follows: A*02 32 (17.77%), A*03 26 (14.44%), B*35 32 (17.02%), B*51 26 (13.83%), DRB1*11 34 (18.28%), DRB1*17 26 (13.98%). Each genotype frequency of these alleles in dead group was compared to the survivors. The genotypic frequency of HLA-DRB1*04 in dead group was statistically higher than the survivors ($p<0.05$). As conclusion, the association was found between the high risk in hematological malignancy and genotype of HLA-DRB1*04 in this study.

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RESULTS OF IMMUNOPHENOTYPING IN NEWLY DIAGNOSED ACUTE LEUKEMIA PATIENTS

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Background: Immunophenotyping is one of the basic parameters used in the diagnosis of acute leukemias. Aim: We retrospectively investigated results of bone marrow immunophenotyping in newly diagnosed 68 acute non-lymphoblastic leukemia (ANLL; 49 men and 19 women) and 44 acute lymphoblastic leukemia patients (ALL; 37 men and 7 women) in our department.Methods: Results were compared with complete blood count values obtained at the time of diagnosis.Results: In ALL group, the most frequent antigens expressed were CD 19, 22, 5, 2 and 7 (57%, 36%, 34%, 33% and 30% respectively) while none of myeloid antigens expressed significantly. Mean white blood cell count in peripheral blood was 31,286±4,86x109/l in ALL patients. White blood cell count was positively correlated just with the expression amount of CD8. At the same time, mean 47,1% blast percentage was correlated with none of the antigens. In ANLL group, the most frequent antigens expressed were CD 33, 13, 11b and 11c (67%, 57%, 36% and 32% respectively) while none of lymphoid antigens expressed significantly. Mean white blood cell count in peripheral blood was 33,982±4,708x109/l in ANLL patients and was positively correlated with the expression amount of CD33 and 45. In this group, mean 50,8% blast percentage was positively correlated with the expression amount of CD45, 7 and 16. We could not demonstrate any correlation between peripheral blood platelet counts and any of the antigens in either group of patients.Conclusions: New studies with more cases might highlight the relationship between immunophenotyping and hematologic malignancies.

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BIOMARKER PROFILES IN CRITICALLY ILL EMERGENCY DEPARTMENT PATIENTS, UTILIZING PROTEINCHIP ARRAY TECHNOLOGY

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Background: Acutely ill patients presenting to the emergency department with suspected sepsis, DIC, or other sepsis related disease processes represent a patient population with proteolytic derangements of coagulation, fibrinolysis, kallikrien kinin, and complement systems. In addition, inflammatory cytokines and other mediators are also upregulated in these patients. Routine analytical methods such as electrophoresis are not capable of detecting these changes. Surface enhanced laser desorption ionization (SELDI) provides a unique approach to profiling biomarkers in the range of 0-150 kDa. As serine proteases contribute to the overall pathogenesis of these patients we hypothesize that protein cleavage products may result in a specific biomarker profile. To test this hypothesis a total of 43 patient samples representing multiple sets were profiled using SELDI technique. Materials and Methods: Patients entering the emergency department (n=8) with multiple medical disorders and cardiovascular/peripheral vascular manifestations were recruited. Blood samples were drawn at the time of presentation and throughout their course of treatment. Plasma samples were tested for inflammatory cytokines CRP, CD40 ligand, MCP1, NO, and a new marker, namely asymmetric 1,3-dimethylarginine (ADMA), using ELISA methods. ProteinChip Array Profile was obtained using Sax-2 chips and the Ciphergen ProteinChip System. Results: Most of the patient samples exhibited upregulation of the inflammatory cytokines and NO. Periodic fluctuations related to treatment and course of disease were evident. Biomarker analysis demonstrated unique profiles in the 11-12 kDa range in 39/43 samples (91%). The control

group did not demonstrate this profile. In addition to the 11 kDa complex another specific biomarker was evident in the 14 and 15 kDa ranges, however only 17/43 (40%) samples exhibited these two peaks. All 17 of those samples demonstrated the 11-12 kDa complex, as well. The relative proportions of the biomarker peaks in the 14 and 15 kDa ranges varied. Conclusion: Plasma samples from acutely ill patients with cardiovascular/peripheral vascular manifestations exhibit marked upregulation of inflammatory cytokines and NO. In addition, wide variations in ADMA levels were noted. Thus, the pathogenesis leading to the observed cardiovascular/peripheral vascular manifestations may involve inflammatory processes. Nearly all of these patients exhibited the 11-12 kDa unique biomarker. Furthermore, a significant number of these samples exhibited additional 14 and 15 kDa biomarkers. This data indicates that the biomarker profiles of these patients, in the molecular weight range below 25 kDa, may be of diagnostic and prognostic value. Furthermore, the identification of these unique biomarkers may be helpful in the understanding of the pathogenesis of sepsis-associated vascular manifestations and to develop therapeutic modalities.

Abstract: 449 Poster: 356

FLOW CYTOMETRIC EVALUATION OF CIRCULATING ENDOTHELIAL CELLS: A NEW METHOD USING A SICKLE CELL MODEL

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In sickle cell disease (SCD), angiogenesis and neovascularisation are undesired and may be associated with a poor outcome of the disease. Circulating endothelial cells (CECs) and endothelial progenitor cells (EPCs) are extremely rare events in normal peripheral blood. However, CECs have been found to increase in peripheral blood of patients with SCD. Different cell surface markers have been used to define these cells and consensus immunophenotyping have not yet been estabilshed. In this study, we tried to improve enumeration technique of CECs and EPCs by means of 4-color flow cytometry using sickle cell model. The study groups consisted of 35 patients with SCD (20 SCD in steady state, 15 SCD in vasoocclusive crisis) and 15 healthy individuals. Sample preparation (with the discussion about washing, and seperating the peripheral mononuclear cells), detection of CECs and EPCs (multiparametric flow cytometry), and the analysis of CECs and EPCs (gating strategy) were revised in our method. Viability stains (propidium iodide and 7-AAD) were used for identification of CECs and EPCs. An analysis of endothelial cells obtained from a saphenous vein sample was performed for the confirmation of the CECs` and EPCs` localization in the diagrams by setting a broad gate around all peripheral neucleated cells. Maturation of the endothelial cells were tested by using an in vitro tube test instead of the cultured mononuclear cells on fibronectin-coated plates because we thought that cultured mononuclear cells don`t reflect in vivo conditions, particularly in SCD which has high endothelial cell turnover rate. We found very remarkable results in regard to activated, resting and total number of CECs and EPCs. We also had an experience in morphological and immunophenotyping characteristics of the endothelial cells while they were in the high endothelial turnover phase in vivo. Some of these findings were different as they were described before. In conclusion, the method used for the enumeration and identification of CECs and EPCs must reflect in vivo condition. We hope that flow cytometric evaluation of CECs` and EPCs` enumeration and their cell kinetics, by using the SCD model which we have described, will give new insights to establish a standardized method. We will discuss the significant points of distinction.

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LPS INDUCED GENE EXPRESSION DIFFERENCES IN PERITONEAL MACROPHAGES FROM WILD TYPE AND G6PD HEMIZYGOUS MICE

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Background: G6PD deficiency is an X-linked recessive disorder causing episodic hemolytic anemia because of decreased ability of red blood cells to deal with oxidative stresses. Lipolysaccharide (LPS), which is found in the outer membrane of cell envelope of Gram negative bacteria is not only a potent inductor of sepsis, but also a potent

inductor of oxidative stress in monocytes, macrophages, and neutrophils. Increased incidence of sepsis and altered monocyte functions in severely injured type A- glucose-6-phosphate dehydrogenase-deficient African American trauma patients have been previously reported by our group (Crit Care Med. 2001:728-36). Our hypothesis is G6PD deficiency is associated with a difference in the innate immune response which results in the susceptibility to sepsis. Aim: The specific aim if this study is to determine the gene expression differences between peritoneal macrophages obtained from wild type mice (y/+) and G6PD deficient mice (y/-) after LPS stimulation. Material and Methods: Peritoneal macrophages were obtained from wild type (n=4) and the G6PD hemizygous mice (n=4). After the extraction of macrophages, half of the macrophages were stimulated with LPS in vitro at T0 hours, the others were non-treated. This made the studied eight mice divided into four groups: LPS stimulated wild type mice (n=2), LPS stimulated mutant mice (n=2), non-treated wild type mice (n=2) and non-treated mutant mice (n=2). At T6 hours total RNA is isolated by Trizol from all the macrophages. The isolated RNA were labeled with Genisphere method and hybridized into CAG-15K mouse arrays which is composed of 70- mer oligonuclotides corresponding to 15,000 mouse transcripts. The experimental design in the two color experimenst was "reference design" in which Cy5 labeled macrophage RNA were compared to Cy3 labeled universal RNA in all the experiments. Results: Comparison of LPS stimulated G6PD mutant and wild type macrophages indicates up-regulation of 211 genes and down-regulation of 56 genes in G6PD mutant macrophages. The EASE analysis indicated ($p<0.01$) the list of up-regulated genes are highly enriched in the genes having role in inflammatory response, cytokine production, wound healing and apoptosis. The genes involved in protein synthesis (particularly ribosomal proteins) are disproportionately prominent among the list of down regulated genes. Conclusion: These results indicate that there is a difference in level of expression in some of the critical genes after LPS stimulation of macrophages in vitro six hours after stimulation. Although these results should be followed in further time points and/or in an in-vivo model, they are highly supportive of our hypothesis there is a different modulation of immune response in G6PD deficiency.

