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1st Balkan Hematology Days

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Turkish Society of Hematology

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Turkish Society of Hematology

1ST BALKAN HEMATOLOGY DAYS

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1ST BALKAN HEMATOLOGY DAYS

Foreword

Dear Colleagues,

In this supplement issue of Turkish Journal of Hematology we are glad to announce the proceedings and the abstracts of the First Balkan Hematology Days.

Turkish Journal of Hematology has come a long way since its first publication. Now it has a new cover, board and online system. Our journal is published quarterly and accepts many types of publications spanning the field of online review articles, research articles, brief reports, case reports, images and letters to the editor. TJH will continue to make a rapid turn-around time for the peer review process a very high priority. TJH considers the arguments made by authors and considers the timely publication of results as a high priority. The success of Turkish Journal of Hematology is depending on you and this is mostly attributed to your research articles.

The supplementary book of Turkish Journal of Hematology involves both the proceedings of the meeting and the scientific works on hematology of the participants of the 1st Balkan Hematology Days' program. There are 15 proceedings under the topics of Thalassemia, Myeloma, Lymphoma, Stem Cell Transplantation and Frequent non Malignant Hematological Problems from the renowned scientists in their field. Also in this supplement, 84 presentations which were submitted to the 1st Balkan Hematology Days' program are published.

We would like to thank to all speakers, participants and contributors for their support, patience and kindness efforts to realize this book project. We hope that this supplementary book of the Turkish Journal of Hematology will be one of the useful resources available in Hematology.

Best Regards,

Aytemiz Gürgey

Editor Chief, Turkish Journal of Hematology

Turkish Society of Hematology

1ST BALKAN HEMATOLOGY DAYS

November 10, 2006, Friday

08:00 - 09:30 **Thalassemia**
Chairs: Yeşim Aydınok, Dimitris Loukopoulos

- Management of Thalassemia
  Yeşim Aydınok, Turkey
- Management of Sickle Cell Disease
  Dimitris Loukopoulos, Greece
- Prevention and Moleculer Genetic Diagno
  Georgi Efremov, Bulgary

09:30-10:00 *Coffee Break*

10:00 - 11:20 **Myeloma**
Chairs: Zafer Gülbaş, Adriana Colita

- Nontransplant Treatmen Approach in Multipl Myeloma
  Meral Beksaç, Turkey
- Myeloma Bone Disease, Current Treatment Approaches
  Julian Rainov, Bulgary
- Primary Amyloidosis as of 2006
  Adriana Colita, Romania

12:45-13:30 *Lunch*

14:45 - 16:15 **Lymphoma**
Chairs: Muhit Özcan, Gerassimos Pangalis

- Hodgkin's Disease in Pediatric Population
  Banu Aygün, USA
- Treatment of Indolent Lymphomas From Watch and Wait to High Dose Therapy
  Gerassimos Pangalis, Greece
- Current Role of Stem Cell Transplantation in Malign Lymphomas,
  Erkut Bahçeci, USA-Georgi Efremov, Macedonia

16:15-16:45 *Coffe Break*

16:45 - 18:00 **Hematology Education in Balkan Countries**
Chair: Emin Kansu
Panelistler

- Sabri Kemahlı, Turkey
- Adriana Colita, Romania
- Julian Raynov, Bulgary
- Gerassimos Pangalis, Greece

1ST BALKAN HEMATOLOGY DAYS

November 11, 2006, Saturday

08:00-09:30 **Stem Cell Transplantation**
Chairs: Osman İlhan, Boris Labar

- A Hard Task, Performing a Transplantation in a Developing Country
  Boris Labar , Serbia and Montenegro
- Current Regulations for Hematopoietic Stcem Cell Transplantation in Turkey and EU,
  Hakan Göker, Turkey
- Stem Cell Transplantation for Multiple Myeloma
  Athanasios Anagnostopoulos, Greece

10:00-11:20 **Frequent Non Malignant Hematological Problems**
Chairs: Çiğdem Altay, Oliver Karanfilski

- Molecular Pathology of VitB12 Deficiency in Turkish Patients
  Çiğdem Altay, Turkey
- Iron Deficiency Anemi, Why Still an Ongoing Health Problem?
  Oliver Karanfilski, Macedonia
- Chronic ITP, Where Are We Now?
  Reyhan Diz Küçükkaya, Turkey

1ST BALKAN HEMATOLOGY DAYS

Clinical management of thalassemia

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Ege University School of Medicine, İzmir, Turkey

The hemoglobinopathies are among the most common monogenic disorders worldwide. The common hemoglobinopathies are the -α and –β thalassemias where there is deficient synthesis of globin protein, and three structural mutations of the β globin chain, HbS (β6 Glu-Val), HbC(β6 Glu-Lys) and HbE (β26 Glu-Lys). This section focuses on the management of β thalassemia major which is caused by a decrease in the production of β globin chains and resulted in a major health problem of the Mediterranean region.