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FLT3-RECEPTOR THYROSINE KINASE ACTIVATION MEDIATES ITS LEUKEMOGENIC EFFECT THROUGH WNT SIGNALLING PATHWAY IN AML PATIENTS

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Human myeloid malignancies are commonly associated with deregulated tyrosine kinase activity. A FMS-like tyrosine kinase-3 (FLT3) gene, an important molecule in acute leukemias, is a class III receptor tyrosine kinase expressed in normal stem cells and blasts of myeloid leukemia. An activating somatic mutation in the form of internal tandem duplication (ITD) of the FLT3 gene, in approximately 30% of patients with acute myeloid leukemia (AML), leads to ligand-independent FLT3 dimerization and constitutive activation. The identification of a second mutation, D835 activation loop domain mutation was found in about 7% of AML patients, has also suggested that FLT3 mutation is the single most common molecular of genetic abnormality in AML. It was also shown previously that WNT signaling cascade also participates in the transforming events emanating from the most prominent member of the other mutation class found in AML, FLT/ ITD. WNTs are secreted signaling molecules implicated in various developmental processes and frizzled proteins are the receptors for these WNT ligands. WNT5a is a member of the WNT family of secreted glycoproteins that play essential organizing roles in development. Similar to other WNT members, WNT5a can upregulate cell proliferation and has been proposed to have oncogenic function. We investigated the expression of WNT5a and frizzled protein FZ5 which is specifically synergizing with WNT2, WNT5a and WNT10b in our AML patient group. Quantitative reverse transcriptase (QRT) PCR, for FLT3, WNT5a and FZ5 genes were done on 29 acute myeloid leukemia (AML) patients at diagnosis, all having FAB M3 phenotype. We have screened a panel of AML patients for the occurrence of FLT3, WNT5a and FZ5 expressions and correlate this to patients` age, sex, sample material (bone marrow/blood), t(15;17) status and FLT3/ITD mutations. We also examined the FLT3/ITD and D835 mutations in our patient group. There was a 56% incidence of FLT3/ITD mutation in the cohort of examined patients. 2 patients (2/29, 6%) showed hemizygous ITD mutations and more than one

ITD repeat was detected in two of the acute myeloid leukemia cases. D385 mutation was not detected in our patient group. No difference was observed in FLT3 expression in between AML patients and controls as well as in the bone marrow samples and in the blood samples. FLT3 expression levels decreased in the adults compare to children. A decrease in FLT3 expression was observed in AML patients having t(15;17). On the other hand, WNT5a and FZ5 expressions showed a decrease in between AML patients and control samples. It was determined an increase in WNT5a and FZ5 expressions in AML patients with FLT3/ITD mutations. Our results indicate that Flt3-ITD mediate their leukemogenic effects in part through the activation of the WNT signaling pathway, possibly defining this signal system as a converging point of leukemogenic events elicited by Flt3 mutations and leuke-mia-associated fusion proteins.

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DECREASED WNT5A AND FZ5 MRNA LEVELS CONTRIBUTES TO ACUTE LYPMHOBLASTIC LEUKEMIA (ALL)

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Wingless type (WNT) signaling provides proliferative signals for the most immature progenitor cells both in the B-and T cell lineages, as well as self-renewal signals for hematopoietic stem cells. WNT signaling accomplishes important regulatory function in haematopoietic progenitors and stem cells during fetal and adult development. In addition to importance of the WNT pathway in normal development, disregulation of the WNT/. catenin pathway can have potent oncogenic effects in different tissues. Therefore in most cases disregulated WNT signaling contributes to leukaemogenesis. WNT signaling has been shown to occur in haemopoietic stem cells and developing T and B cells. Some studies imply that WNT signaling might be differentially regulated between T and B cell lines. WNT5a can act similar to other WNT family members and bind with frizzled (FZ) receptors located on the cell surface. WNT5a has been previously shown to have growth enhancing or oncogenic potential. WNT5a protein also functions as a tumor suppressor in haematopoietic tissues. Deletion of the WNT5a gene and loss of WNT5a expression is observed in mouse and human B and pre B cell leukemias. In this study, we investigated the WNT5a and FZ5 expressions in pre B, B ALL and T-ALL patients. To examine whether WNT5a might also function as a tumor suppressor in human malignancies, we performed quantitative real time reverse transcription PCR (QRTPCR) to analyze the expression of WNT5a and FZ5 in pre B (n=22), B-ALL (n=27),T-ALL (n=29) at diagnosis and control bone marrow samples (n=5). Levels of WNT5a and FZ5 mRNA`s were lower in ALL patients than in controls. There was a trend towards lower levels of WNT5a in those with B-cell, T-cell and pre B ALL. Expression pattern analysis is also performed on blood or bone marrow samples. A decrease of WNT5a and FZ5 expression was observed in the bone marrow samples compare to blood samples. In the future, it will be important to investigate the expression of WNT5a and FZ5 according to the different clinical parameters as such, leucocyte number, relapse status and t(4;11), t(12;21) and t(9;22). Our result is in concordance with the hypothesis that WNT5a is a tumour suppressor gene with potential clinical applications. The expression of the WNT5a and FZ5 genes were found to be suppressed in ALL patients. This might be explained by the increase of B cell, pre-B cell and T cell proliferation due to the absence of WNT5a gene expression. This study provides additional support to the hypothesis that the WNT signaling pathway is involved in leukemogenesis. HODGKIN`S LYMPHOMA

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MALIGNANCY ASSOCIATED HAEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS: A MULTICENTER STUDY FROM TURKEY

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Purpose: Secondary Haemophagocytic Lymphohistiocytosis (sHLH), which implies an acquired cause of HLH, is the result of strong immunologic activation, commonly occurring in an immunocompromised host. One of the common triggers of secondary HLH is malignancy which became one of the hot topics of pediatric oncology very recently. Although the mortality rate in children

diagnosed with primary HLH is high, little has been described about the nature of secondary form. This abstract evaluates unfavorable events in children with secondary HLH related with malignancy to suggest methods of improving outcomes. Methods: Charts of patients who met diagnostic criteria for sHLH associated with malignancy at the 6 different Hospital of Turkey between January 2000 and January 2005 were retrospectively reviewed. The diagnosis of HLH achieved by bone marrow aspiration in 23 patients while in two, CSF and liver biopsy were the material showing HLH. Results: Twenty five children were diagnosed with secondary HLH related with malignancy. 18 cases (17 ALL and 1 AML M2) with acute leukaemia, 8 girls/10 boys, median 8 years (3 - 14 years) were found having sHLH. No case had a history of consanguineous parents except one. Fourteen ALL patients were diagnosed with sHLH during therapy (therapy-malignancy related) and 4 at the beginning of the disease during diagnostic evaluation. Fever, cytopenia were common in each case. All of the children were anemic (Hb values < 11g/dl, 4 of them having values under 8g/dl, 12 having values between 8-10g/dl). Except 4 patient, all of the cases had thrombocytopenia (< 100.000/mm3). Secondary HLH patients found during chemotherapy all had leukopenia, in contrast the cases diagnosed during the evaluation had leukopenia in % 50 of sHLH. Laboratory results revealed abnormality in patients diagnosed as sHLH during diagnostic period. Hepatosplenomegaly was detected in none with therapy-malignancy related cases. When the therapy -malignancy related cases were documented, the HLH period falls within the period of days 50 to 64 of induction therapy (Protocol I, Phase 2 of BFM regimens) in 11 cases and between days 40 to 50 in consolidation therapy (Protocol II, Phase 2 of BFM regimens) in 2 cases and one after the first high risk block just after induction regimen. During these periods, cytokine storm may be out of control after corticosteroid withdrawal. Transfusions given in the name of supportive therapy might also trigger the defective cytotoxic response of the immune system, causing HLH. All ALL patients were treated for HLH, which was detected during therapy, with either dexametazone (3) or IVIG (2) or both (9) with no sequaele, strikingly different from the prognosis of the patients diagnosed as sHLH during the evaluation period which was dismal. These four ALL cases deceased very early, after diagnosis within 15 days. Neither HLH mutations nor NK functions were investigated in these sHLH cases. These deaths were attributed to progressive HLH and invasive infections. Other than leukaemia, 7 patients with different solid tumors have been demonstrated to have sHLH. Two cases of rabdomyosarcoma, neuroblastoma and NHL, one case Hodgkin disease were diagnosed as having concomitant haemophagocytosis at the initial evaluation of the tumor. Of 7 patients, with solid tumors and sHLH, all were lost either on the cancer treatment or after relapsed, except for one case (the patient with neuroblastoma and transplanted with autolog pheripheric stem cell). Conclusions: Secondary hemophagocytic lymphohistiocytosis may resolve, with or without specific treatment, in particular if prior immunosuppressive therapy is completed or underlying malignancies are controlled. However, mortality in sHLH is reported to be high just as our results. The overall mortality rate was 40 % in our series of 25 children with sHLH; 16% of deaths were directly attributable to HLH. Our recommendation at present that, patients with severe, persistent or recurrent HLH bystander with the malignancy must be treated with great care. Even the patients that responded well to initial therapy succumbed later, reflecting the dismal prognostic characteristics of sHLH. But the patients diagnosed during chemotherapy could be succesfully treated with steroids, IVIG or both.

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HEMOPHAGOCYTIC LYMPHO-HISTIYOCTOSIS SECONDARY TO CHEDIAK- HIGASHI SYNDROME AND GRISCELLI SYNDROME IN TURKEY

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Chediak-Higashi syndrome (CHS) and Griscelli syndrome (GS) are similar diseases which can be distinguished from the other by clinical and laboratory findings. Both of them cause secondary hemophagocytic lymphohistiyocytosis. CHS is a rare autosomal recessive disorder that is characterized by decreased pigmentation of hair and eyes (partial albinism), photophobia, nystagmus, large eosinophilic, peroxidase-positive inclusion bodies in the myeloblasts and promyelocytes of the bone marrow, neutropenia, abnormal suscep-

tibility to infections. About 85 to 90% of CHS patients eventually develop a strange lymphoproliferative syndrome, the so-called `accelerated phase` of the disorder, characterized by generalized lymphohistiocytic infiltrates, fever, jaundice, hepatosplenomegaly, lymphadenopathy, pancytopenia, and bleeding. GS is also a rare autosomal recessive disorder that results in pigmentary dilution of the skin and hair, the presence of large clumps of pigment in hair shafts, and an accumulation of melanosomes in melanocytes. Most cases described are from Turkey and Mediterrannean countries. Most patients also develop hemophagocytic syndrome, leading to death in the absence of bone marrow. In this paper five patients with CHS and four patients with GS from Turkey have developed hemophagocytic lymphohistiyocytosis secondary to these diseases presented. In this study clinical and laboratory findings of these patients were documented from five centers retrospectively. All the patients were diagnosed with clinical and laboratory findings, no mutational analysis was performed. For CHS, the mean age at diagnosis of hemophagocytosis is 3,4 year (0,2-6), for GS is 6 year (0,2-14,5). Male/female ratio for CHS was 4, for GS was 1. There was consanguinity within 44% of all patients. Previous childhood deaths in these families or relatives was 44% in all cases. Mean count of previous childhood deaths was 2,5 (1-5). They were treated with HLH-94, HLH-2004 protocols or alternative regimens. Cure ratio of all patients from hemophagocytic lymphohistiyocytosis was 12%, mortality ratio was 44%, other patients (44%) were under treatment.

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PLASMA CONCENTRATIONS OF NT-PRO-BNP AND CARDIAC TROPONIN-I IN RELATION TO DOXORU-BICIN-INDUCED CARDIOMYOPATHY AND CARDIAC FUNCTION IN CHILDHOOD MALIGNANCY

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Objective: Anthracyclines are well established as highly efficacious antineoplastic agents for childhood malignancy, but they frequently cause dose-related cardiotoxicity. For this reason, children who have received anthracyclines need periodical cardiac evaluation. The plasma levels of B-type natriuretic peptide (BNP) have been shown to increase in proportion to severity of cardiac dysfunction. N-terminal BNP (NT-pro-BNP) is secreted from the cardiac ventricles in response to volume expansion and pressure overload. The aim of our study was to investigate whether plasma levels of NT-pro-BNP and cardiac troponin I (cTnI) can be used as specific markers for doxorubicin-induced cardiotoxicity in children with malignancy. Methods: The study was performed in Dicle University Hospital pediatric hematology-oncology clinic. Plasma NT-pro-BNP and cTnI were measured in 31 patients (14 boys, 17 girls) who received doxorubicin-containing chemotherapy for their malignancy at cumulative doses of 30-600 mg/m², between October 2000 and December 2004. Cardiac evaluation of the patients included recording of electrocardiography, and assessment of systolic and diastolic functions of the heart by echocardiography. Results: Of the 31 patients, 4 (12.9%) had left ventricular dysfunction as assessed by echocardiography. Plasma NT-pro-BNP levels in these patients were significantly elevated in comparison with healthy controls ($p<0.001$). Plasma NT-pro-BNP levels were significantly elevated in patients with cardiac dysfunction when compared with normal cardiac function ($p<0.008$). cTnI levels were found under normal value in all patients. Conclusion: Measurement of NT-pro-BNP level may be easy and practical tool, during treatment may allow earlier-identification of individuals at risk for monitoring cardiac damage. Plasma NT-pro-BNP concentration may be as an useful and sensitive indicator of cardiac dysfunction in children receiving doxorubicin therapy.

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SECONDARY HEMOPHAGOCYTIC SYNDROME; A PRESENTATION OF NINE PEDIATRIC CASES

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BACKGROUND: Hemophagocytic syndrome (HS)is a rare, heterogeneous disorder in infants and children by characterized by a group of clinical, laboratory and histopathological findings such as fever, hepatosplenomegaly, cytopenia, hypertriglyceridemia and hemophagocyto-sis in the bone marrow, spleen, and lymph nodes. AIMS: to report a series included 9 pediatric patients, to present the features of syndrome in these

pediatric patients. METHODS: Nine pediatric patients with pathologically proved hemophagocytic syndrome (HPS) from a single institution during 1993 and 2001 were retrospectively analyzed. All patients met the diagnostic criteria according to the hemophagocytic lymphohistiocytosis protocol (HLH-94) of the Histiocyte Society. RESULTS: There were 6 males and 3 females (ages between 3 months-18 years). There wasn`t a family history of the disease in any of the cases. Fever, hepatomegaly and splenomegaly was present in all cases, and rash in 4 cases, lymphadenopathy in 3 cases, jaundice in two cases and neurologic symptoms in two cases. The diagnosis was carried out by bone marrow aspiration-biopsy in all of the patients (in one case additional histopathologic examination of spleen revealed histiocytic hemophagocytes). Laboratory tests showed pancytopenia/bicytopenia and elevated lactate dehydrogenase in all cases, elevated triglycerides in 8 cases, elevated liver enzymes and ferritin in 7 cases. HPS was related to viral infection in seven patients (5 cases associated with cytomegalovirus and 2 cases with Epstein-Barr virus) and to neoplastic disease in two cases (T cell nonhodgkin lymphoma and malignant histiocytosis). Herpes simplex virus Tip I infection were detected in the patient with T cell nonhodgkin lymphoma. Death occurred in the two patients with associated malignancy. The recovery was complete in the seven surviving patients with supportive therapy, antiviral therapy (gancyclovir, CMV hyperimmuneglobuline) and intravenous immunglobuline. CONCLUSIONS: Due to its confusing clinical manifestations, HPC may be underdiagnosed. The combination of fever, cytopenia, elevated serum LDH and triglyceride levels, and/or hyperferritinemia is a clue to the diagnosis of HPS; bone marrow biopsy is valuable in establishing the diagnosis. Two forms of HPS have been well characterized; primary/familial hemophagocytic lymphohistiocytosis and secondary/reactive HPS. The familial form is a lethal disorder. Reactive HPS is usually secondary to infections, mostly cytomegalovirus and Epstein-Barr virus like our cases. Because infections were the leading cause, the need for advanced microbiological investigation is important in these patients.