Pathophysiology of β thalassemia major

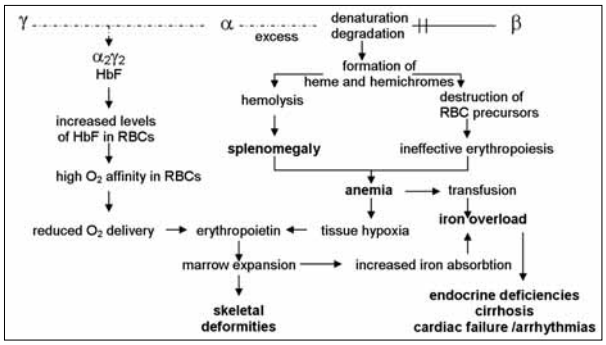

Figure 1. Pathophysiology of β thalassemia major and its main clinical consequences.

Any of more than 200 point mutations and rarely, deletions of β globin gene lead to either an absence of β globin chain production (β^0) or variable reduction in their output (β^+). The management of β thalassemia major is mainly based on the management of major clinical consequences of the pathophysiology of β thalassemia (Figure 1) (1). In severe β thalassemia, oxidation of excess α subunits leads to formation of heme and hemichromes. Hemichromes are precipitated and modify red cell membrane by binding to membrane proteins. After precipitation of hemichromes, heme disintegrates, and iron dependent oxidation of membrane proteins are occurred by releasing toxic, non-transferrin bound iron species. The membrane changes produce rigid and deformed thalassemic red cells resulted in accelerated premature destruction in marrow which calls as ineffective erythropoiesis and entrapment in the spleen. Ineffective erythropoiesis and hemolysis are underlying causes of severe anemia. Anemia, stimulates the synthesis of erythropoietin leading to expansion of ineffective marrow as much as 30 times the normal level. Marrow expansion results in characteristic bone deformities of the skull and face as well as osteopenia. Marrow expansion also leads to increased iron absorption which ultimately contributes to iron overload (2,3). Further understanding of the pathophysiology of β thalassemia may gain further insights in future management of the disease. In fact, increased fragmentation and decreased deformability of thalassemic red cells have been ameliorated during exposure to agents that bind membrane iron in a mouse model (4). Although, hepcidin levels normally increase and inhibit iron absorption in small bowel when iron stores are elevated, hepcidin levels were found to be inappropriately low in patients with thalassemia (5). In that case, administration of hepcidin or agents that increase hepcidin expression may be useful for the inhibition of gastrointestinal iron absorption. While in thalassemia intermedia patients who are not receiving transfusions, increased gastrointestinal iron absorption, depending on the severity of erythroid expansion, results in increases in iron burden, in thalassemia major, iron loading pre-

dominantly derives from blood transfusions but excess iron absorption may also contribute. The combination of iron overload and increased outpouring of catabolic iron from the reticuloendothelial system overwhelm the iron binding capacity of transferrin, resulting in the emergence of toxic non-transferrin bound plasma iron (NTBI). NTBI promotes the formation of free hydroxyl radicals and accelerates the peroxidation of membrane lipids. Both lipid peroxidation and TGFβ-1 expression resulted from iron overload may promote hepatic injury and fibrogenesis (6). It is reported that, transferring-iron uptake by heart cells is inhibited at high tissue iron concentrations whereas NTBI uptake is increased and resulted in myocardial lipid peroxidation and abnormal contractility (7). In other tissues, similar mechanisms are likely to be involved and resulted in hypogonadism, diabetes, hypothyroidism and hypoparathyroidism without an effective iron chelation therapy.

Transfusion and Splenectomy

Transfusion Decision: Although life long transfusion therapy is the cornerstone of the treatment for most patients with homozygous β thalassemia, the decision for initiation of regular transfusion should be taken carefully. Regular transfusion regimen should only be started if the patient can not maintain hemoglobin level of 7g/dl or higher and/or suffered from growth impairment and/or progressively increased in spleen size. Genotyping is only rarely helpful in this decision.