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PROGNOSTIC SIGNIFICANCE OF SOME CLINICAL PARAMETERS IN HODGKIN`S DISEASE: A SINGLE INSTITUTION EXPERIENCE

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BACKGROUND: The optimal therapeutic strategy of patients with Hodgkin`s disease is usually determined on clinical staging and other prognostic parameters. The AIM of the study was to identify prognostic factors related to survival of patients with Hodgkin`s disease in the population of Plovdiv district (South Bulgaria). PATIENTS AND METHODS: The retrospective study involved 151 patients with Hodgkin`s disease, diagnosed and treated in the University Clinic of Hematology, Plovdiv from 1995 through 2003. The prognostic value of the following parameters related to survival was analyzed:1. Age,2. Sex, 3. Ann-Arbor clinical staging, 4. ECOG Performance status, 5. Histological type, 6. Extranodal localization, 7. B symptoms, 8, Mediastinal lymphadenopathy, 9. Abdominal lymphadenopathy and 10. Infradiaphragmatic disease. The patient`s survival was analyzed by using the Kaplan-Meier method. The log-rank test was used to comare cumulative survival functions.RESULTS: The mean survival period of patients was 14,17 years (95% C.I. 11,01-17,33). The most important prognostic factors for survival were: age (p<0,001), ECOG Performance status (>2, P<0,0001), Ann-Arbor clinical stages (III+IV, P=0,018), extranodal localization (>1 site, P=0,0001), abdominal lympadenopathy (P=0,0001) and histological type (lymphocyte predominance+nodular sclerosis versus mixed cellularity+lymphocyte depletion, P=0,02). CONCLUSION: Prognostic factors identified in the present study can be easily applied in the routine clinical practice and may be used as a basis for mathematical prognostic models.

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LANGERHANS CELL HISTIOCYTOSIS- A RETROSPECTIVE STUDY FROM A PEDIATRIC HEMATOLOGYONCOLOGY CENTER

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The aim of this study was to evaluate the clinical, laboratory findings and treatment results of Langerhans cell histiocytosis (LCH) cases referred

in a faculty clinic in İstanbul. Material and Methods: Forty-three cases of newly diagnosed LCH were retrospectively evaluated: 30 of the cases were male, 20 girl (M/F:1.5). Age at diagnosis was between 1.5 months and 14 years (median 3 years). The duration of follow up period ranged from 6 months to 10 years. DAL-HX, LCH I,II and III protocols were administered. Results: The patients were stratified as single system disease (SSD) in 30 cases with 13 as multiple site and 17 as unifocal site-disease, Multi-system-disease was noted in 7 low risk and 13 high-risk group. Initial presentations were as bone in 32 cases (64%), palpable lymph node in 25 cases (50%), palpable liver in 21 (42 %), soft-tissue in 17 (34%), skin in 15 cases (30%), palpable spleen in 13 cases (26%), bone marrow infiltration in 5 cases (10%). CNS infiltration was noted as initial finding in the hypophyse in 4 cases and parenchymal in 1 case and diagnosed as a relapse finding in 5 cases (total 10/50). Lung infiltration was noted in 5 cases (10%). Liver dysfunction was found in 11 patients (22%). Multiple organ dysfunction was present in 8 patients (16%). Anemia was present in 34% of the patients. Leucopenia was noted in 2 cases and thrombopenia in 2. Twelve cases were treated by surgery alone and 5 with surgery plus local steroid injections. Radiotherapy was given in 4 cases. Systemic 2-3 or 4 drugs-chemotherapy was applied in 23 cases. Relapse occurred in 15 of 50 cases (% 30) and was predominantly in bones, CNS, bone marrow, soft-tissue and liver. Six patients with multi-system high-risk disease were lost with disease progression. Ten patients were lost to follow-up (20%). Conclusion: Histiocytosis is a disease with high overall survival with the exception of some malignant course patients. Thirty-four of our patients are under follow-up and the overall survival is 86 % at 6 years. Relapse occurred mostly in MSD and SSD multiple site patients.

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A RARE CAUSE OF CERVICAL LYMPHADENOPATHY IN CHILDREN; NASOPHARYNGEAL CARCINOMA

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Background: Nasopharyngeal carcinoma (NPC) is rare malignancy in children. Incidence varies widely around the world. NPC is strongly associated with Epstein-Barr virus (EBV) infection. It represents a locally advanced undifferentiated tumor with varying clinical features included cervical lymphadenopathy, torticollis, trismus, epistaxis, change in voice quality, unilateral otitis media with effusion and a nasopharyngeal mass demonstrable in physical examination or CT/MR scans. The metastasis to cervical lymph nodes represents a frequent initial manifestation of NPC. Lymhadenopathies of infectious causes are common in children and are often indistinguishable from metastatic nodes arising of nasopharyngeal carcinoma with clinical examination alone. Aims: to describe a patient with nasopharyngeal carcinoma who initially presented with cervical lymphadenopathy, to alert the physicians to the potential for malignant causes of cervical lymphadenopathy in children. Methods: We evaluated retrospectively clinical, laboratory and radiological data of the patient. Results: A 12 years old boy was admitted to a pediatric outpatient clinic of a local hospital with cervical lymphadenopathy. After two weeks of per oral antibiotic therapy, it was found nonresponsive and fine-needle aspiration biopsy was performed. Cytologic specimens was found nondiagnostic. After that, excisional biopsy of the lymphadenopathy was performed, histologically the tumor was NPC. Two months after the first medical attention he was referred to our clinic. Large tumoral mass was noted on CT scans of the nasopharynx. EBV serology was found negative. Following staging procedure, neo-adjuvant chemotherapy was started. Conclusions: Early symptoms of nasopharyngeal carcinoma, like cervical lymphadenopathy can often be confusing. This presentation illustrates that refractory lymphadenopathy, despite adequate treatment of the suspected infection, should prompt a search for underlying disease. Although it is a rare condition in under 20-year-olds, the possibility of metastatic nasopharyngeal carcinoma should always be considered in children with enlarged cervical lymph nodes

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SECONDARY HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS (NOT MALIGNANCY ASSOCIATED): A STUDY FROM FIFTEEN CENTERS IN TURKEY (TURKISH HLH STUDY GROUP)

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Secondary hemophagocytic lymphohistiocytosis (secondary HLH) is characterized by lympohistiocytic proliferation with hemophagocytosis may develop as a result of and secondary to strong immunologic activation, such as a severe infection. Secondary HLH is largely associated with infection by viruses, bacteria, fungi, or parasites, but also occurs as autoimmune disease, or metabolic disease-asso-ciated forms. In this study eighty six patients with secondary HLH were documented from fifteen centers in Turkey, retrospectively. The cases with malignancy-associated HLH were not included of the study. The patients were diagnosed according to the diagnostic guidelines for HLH-2004. 45 (52%) of the patients were male. The age of the patients was between 2 days and 17years, mean age was 6,2 years. 40% of the patients were 0-2 year old. There was consanguinity within 34% of the families. Previous childhood deaths in these families was 17%. At physical examination of the patients; fever in %85, hepatomegaly in %84, splenomegaly in 71%, rash in 24%, lymphadenopathy in 23%, neurologic findings in 21 %, hemorrhage in 19% were documented. Secondary HLH of the patients were associated with viral infections (EBV, CMV, HSV, HPV, HAV, Adenovirus) in 35%, tuberculosis in 2,3%, brucellosis in 10.5%, visceral leishmaniasis in 4.7%, others bacterial infections in 7%, fungal infections in 2.3%, others causes in 11.6%, and unknown causes in 27.6% (Table 1). In this study, the treatments were specific to the associated agents. These were antibiotics, antiviral, antiparaziter or antifungal drugs. In addition, the various combinations of intravenous immunoglobuline, steroids, vepesid, cyclosporine A and G-CSF was additted to specific treatments. Most of the previously identified patients with familial HLH have belonged to Turkish population. This study demonstrated that secondary HLH also frequently has been diagnosed in Turkish population.

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POLYMYALGIA RHEUMATICA AS A PRESENTING FEATURE OF HODGKIN`S LYMPHOMA: A CASE REPORT

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Polymyalgia rheumatica (PMR) is a relatively common disorder characterized typically by morning stiffness and aching of the shoulder and hip girdles, neck and torso in patients over the age of 50. Cutaneous vasculitis, seronegative arthritis, and polymyalgia rheumatica are the most common findings associated with lymphoid malignancies. Here, we report a Hodgkin`s lymphoma case presented with PMR. Case: A 75-year-old man presented with a 3-week history of progressive pain and moderate stiffness in his shoulder, cervical and hip girdles, limitation of mobility, bilaterally swelling of wrists and knees. Medical history revealed weight loss of 10kg over 3 months and a mass on left inguinal area that not progressed about twenty years. Physical examination revealed a temperature of 37.6/C, tenderness and limitation of shoulder and synovitis of wrist, enlarged, non-tender left inguinal lymph node of 5x5 cm in size. There was no lymphadenopaty and no signs of temporal arteritis. Laboratory data showed high erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Renal and liver profile was normal. Viral and bacterial infections, autoimmune disorders were ruled out. Chest radiography and two-dimensional echocardiography were normal. Abdominal ultrasonography and computerized tomoghraphy (CT) scan confirmed enlarged lymph node on left inguinal area. Gastric, duodenal, prostate, bone marrow malignancies were ruled out by their biopsies. Biopsy of the left inguinal lymph node was reported as granulomatous lymphadenitis but no caseification necrosis. As differential diagnosis of granulomatous infections revealed negative result; 25 mg of prednisone daily initiated. A week later, he felt well as pain and stiffness had apparently regressed. ESR was decreased, but the size of the

lymphadenopathy did not differ. By the dose reduction of steroids PMR reoccurred with dose reduction. Four months after discharge, the patient presented with similar complaints in first admission and with right inguinal pain, easy fatigue. It was learned that the patient was no longer on steroids by his own decision. On examination, weight loss, paleness and pain and limitation of shoulder and wrist mobility, enlarged posterior cervical and right inguinal lymph nodes were observed. Left inguinal lymph node was progressed as 7x7 cm in size. No hepatosplenomegaly was observed. Except anemia, high CRP, LDH and ESR all biochemical parameters were normal. Abdominal CT revealed enlarged paraaortic multiple lymph nodes. A second right and left inguinal lymph node biopsies revealed as Hodgkin`s lymphoma, mixed cellular type. The Ann Arbor clinical staging was IIIB. He received the combination treatment ABVD of 4 cycles. Conclusion: Hodgkin`s lymphoma should be considered in the differential diagnosis of atypical PMR. Awareness of these musculoskeletal syndromes should alert the physician of possible paraneoplastic manifestations of an evolving neoplasm

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UNEXPECTED PRESENTATION OF HODGKIN`S LYMPHOMA: JUGULAR VENOUS THROMBOSIS RELATED TO FACTOR V LEIDEN MUTATION

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A 47-year-old woman was admitted to hospital because of dyspnea for 2 months In her physical examination, left jugular venous distention and left servical 1.5x 1.5 cm lymphadenopathy was observed. Jugular venous doppler ultrasonography demonstrated venous subacute thrombosis in proximality of left external jugular vein, left subclavian vein and left brachiocephalic vein. There was no prior thromboembolism, using oral contraseptives or a strong family history. Thrombosis etiology was investigated and thorax CT showed anterior mediastinal multiple lymphadenopathies in upper mediastinum and on DNA analysis, a heterozygote Factor V Leiden mutation established. Histological examination of left servical lymphadenopathy confirm the diagnosis of the nodular sclerosing type of Hodgkin`s Lymphoma (HL). Malignancies as well as HL and others increased the risk of thromboembolism without a well recognized inherited thrombotic disorder. Factor V Leiden mutation is the most common inherited thrombotic disorder. This case report demonstrated an unexpected presentation of HL with juguler venous thrombosis related to factor V Leiden mutation.

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OPTIONS FOR UNMANAGEABLE HODGKIN`S DISEASE PATIENTS: SEQUENTIAL HIGH-DOSE CHEMOTHERAPY FOLLOWED BY AUTOGRAFT

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In Macedonia, we have the option of bone marrow and stem cell transplantation for the treatment of hematological malignancies since 2000, and so far, almost 100 transplants have been performed in patients with differing entities. Since 2001, we adopted the GHLSG (Josting, Diehl et al.) protocol - sequential high-dose chemotherapy, followed by BM or PBSC autograft, as standard in the treatment of those HD patients in whom this approach was considered the only choice. According to this protocol, we have treated a total of six HD patients until now, three male and three female. Their age varied from 21 to 42. Indications for using this approach were: multiple relapses in two cases, quick relapse after initial treatment (<12 months) in one, and initial treatment failure with disease progression in three. Transplants consisted of harvested peripheral stem cells in five cases and in one patient a combined graft (PBSC + BM) was administered, due to insufficient quantity of peripheral cells harvested. In all six patients, marked response to treatment was observed, resulting in tumor mass reduction and symptom loss, i.e. in disease control. In three patients residual tumor mass was noted after completion of the procedure, for which additional involved site radiotherapy was performed. Following protocol cessation, progression of disease was observed in two patients so far, following 6 and 28 months of disease-free period. One patient was treated with salvage chemotherapy intermit-

tently and radiotherapy, until the fatal outcome (cardiovascular complications), one year after autografting. The other is currently under salvage chemotherapy. The other four patients are in a stable state, without disease progression, 3-14 months after completion of the procedure. Problems encountered in the procedure consisted most often of profound leuko- and thrombocytopenia, managed fairly easily by adequate substitution and stimulation therapy; still, these complications are accountable for prolonging the time schedule of the protocol in some instances. Due to previous bone marrow "exhaustion" PBSC harvests often resulted in low cell yield, thus requiring more sessions or alternative methods. Infective and hemorrhagic complications were extremely rare and managed with standard counter-measures. Two of the patients received Epo complimentary to the protocol, thus diminishing erythrocyte transfusion requirements. In one patient toxic affection of the liver was noted during the sequential HDCT course, requiring hepatoprotective treatment and postponement of the procedure. Our impressions from implementing the GHLSG protocol in the management of poor prognosis, previously treated, HD patients, are certainly positive. We adopted it as standard procedure in such patients. Personal experiences allow us to practice progressively greater latitude in diminishing the markedly long proportional in-hospital stay, one of the protocol requirements quite uncomfortable to patients.

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MUTUAL AND DIVERSE PROGNOSTIC FEATURES OF HODGKIN`S DISEASE IN EXTREMELY UNFAVORABLE STAGES

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Upon the observation that patients with both clinical stage IIIB and IV Hodgkin`s disease (HD) follow an almost identical survival curve, we underwent this study in order to define similarities in their characteristics and determine the parameters that influence their outcome. In a longer than twenty-year period, out of the total number of observed patients with HD, 172 manifested extremely unfavorable clinical stages at disease presentation. Their actuarial survival is understandably quite poor, reaching 42% at the 10-year mark, and somewhat over 32% at the 20-year observation point. Univariate statistical analyses, performed separately on each stage subgroup, as well as in the subpopulation as a whole, pointed out several parameters that manifested prognostic significance regarding the vital statistics, some marginal and fewer true. Four of these are International Prognostic Index (IPI) parameters, but only one manifests true prognostic significance. Not surprisingly, some of the prognostic factors incorporated in the IPI, showed no significance at all. Since the whole studied subpopulation already fulfills one criterion among the adverse prognostic factors, it is also understandable that the majority of these patients had an unfavorable IPI score (>=3) also: 70.93% of the subpopulation, mostly attributable to the 93.51% of the stage IV patients. Multivariant statistical analysis left only one of the IPI parameters with significant prognostic influence: abnormal WBC levels (p= 0.020061). However, it also revealed the prognostic significance that the diagnostic and treatment periods carry for the vital statistics of these patients. Development of the disease, lacking treatment, in a period of more than three months, carries a significant negative prognosis (p= 0.045212). Among the management parameters, meticulous fulfillment of treatment administration in terms of dosage, accounts for significantly more favorable prognosis (p= 0.033629). The analysis shows a x2 value of 12.2728 for three degrees of freedom (p= 0.00651). Although representing an extremely delicate and tenacious population in terms of management, we need to keep in mind that disease characteristics have already classified such patients as prognostically unfavorable. Not being in the position to alter those parameters, we still need to instigate our efforts, skills and knowledge in two domains, in order to reverse the odds and accomplish more favorable prognosis for these patients: quick, precise diagnostic procedures and proper, meticulous, relentless treatment.

Abstract: 465 Poster: 372

A CASE OF POSTERIOR LEUKOENCEPHALOPATHY IN ASSOCIATION WITH TUMOR LYSIS SYNDROME

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Background: Posterior leukoencephalopathy (PL) is a reversible clinical and radiological syndrome characterized by headache, altered level of consciousness, seizures and occipital blindness in association with transient parietooccipital abnormalities on neuroimaging studies. It has been most commonly described in association with severe hypertension, systemic lupus erythematosus and immunosuppressive and cytotoxic drugs. However, little attention has been paid to tumor lysis syndrome as a contributory factor for PL. Case Presentation: A 21-year-old woman who was diagnosed as having Burkitt lymphoma received the induction therapy, including cyclophosphamide, vincristine, doxorubicine and dexamethasone. At the time of hospitalization, her hematological and biochemistry tests were normal except for hemoglobin (10 g/dl) and lactate dehydrogenase (1327 UI/l) levels. The cerebral CT scan and whole spinal MRI revealed no abnormality. However, just before the initiation of induction chemotherapy serum creatinine (Cr) and uric acid (UA) levels gradually increased to 3 mg/dl and 14.4 mg/dl respectively albeit prophylactic management with hydration, urinary alkalinisation, frusemide and allopurinol. Since day 4 of induction therapy she developed blurred vision, headache and then generalized clonic convulsions which were only controlled with phenytoin infusion. At that time- Cr was 1.2 mg/dl, UA 2.1 mg/dl, K 2.6 mg/dl, Ca 6.9 mg/dl and P 5 mg/dl. A cerebral MRI revealed abnormalities relevant to vasogenic edema in bilateral parietooccipital and frontal regions involving both cortex and subcortex. Examination of cerebrospinal fluid revealed no abnormal cells suggesting infection or central nervous system involvement with lymphoma. There was also no preceding viral illness or vaccination to suggest a post-infectious acute disseminated encephalomyelitis. Her mental status remained abnormal for 7 days. Her symptoms were completely resolved within 2-weeks, and in this period her blood pressure remained within its normal limits. Follow-up MRI, on day 14, showed significant improvement even though some minimal residual abnormalities remained in the same regions. Subsequent chemotherapy, commenced on day 31, with methotrexate and cytosine arabinoside was performed without any neurologic complications. Conclusion: Although the pathogenesis of PL might be multifactorial in our patient, it is likely that tumor lysis syndrome exacerbated by combination chemotherapy played a major role in the development of PL. Subsequent chemotherapy given without any neurologic complications also supports our theory. Physicians employing combination chemotherapy especially for lymphoid malignancies should remain alert to PL in association with tumor lysis syndrome. Prompt diagnosis, symptomatic and supportive therapies are the mainstays of treatment, and chemotherapeutic regimens can be continued after complete recovery from PL and tumor lysis syndrome.