Transfusion Therapy: It was shown that increased erythroid activity over 5 times normal with a high degree of ineffective erythropoiesis is associated with increased iron absorption and progressive iron loading (8). These degrees of erythroid expansion are also associated with impaired bone production (9). Once transfusion decision is established, regular transfusion program by using packed RBCs is initiated not only for correction of anemia but suppression of erythropoiesis which is resulted with the prevention of skeletal deformities and splenomegaly and inhibition of increased gastrointestinal absorption of iron. “Hypertransfusion” and “Supertransfusion” regimens, in which pretransfusion hemoglobins are maintained above 10 and 12 g/dl, respectively, were shown to be associated with achievement of these goals (10,11) but are also associated with substantial iron loading. Ultimately, moderate transfusion regimen with a target pretransfusion Hb>9–9.5 g/dl are found to be associated with both adequate marrow suppression up to two to three times of normal and relatively lower rates of iron accumulation (12). Although neocyte (young red cells) transfusion can reduce the blood requirements and the transfused iron in the patients with thalassemia major (13), it has not been implemented widely.

Complications of Transfusions: The patients with thalassemia major are under the risk of complications of blood transfusions like other transfusion dependent anemias. The main complications of blood transfusions are blood-borne infections, transfusion reactions and haemosiderosis.

Blood-borne infections: The safety problem of blood supply has been reported by the World Health Organization that each year 8-16 million people acquire hepatitis B and 2.3 – 4.7 million acquire hepatitis C, and 80.000 – 160.000 get infected by HIV by means of infected blood on a worldwide basis (14). HBV becomes chronic in about 5% of the population infected.

Thalassemia major patients should be immunized for HBV, before infection, by vaccination. HCV becomes chronic in 70 -80% of infected individuals and 20% of chronically infected patients will develop cirrhosis within 10 years. HCV antibodies are found in 85% of multitransfused Italian patients (15) and a one third of thalassemia major patients have anti-HCV in USA (16). According to data collected from 12 thalassemia centers covering 1000 patients with thalassemia major in Turkey, the overall frequency of the patients with HBsAg and anti-HCV were found 2.5% and 12.6% respectively. The presence of HCV antibodies was found in much lower prevalence than that reported in Italy and USA. This may be a result of the relatively young age of our patients who were born after the screening of blood products for HCV (1996). The confirmed anti-HIV positivity was appeared 0.2% in the study group and consistent with the low frequency of the disease among blood donors in Turkey (17). Furthermore, a recent epidemic of transfusion transmitted West Nile virus in US indicates that the emergence of new pathogens is always possible (18).

The improvement of safety of blood supply by donation programs providing recruitment of enumerated donors, the adoption of questionnaires containing direct questions on risk factors for transfusion transmissible infections, screening

tests for major blood-borne viruses (HBV, HCV and HIV) would decrease the risk of transfusion transmitting viruses through blood transfusions. Haemovigilance program has a key importance in follow up blood safety.

Continuous monitoring of transfusion transmissible infections in patients should be performed at 6 monthly intervals. Treatment with IFN shows treatment effectiveness in 25-30% of thalassemia patients with chronic HCV hepatitis. Patients with thalassemia have generally been excluded from receiving optimal combination therapy (IFN/RBV or PegIFN/RBV) because of concern about their anemia being exacerbated by RBV. At this time, the combination therapy (IFN/RBV) the combination therapy is considered in patients with thalassemia who fail to respond to IFN or relapse after therapy, have advancing fibrosis or are scheduled for BMT (19)

Transfusion reactions: The patients with thalassemia major should be transfused with leukocyte-depleted packed RBCs for reducing incidence of febrile non-hemolytic transfusion reaction (FNHTR). The patients who suffer from severe allergic reactions related with sensitivity to plasma proteins of donor blood should have pre-medication prior to transfusions and who have sustained allergic reaction despite pre-medication or develop anaphylactic reactions should be transfused with washed packed RBCs. Alloimmunization is another common transfusion reaction resulted with delayed haemolytic transfusion reaction (DHTR) in thalassemia major. The specificity of most common alloantibodies found in the patients with thalassemia major are as follows; CcDEe, Kidd, Duffy, Kell and MNS. It is suggested that the patients who were started regular transfusions after 12 months old are more readily to develop alloantibodies and better-match policy in transfusion programmes, including at least Rhesus (CcEe) and Kell antigens, is recommended for all thalassemics who start to transfusion therapy after 12 months (20).