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CLINICOPATHOLOGICAL FEATURES OF PATIENTS WITH EXTRANODAL LYMPHOMA:

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Purpose: To study the main clinicopathologic features and outcome of patients with non-Hodgkins lymphoma which was presented in extranodal involvement, retrospectively. Material-Methods: A total of 40 patients with extranodal non-Hodgkin lymphoma (NHL) diagnosed in the period between 2001 and 2004, were used to describe the clinicopathological of extranodal lymphomas. Results: Male/female ratio was 26/14. Median age was 50 years (range, 9-79 years). The most common lymphoma observed was diffuse large B-cell lymphoma (60%) followed by marginal zone lymphoma (10%). The most common extranodal sites were Waldeyer`s ring (25%), maxillo-orbital region (15%), stomach (17.5%) and bone (12.5%). The rates of early stage disease (stage 1-2) and advanced disease (stage 3-4) in the patients were 75% and 25% according to Ann-Arbor staging, respectively. The presence of B symptoms was found in 13 patients (32.5%). Twenty eight patients (70%) was treated anthracycline-based chemotherapy with. Nine patients (22.5%) were treated with anti-CD20 monoclonal antibody.Overall response rate was 85 % (complete response 47.5%, partial response 37.7%). Twenty one patients (52.5%) received involved-field radiotherapy. The median follow-up time was 16 months (range 2-65 months). Four patients were lost the follow up and 2 patients died due to

disease progression. In patients having complete response, the median disease-free survival was 18 months (r,4-64 months). The median overall survival times were 15.5 months (r,2-64 months) and 24 months (r,4-64 months) in all patients and in the complete responders, respectively. Conclusion: In the previous studies it was determinend that the most common extranodal site was gastrointestinal tract on the contrary in our small series Waldeyer`s ring was found the most common primary region as it was interesting.

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RETROSPECTIVE ANALYSIS OF ADULT ONSET HISTIOCYTOSIS X SYNDROMES

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Background and aim: Histiocytosis X syndromes have been described as the range spectrum disorders of Langerhans Cell Histiocytosis such as Eosionophilic Granuloma, Letterer-Siewe syndrome and Hand-Schull-er-Christian Disease. These disorders have been diagnosed mostly at early ages of life and rare entities in adult age groups. There is no standardized treatment modality for these diseases at adult onset although 2-chlorode-oxyadenosine (Leustatin) has been found to be effective in the treatment of patients especially with systemic organ infiltration. In this study, we aimed to evaluate retrospectively the patients with Histiocytosis X followed-up in Ege University Hospital. Methods: Seven patients (4 male and 3 female) patients have been treated at our center between 1995 and 2005. Mean age of patients was 27.5 (18-40) years. Main complaints were classified as bone pain in multiple sites (100%), polydipisa (28%), lung infiltration (14%), oral mucosal infiltration (14%), and cranial nerve infiltration (14%). Two patients were diagnosed as Hand-Schuller-Christian Disease, other were accepted as Eosinophilic Granuloma. There was no bone marrow and any other organ infiltration except lung infiltration in one patient. Bone infiltration was the prominent sign in all patients with ratio of minimum 1 to maximum 7 different sites. Results: All patients have been alive during follow-up period. All patients were treated with radiotherapy except one patient treated with chemotherapy regimen started with vincristine plus dexamethasone and continued with leustatin. Two of 7 patients were treated with combined modality of radiotherapy and chemotherapy. Remaining 4 patients were treated with only radiotherapy. After treatment all patients became asymptomatic compared to diagnostic stage. There was no grade 3-4 hematological or systemic side effects of treatment. Relapses were detected only in 2 patients as new bone infiltrations which responded completely to radiotherapy. Conclusion: Histiocytosis X syndromes have relatively benign course in adult patients and could be treated with either radiotherapy or chemo-radiotherapy successfully.

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THE ANALISIS OF THE BASIC BY-EFFECTS OF A CHEMOTHERAPIC PART OF THE PROTOCOL DAL-HD-90 AT ADOLESCENT AND YOUNG ADULTS WITH HODGKIN`S LYMPHOMA

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Background: the protocol DAL-HD-90 in our research has shown the high efficiency in treatment of adolescent and young adults with Hodgkin`s lymphoma. The complete remission has been achieved at 92.5 % patients. 5y-OS had 95 %. The probability of 5y-EFS has made 79 %. Aims: to study of the basic toxic complications of a chemotherapeutic part of the protocol DAL-HD-90 at adolescent and young adults with Hodgkin`s lymphoma. Patients and treatment: during 1998 - 2003 in this study of 99 patients in the age of from 15 till 29 years with primarily diagnosed Hodgkin`s lymphoma had been included. Patients were allocated into separate groups in conformity with the years: 43 patients (m-22, f-21) -in group of 10-14 years; 36 (m-16, f-20) -15-18 years and 20 (m-6, f-14) -19-29 years. All the patients had received therapy within the program DAL-HD-90 according to group of risk. The toxicity estimation was spent according to scale CTC-NCIC. Results: 3/4 degree of a neutropenia (ANN < 1000/μL) was observed in group of 10-14 years (81.3 %) that is in 1.6 times more often in comparison with group of 15-18 years (51.4 %, ğ=0.038) and in 4.1 times is more often in comparison with group of 19-29 years (20.0 %, ğ=0.001). Average duration of epi-

sodes of a neutropenia of 4 degrees (ANN < 0.5x10 9/L) was 17.3 ± 11.2 days (10-14 years) vs. 11.6 ± 9.6 days (15-19 years) vs. 14.0 ± 2.8 days (20-29 years). Cases of a febrile neutropenia were registered only at 15.3 % of patients developed a neutropenia of 4 degrees. Replaceable therapy by components of a blood was required in infrequent cases (13.3 %). G-CSF has been used in 5.1 % of cases. Mucositis of 1/2 degrees prevailed among all infectious complications (51.1 %, duration 6.3 ± 5.8 days, group of 10-14 years; 80.0%, 7.0 ± 2.8 days, 15-18 years; 83.3%, 10.2 ± 5.0 days, 19-29 years). The Herpes infection has developed in 15 cases, including at younger teenagers (11.6%, duration 5.2 ± 1.1 days) and at the senior teenagers (22.8%, 5.9 ±0.4 days) and at young adult (5.0%, duration of 6 days). Acute respiratory virus infections arose at adolescents of 10-14 years (25.6 %, duration 7.9 ± 4.9 days) in comparison with adolescents of 15-18 years (17.1 %, duration 6.7 ± 2.4 days) and with patients of 19-29 years (10.0 %, 5.3 ± 1.2 days) more often. Neurological by-effects (polineuropatia, obstipation and giddiness) and cardiovascular events (arrhythmia) did not exceed 1 and 2 degrees. Any patient had not developed a renal toxicity. Summary: treatment of adolescents and young adults with a Hodgkin`s lymphoma to program DAL-HD-90 is well transferred. The hematological and not hematological toxicity of 3/4 degrees develops extremely seldom. Therapy can carry out in polyclinic conditions. It promotes a social after treatment of patients and raises economic profitability of program.

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GEMCITABINE FOR RELAPSED, REFRACTORY, ADVANCED HODGKIN`S DISEASE

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Gemcitabine is a nucleoside analog that exhibits ant tumor activity. Gemcitabine has been reported to be active in advanced phases of Hodgkin`s disease. The aim of this study was assessment of the effects that Gemcitabine administration had in patients (pts) with advanced refractory Hodgkin`s disease. We present 7 patients previously treated with chemotherapy, some also with radiotherapy and auto grafting. Prior to Gemcitabine administration, pts received between 1 and 5 (average 3) different chemotherapy regimens (COPP, ABVD, CVP, BEACOPP, DHAP, BEAM, PBSCT). For an initially early stage disease 5 pts also received prior radiotherapy in 2-5 sessions. The period of treatment preceding Gemcitabine administration varied between 1 and 13 years (average 7 years). At the time of commencing Gemcitabine treatment all pts had confirmed stage IV HD (bone involvement-3pts, liver-1pt, pleura-3pts, renal involvement -1pt). Gemcitabine was administered in a 30 min. i.v. infusion 1000mg/sq.m. - day 1, 8, 15, in combination with steroids. Cycles were repeated every 28 days. The total number of cycles administered varied between 3 and 9 (average 6). After 3 cycles of therapy, fatal outcome occurred in 1 pt, caused by acute gastrointestinal bleeding. 3 pts achieved complete remission, 2pts responded partially, manifesting disease progression after cessation of Gemcitabine treatment and the consequentially administered other chemotherapeutic regimens.1/2pt manifested progression of the disease after high dose chemotherapy and PBSCT, and Gemcitabine treatment resulted with stable disease for next 5 mounts Observed toxicity was low, mainly hematological, 1pt developed gr. III thrombocythopenia, 1 pt gr. II, and 4pts gr. I. 2pts manifested leucopoenia gr.II and 1pt gr.III. No infectious complications were observed. We support the assertions that Gemcitabine shows potential in pts with refractory, relapsed HD, as well as in pretreated pts, who did not respond positively to standard chemotherapy options.

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DAL-HD-90 PROTOCOL FOR ADOLESCENTS AND YOUNG ADULTS HODGKIN`S LYMPHOMA: SINGLE CENTER EXPERIENCE

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Background: the analysis for the first time protocol DAL-HD-90 used in Russia for treatment of adolescents and young adults with Hodgkin`s lymphoma is submitted in the present research. Feature of this protocol is reduction of chemotherapy and radiotherapy to minimize toxicity and the risk of late effects. Aims: to study therapy efficiency of adolescents and young adults with Hodgkin`s lymphoma under protocol DAL-HD-90. Methods: during 1998 - 2003 99 patients in the age of from 15 till 29 years with primarily diag-

nosed Hodgkin`s lymphoma have been included in this study. Patients have been allocated in conformity with the years on groups: 43 patients (m-22, f-21) were in age group of 10-14 years, 36 (m-16, f-20) -15-18 years and 20 (m-6, f-14) -19-29 years. All patients have received therapy under protocol DAL-HD-90 according to group of risk. Results: The complete remission (CR) has been achieved at 86/93 (92.5 %) patients. The highest interest of CR has been achieved at adolescents of 10-14 years (97.6 %) that is a little bit less than at the senior adolescents of 15-18 years (90.9 %). Young adults 19 -29 years worst of all have answered (82.4 %). The probability of CR achievement was authentically above in group of 10-14 years in comparison with groups 15-18 (ğ=0.037) and 19-29 years (ğ=0.004). At younger adolescents of a tumor progression on a background of treatment it has not been registered while at grown-ups it has been seen in 6 (12.0 %) cases. During too time the number of relapses at younger adolescents (14.2 %) in 2 times exceeded those at the senior adolescents and young adults (6.6 % and 7.1 %, accordingly). The 5-years overall survival (5y-OS) in all researched groups had made 95 %. 5y-OS (median of observation -37.8 months) was the highest at the senior adolescents (100 %). In two other groups 5y-OS appeared below and had made at adolescents 10-14 years -93 % (a supervision median of 70.3 months) and at persons of 19-29 years -91 % (a supervision median of 19.3 months). 5-years event free survival (5y-EFS) in all age groups has made 79 %. The probability of 5y-EFS in I (84 %) and III therapeutic groups (82 %) had practically identical. The lowest it had at patients from II therapeutic group -74 % (ğ=0.4). Conclusion: results of our study have proved high efficiency of protocol DAL-HD-90 in treatment of adolescents and young adults with Hodgkin`s lymphoma.

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SIMULTANEOUS OCCURRENCE OF HODGKIN`S LYMPHOMA AND TUBERCULOSIS: A CASE REPORT

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BACKGROUND: Tuberculosis (TB) has been extensively described in association with malignancies including Hodgkin`s lymphoma (HL). Constitutional symptoms of fever, fatigue and weight loss can present either due to TB or HL which may coexist. The signs and symptoms of the two disease are similar, causing the late recognition of either one of these diseases. We report here a female patient of simultaneous HL and TB infection. CASE REPORT: A 21-year-old female was admitted with 6 months history of progressive right cervical and supraclavicular lymphadenopathy. She also reported the loss of 10 kg of body mass in a period of 3 months, skin itching, fatigue, and drenching night sweats. She complained of fever, chills and cough for 3 weeks before admission. The physical examination revealed painless lymphadenopathy (1-3 cm) in the right cervical and supraclavicular regions. The erythrocyte sedimentation rate was 71 mm/h. Thorax CT showed mediastinal multiple lymph node enlargement up to 2 cm in size, and diffuse infiltrations within the right lung. Abdominal CT disclosed hepatomegaly, and paraaortic lymph nodes up to 2 cm in size. The biopsy of the right cervical lymph node revealed nodular sclerosis HL. The patient was clinical stage III B according to Ann Arbor classification. TB was suspected, and sputum specimens, bronchoalveolar lavage fluids were also evaluated for acid-fast bacilli. The diagnosis of TB was based on smear positivity for acid-fast bacilli, and after culture was confirmed as Mycobacterium tuberculosis. The patient started treatment with ABVD chemotherapy regimen consisting of adriamycin, bleomycin, vincristine, dacarbazine, and she received anti-TB regimen consisting of isoniazid, rifampicin, streptomycin and ethambutol. The patient presented good tolerance to chemotherapy and tuberculostatic drugs. After 3 courses of ABVD, complete remission (CR) was obtained. Three additional courses of ABVD were administered and anti-TB treatment was continued for 9 months. At present, the patient remains in CR. CONCLUSION: The association between TB and neoplasm is well known, but now is very rare. HL is accompanied by changes in the cell immunity, mainly of delayed type. TB can precede HL or complicate its evolution during a simultaneous development of both diseases. The clinical presentation is misleading because the signs and symptoms of both diseases, such as fever, weakness, night sweats and weight loss, are resembling. X-ray or CT imaging are the first choice in visualization of the lesions, but are not specific for the differentiation between TB and lymphoma. An essential factor in deciding both therapy and prognosis of the malignant lymphoma is to diagnose the presence of a TB, for the concurrent treatment of primary and secondary disease provides the only chance of cure. We conclude that the possibility of concomitance TB should be kept in mind in patients with a lym-

phoma, especially in countries where TB is highly prevalent.

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TWO CASE REPORT OF PRIMARY EXTRANODAL LYMPHOMA EXTENDING ORBITAL WALL

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Non-Hodgkin`s Lymphoma (NHL) is the second most common neoplasm in the head and neck region after squamous cell cancer. In, NHL, the head and neck region is the second most common site of extranodal lymphoma after the gastrointestinal tract. The common lymphomas observing in the nasopharynx and waldeyer` s tonsillar ring are the follicular lymphoma, mantle cell lymphoma (MCL), small lymphocytic lymphoma. We are reporting primary MCL of nasopharenx displaying orbital wall involvement and marginal zone B-cell lypmhoma of the lacrimal gland of the orbita in two patients. Case 1: A 63-year-old man was referred to hematology clinic at Celal Bayar University for evaluation and treatment of left orbital swelling from surgical services after results of biopsy from hard plate was made MCL. On physical examination, he had bilateral tonsillar hypertrophy and multiple lympadenopathy in the abdomen, submental, axillar region.The patient was admitted stage IIIE. The treatment in this patient was given 8 cycles of CHOP chemotherapy. After the chemotherapy, he was accepted partial remission. Then he will be radiotherapy. Case 2: A 63-year-old woman was referred to eye disease clinic at Celal Bayar University for evaluation because of right orbital swelling. Magnetic resonance (MR) was demonsrated a mass located in lacrimal gland of the right orbita. At the result of biopsy, marginal zone B-cell lymphoma was revealed. The patient was admitted stage IE. Chemotherapy and radiotherapy have been planned. According to authors` experiences, In the head and neck lymphomas may be given chemotherapy,radiotherapy or combined treatment modality. But there is no consensus about which treatment will give. We discussed here treatment modality of these patients.