Hemosiderosis: A unit of packed RBC contains approximately 200 mg iron and in the absence of chelation therapy, regular transfusion program in thalassemia major causes accumulation of iron at the rate of 0.4 mg/kg/d which is approximately 10 g iron per annum for an adult patient who weighs 70 kg. The consequences and treatment of hemosiderosis will be discussed in details.

Splenectomy: Because of the risk of post-splenectomy infection, splenectomy should not be considered before 5 years of age. The adoption of more effective transfusion regimens in β thalassemia major has delayed the development of splenomegaly and hypersplenism; thus, splenectomy is currently postponed until the second decade of life or even later. Splenectomy has been recommended when blood consumption required to maintain pretransfusional haemoglobin of 9-9.5 g/dl, is more than 200-250 ml/kg/year of packed RBC (21,22). Before attributing the increment in blood consumption to hypersplenism, the presence of DHTR and poor quality of transfused units should be excluded as possible underlying mechanisms of increased blood consumption. Splenic embolization (23) and partial splenectomy (24) have been followed by recurrence of hypersplenism, and total splenectomy by open technique or laparoscopic approach is recommended at present. The laparoscopic splenectomy is associated with quicker return of bowel function, a shorter hospital stay and improved cosmetic result without a higher financial cost and is recommended whenever possible (25). The accessory spleens should be removed and cholecistectomy should be considered when indicated during the splenectomy procedure. Liver biopsy should also be obtained for histopathological evaluation as well as estimation of liver iron concentration (LIC) in biopsy specimen. The splenectomized thalassemic patients have increased susceptibility to overwhelming post-splenectomy infections with a higher mortality rate than patients with post-traumatic splenectomy (5.1% vs 1.1%) (26). Encapsulated microorganisms including Streptococcus pneumoniae, Haemophilus influenza and Neisseria meningitides are most frequently responsible for the sepsis. A set of Guidelines published by British Committee of Standards in Hematology for the prevention of post-splenectomy infections included immunoprophylaxis, antibiotic prophylaxis and patient education. Polyvalent anti-pneumococcal vaccine, anti-Haemophillus influenza type B and influenza vaccines and optionally anti-meningococcal vaccine should be given 2 weeks before splenectomy or as soon as possible after it. Re-immunization with anti-pneumococcal vaccine should be performed every 5 years and every 3 years in patients younger than 10. Antibiotic prophylaxis with penicillin, amoxicillin or erythromycine is suggested for the first 2 years after surgery and until 16

years of age for the younger children. Early antibiotic therapy is initiated in the patients suffered from fever and malaise. Parenteral antibiotherapy should be administered to the patients with high fever of unknown origin (27). Splenectomy is usually followed by mild, symptomless thrombocytosis in 75% of the patients, reaching 1000 $x10^3$/L in 15% of them. Splenectomised patients have higher prevalence of thrombotic events like pulmonary embolism, deep venous thrombosis and portal vein thrombosis and should have prophylaxis when they are exposed to thrombotic risk factors (surgery, prolonged immobilization and delivery). The high platelet count after splenectomy makes the antiaggregant long-term treatment (low dose aspirin) worthwhile (28).

Iron chelating therapy

Iron accumulation is about 0.4 mg/kg iron per day in splenectomised and 0.5 mg/kg per day in non-splenectomised patients with transfusion dependent anaemia. The aims of iron chelation therapy for iron overload are to induce sufficient iron excretion to achieve safe tissue iron levels which are not harmful and to detoxify the excess iron until the first objective is achieved. In that case, the management of iron chelation therapy demands accurate assessment of iron burden.

Assessment of iron burden: Serum ferritin and liver iron have been the favored surrogate markers of total body iron. The serum ferritin concentrations are convenient but highly unreliable surrogate marker of iron burden, particularly in thalassemia intermedia or associated liver disease. Since most of the total body iron is found in the liver (29) the liver has become the ideal target organ for monitoring the chelation treatment of the patients. Direct measurement of liver iron in the biopsy specimen has been accepted as the “gold standard” reflecting total body iron. Furthermore, liver biopsy gives an opportunity for assessment of histopathology. However, the liver iron content in biopsy specimen obtained from the patient with moderate to severe fibrosis and cirrhosis is underestimated since iron is not stored in scar tissue. Furthermore, the accuracy of liver iron assessed in the tissue less than 0.4 mg dry weight is unreliable (30). Therefore, an accurate and non-invasive measurement of liver iron would be preferable to biopsy. Super-conducting Quantum Interference Device (SQUID) provides a direct measurement of hepatic storage iron but is a barely available technology, expensive and very difficult to operate. It is, therefore, not practical for wide clinical use (31). A magnetic resonance imaging technique (R2 MRI) has now been developed that enables safe non-invasive measurement of liver iron concentrations (LIC) greater than 1.8 mg/g dry weight (32). Iron induced cardiac failure remains the leading cause of death in thalassemia patients. Hepatic iron concentration may not accurately represent iron deposition in the heart. Therefore, non-invasive techniques for the measurement of myocardial iron may help to be gained further insights into chelation therapy of the patients. The application of myocardial T2* which can be measured with gradient echo images that are more sensitive to iron levels appears to be useful for measurement of myocardial iron in thalassemia (33).

Initiation of Chelating Therapy: It is advised to initiate iron chelation therapy based on LIC obtained after 1 year of regular transfusions. R2 MRI is preferable to biopsy as a non-invasive method. Normal concentration of liver iron is less than 1.6 mg/g dry weight. Iron chelation therapy is initiated when LIC is above 3.2 mg/g dry weight. If R2 MRI is not available, direct measurement of LIC in liver biopsy is recommended for the decision of the initiation of the therapy. However, iron chelation therapy is initiated after 1 year regular transfusions and when serum ferritin reaches around 1000 μg/L, in practice by most clinicians. In that case, measurement of LIC could then be reserved when necessary in the management of iron chelation (etc. suspicion about reliability of serum ferritin concentrations in reflecting actual body iron burden in individual patient).

Management of iron chelating therapy: Because the magnitude of the body iron burden seems to be the principal determinant of clinical outcome, the prime goal of iron chelating therapy in patients with thalassemia major is to maintain an “optimal” body iron corresponding to hepatic iron concentrations. Chelation therapy should not be aimed at maintenance of a normal hepatic iron (0.2-1.6 mg/g d.w.), as this greatly increases the probability of chelator related toxicity (34). On the other hand, LIC exceeding 15 mg/g dry weight is associated with an increased risk of cardiac disease and early death (35). Concepts about desirable target ranges of tissue iron with chelation therapy have been based partly on observations in patients with heterozygous hereditary hemo-

Table 1. Management of DFO therapy in Thalassemia Major

Age (years)	*Iron Burden*		*Chelation Days (x / wk)*	*DFO dose (mg/kg)*
	Ferritin (µg/L)	*LIC (mg/g d.w.)*		
<5	<500	<3.2	-	-
	500 - 1500	3.2 – 7.0	X5	25
	1500 - 2500	7.0 -15.0	X6	35
	>2500	>15.0	X7	35
5-10	<500	<3.2	-	-
	500 - 1500	3.2 – 7.0	X5	40
	1500 - 2500	7.0 -15.0	X6	40- 50
	>2500	>15.0	X7	50
>10	<500	<3.2	-	-
	500 - 1500	3.2 – 7.0	X5	40
	1500 - 2500	7.0 -15.0	X6	40 -50
	>2500	>15.0	X7	50 - 60

chromatosis enjoy a normal life expectancy while having mildly increased LIC (3.2 – 7.0 mg/g d.w) (36). The patients with homozygous hereditary hemochromatosis have higher iron burdens about 7-15 mg/g liver d.w. and are considered to be at an increased risk of hepatic fibrosis, diabetes mellitus and other complications of iron overload (37). Therefore, a realistic goal with chelation therapy is to achieve LIC in the range of 3.2 -7.0 mg/g d.w. The serum ferritin concentration corresponding to the optimal range of LIC in thalassemia patients are less clearly defined. The maintenance of serum ferritin concentrations between 500–1000 µg/L is recommended to prevent iron induced complications. Cardiac dysfunction is mostly attributable to myocardial iron deposition. Myocardial T2* levels less than 20 ms indicate iron overload, and this is considered severe when T2* is less than 10 ms. Most recorded cases (89%) of heart failure in thalassemia have occurred in patients with very low T2* values. However, cohort studies have failed to show any significant relationship between liver R2 and heart T2*MRI. In accordance with this finding, there is no significant relationship between LIC or ferritin and LVEF (38,39). These finding suggest that estimation of myocardial iron by using liver iron content or serum ferritin is not sufficiently reliable for clinical management decisions relating to cardiac risks. The assessment of myocardial iron by using T2* MRI as well as monitoring LIC by using R2 MRI at 1-2 yearly basis can give advantages to keep body iron burden at determined safe levels and to monitor chelation therapy. Meanwhile, serum ferritin levels measured at 3 monthly intervals can be useful for monitoring compliance of the patients to the advised chelation regimen.