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A CASE OF POSSIBLE RECURRENT SECONDARY HEMOPHAGOCYTIC SYNDROME

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Introduction: Hemophagocytic syndrome (HPS) is characterized by the activation of the mononuclear phagocytic system with prominent hemophagocytosis in the bone marrow and reticuloendothelial systems. This may result in pancytopenia, fever, organ enlargement, neurological dysfunction and disseminated intravascular coagulation. Two forms of HPS have been well characterized, primary/familial hemophagocytic lymphohistiocytosis and secondary HPS. Secondary HPS is usually associated with a variety of infections, malignancies, drugs, autoimmune diseases, and various immundeficiencies. What we describe here may be a recurrent and self-limiting secondary hemophagocytosis. Case: The patient is a 5.5-year-old girl complaining of fever that lasted for 1 week, fatigue, epistaxis and cough. When she was 7-months-old she was admitted to another hospital for febrile convulsion and her transaminase levels were possible malign transformation. In case of a progression established very high, platelets count low. Her liver like in our patient, malign transformation must be kept biopsy was determined normal. But with every episode in mind. of infection she has recurrent elevated liver enzymes and thrombocytopenia. In admission her physical examination showed pallor, petechiae in the mouth, red tympanic membrane, and hepatomegaly (5.5 cm below costal margin). Her leukocyte count was 900/mm3, hemoglobin (Hgb) 11.1g/dl, and platelet count 21000/mm3. Blood chemistry revealed aspartate aminotransferase (AST) 2591 U/L, alanine aminotransferase (ALT) 849 U/L, lactic dehydrogenase (LDH) 5892 U/L, triglyceride 460 mg/dl. Fibrinogen assay was 213 mg/dl, and ferritin was 20502 mg/dl. Her Hgb level detoriated to 4.8 g/dl in two days. Bone marrow aspiration showed marked histiocytic hemophagocytosis, affecting all 3-cell lines. Bacterial cultures and viral serology was normal. The chest radiograph showed infiltration in the basis of the right lung. The patient received only supportive care, including replacement of blood components and antibiotic therapy for otitis media and secondary pneumonia. On day 11 fever was under control and hepatomegaly regressed (2 cm). ALT, AST,

triglyceride, ferritin was decreased (142 U/L, 87 U/L, 124 mg/dl, 152.9 mg/dl), Hgb (11 g/dl), WBC (4300/mm3) and Plt (280 000/mm3) count was also normalized. In 2nd month hepatomegaly was persisted (3 cm) but Hgb (12.4 g/dl), WBC (6400/mm3), Plt (223 000/mm3), ALT (30 U/L), AST (41 U/L), ferritin (28 mg/dl) triglyceride levels (89 mg/dl) were normalized. Conclusion: HPS is a rare but fatal disorder of the child. Differentiation between primary and secondary HPS is extremely important. As in our case, a patient with secondary infection associated HPS could be managed with efficient supportive care and treatment of the underlying diseases. During investigation of the family history, we found out that the patient`s aunt had similar symptoms, though much milder; which lead us to consider the possibility of another subtype of HPS, that aggravates after an infection, and could be due to a different mutation that is not yet defined.

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MULTICENTRIC HYALIN VASCULAR TYPE CASTEL-MAN`S DISEASE CONVERTING TO LARGE B CELL LYMPHOMA

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Castleman`s disease can histopathologically be classified into three groups such as hyalin vascular, plasma cell and mix types. It can also be grouped as localised and multicentric clinically. Even though generally it has benign ongoing, in multicentric types the risk of malign lymphoma transformation increases. The total excretion of the mass in hyalin vascular type may provide total cure, whereas the mortality rates increase if malign lymphoma develops. Bilateral servical mass which was progressively growing and systemic symptoms were detected in a 66 year old female patient who had been diagnosed as hyalin vascular type Castleman`s Disease. The histopathological examination of the excisional biopsy was harmonious with large B cell lymphoma. After administration of RT to the servical area, the mass had resolved. Finally, although castelman`s disease is rarely seen it must be evaluated and followed carefully because of a Immunohistochemically CD20 positive areas 4x

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MICROALBUMINURIA IN HODGKIN`S DISEASE

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In some malignant disorders it was reported that urinary albumin excretion (UAE) was correlated with the prognosis and extent of the disease. In this study 24 hour UAE was determined in 34 Hodgkin`s disease patients without prior treatment and 19 healthy controls. Microalbuminuria (MAU) was defined as UAE >20 µg/min. In patients with MAU, UAE was determined again after the treatment. Mean UAE was 31.2 µg/min in the patients group and 5.6 µg/min in the controls (p=0.005). While MAU frequency was 47% in the patients, there was no MAU in the controls. Mean UAE tended to be higher in advanced stage patients compared to early stage (p=0.051). Also MAU frequency tended to be higher in the advanced stage group compared to the early stage (p=0.196). In four patients that remission could not have been achieved, although UAE was reduced, MAU was not disappeared. In conclusion UAE was increased in Hodg-kin`s disease. However there is no significant correlation between the UAE and the disease extent.

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HODGKIN`S DISEASE PRESENTATION IN A THALASSEMIC PATIENT

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Thalassemia and malignant lymphoprolipherative diseases have many common symptoms such as splenomegaly, hepatomegaly or sometimes lymphadenopathy which the last two are mostly due to the extramedullary hematopoetic nature and sometimes to secondary hemosiderosis in thalassemia disease. These symptoms usually do not raise suspicion of malignancies in thalassemic patients, especially because there are rare thalassemic cases having coexisting malignant diseases.

So far in the worldwide literature only 5 thalassemia and coexisting lymphoma have been reported. Here we describe a case of mixed cellularity hodgkin disease with bone marrow involvement and B symptoms which occured in a 22 years old homozygous beta thalassemia patient with mutation type IVS 1-1/IVS 1-6. One of the cases in the literature was described with the interaction of hydroxyurea usage in a sickle cell patient. Our case did not have any drug interaction including hydroxyurea which would cause susceptibility to lymphoprolipherative diseases. She was splenectomised long before and suffered from generalised lymphadenopathies since 3 years and refused a lymph node biopsy thinking that the most common reason for these was extramedullary hematopoiesis, though warned about the possibility of other diseases also. Hodgkin`s disease was diagnosed from cervical lymph node biopsy which she accepted after it was noticed in pelvic ultrasonography that she had conglomerated lymph nodes in the pelvic region as well (Figure 1). Her treatment with 8 ABVD cycles produced greater than 75 % recovery in the disease with only some residual mediastinal lymphadenopathies which are going to be tested with PET CT for active disease in these regions. Our future plan for this patient would be involved field radiation to the residue sites, or search for a HLA identic unrelated stem cell donor. This case should suggest for careful diagnostic assesment of lymph node enlargements in thalassemic patients and may point out to the significance of case-control studies for lymphomas in thalassemic patients. Figure 1. HE Staining of the cervical lymph node.

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ALK-POSITIVE ANAPLASTIC LARGE CELL LYMPHOMA WITH LEUKEMIC PERIPHERAL BLOOD INVOLVEMENT AT PRESENTATION: CASE REPORT

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Although anaplastic large-cell lymphoma (ALCL) frequently presents with disseminated disease and extranodal involvement, leukemic peripheral blood involvement is uncommon and leukemic presentation is very rare. Leukemic involvement may occur at the time of initial diagnosis or develop during the course of disease. We describe a 24-year old female presented with weigth lost, dispnea and fever. Physical examination revealed bilateral axillary, left supraclavicular, bilateral inguinal painful lymphadenopathies and hepatosplenomegaly. Complete blood count showed that hemoglobin was 9.7 g/dL. She had leukocytosis (54x109/L) and thrombocytopenia (78x109/L). Examination of peripheral blood smear revealed 63% of leukocytes were atypic lymphoid cells. Flow cytometric examination of peripheral blood revealed that these cells were positive for CD45, CD2, CD7, CD8 and HLA-DR. CT scans showed generalized lymphadenopaties, hepatosplenomegaly and bilateral pleural effusions. Microscopic examination of axillary lymph node revealed a diffuse neoplastic infiltrate composed of pleomorphic large cells with prominant nucleoli and eosinophilic cytoplasm. These cells were positive for CD43, CD30 and ALK by immunohistochemistry. Based on these findings, a diagnosis of CD30-positive anaplastic large T-cell non-Hodgkin`s lymphoma with leukemic peripheral blood involvement was made. Hyper-CVAD chemotherapy was given. But shortly after chemoterapy, the patient died due to pulmonary infection.

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A PATIENT WITH DIFFUSE LARGE B-CELL NON-HODGKIN`S LYMPHOMA AND AA-TYPE AMYLOIDOSIS

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The association of non-Hodgkin`s lymphoma (NHL) with AL amyloidosis is well known. However, AA amyloidosis with diffuse large B-cell (DLBCL) is extremely infrequent. Here, we reported a 51-year-old woman with AA-type amyloidosis and DLBCL. She had diabetes mellitus type 2 and thalassemia trait. Physical examination revealed hepatosplenomegaly but there was not any peripheral lymphadenopathy. Laboratory tests showed hypocrom microcytic anemia and leukocytosis. Beta-2-microglobulin level and erythrocyte sedimentation rate were slightly ele-