Management of Iron Chelating Therapy in Thalassemia Intermedia: Iron loading secondary to increased gastrointestinal iron absorption in patients with thalassemia intermedia is less accelerated than that of transfusional iron overload in thalassemia major (40) and may be similar to that found in homozygous for hereditary hemochromatosis. Serum ferritin concentrations are not reflecting the degree of iron accumulation in thalassemia intermedia and determination of LIC is indicated at modestly elevated serum ferritin levels up to 1000 µg/L. Chelation therapy should be initiated if hepatic iron concentration exceeds 7 mg/g dry weight liver tissue. Usually in untransfused thalassemia intermedia patients, the reduction of LIC is rapid, and desferrioxamine therapy (25-35 mg/kg/g) is rarely required for more than 18 months. Deferiprone has been shown to be rapidly effective in reducing body iron stores in thalassemia intermedia (41)

Iron chelators

Desferrioxamine (DFO, Desferal); has been the standard iron chelator since the 1970s. With the introduction of slow subcutaneous daily infusions over 10-12 hours by using a battery operated pump of this drug in the mid 1970s, the life expectancy of patients with thalassemia major improved dramatically (42). Although, it is both safe and effective iron chelator, the need for painful parenteral administration causes compliance

problems with the chelator. The UK data indicated that about 50% of the adolescent and young adults are not able to adhere to DFO chelation which limits the life expectancy of these patients (43). DFO chelation in patients with thalassemia should be administered at dosages and frequencies adjusted according to the degree of iron overload presented in Table 1. Twenty-four hour continuous infusion of DFO (50-60 mg/kg) in patients with cardiac complications of iron overload can reverse the complications in most patients (44).

DFO is remarkably non-toxic however; DFO toxicity including retinopathy, sensorineural hearing loss in high-frequency and decrease in growth rates is observed primarily in the presence of low iron burden. These adverse effects are preventable if proper monitoring is practiced to detect early signs of toxicity. If LIC is not regularly assessed, a "toxicity index" which is defined as mean daily dose of DFO (mg/kg) divided by the serum ferritin (μg/L), is calculated to prevent DFO toxicity and the ratio should not exceed 0.025 (45). Fundoscopy, electroretinography, audiometer and bone age should be investigated yearly. Sitting and standing height should be measured 6 monthly for monitoring growth velocity and ensuring proportionate growth. Reducing DFO dosage is sufficient in mild audiovisual toxicity but DFO should be interrupted in symptomatic and severe toxicity. The patient is followed 3 monthly until becoming normal or stable then DFO is restarted with dose adjustment. A quick resumption in growth follows reduction in DFO dosing, without the need to stop treatment (46). There is increased risk of Yersinia infection in iron overload and this risk further increase with DFO chelation. It is advised to withhold DFO in febrile patients until the source of the fever has been identified (47). Systemic reactions, with fever, muscle aches and arthralgia are uncommon and anaphylaxis may occasionally occur. Desensitization regimens are described and successfully applied in anaphylactic reactions (48).

Deferiprone (DFP); is an orally active chelator first used in humans in 1987. DFP is widely used regimen of 75 mg/kg per day at three divided doses, up to 100 mg/kg daily. DFP has been licensed in India since 1994. The European Union granted marketing approval for DFP in 1999 under the "exceptional circumstances" policy that requires further studies. DFP achieved full marketing authorization in Europe in April 2002 since that date; DFP can be used when standard therapy is inadequate, intolerable and unacceptable. DFP has been registered in Turkey since January 2004 and has recently been accepted as first line therapy by the MOH of Turkey.

Effectiveness of DFP: Clinical studies with DFP revealed that DFP was capable of decreasing serum ferritin levels and the decline was found significant in most of them (49). However, in a multicenter prospective study, 25% of the patients receiving DFP at 75 mg/kg daily during an observation time of 4 years discontinued therapy because of concerns regarding the long term effectiveness of the chosen dose assessed by serum ferritin levels and/or liver iron concentrations (50). In a 3-year large-scale study performed in Italy, serum ferritin improved in 61%, remained stable in 21% and worsened in 18% of patients (51). In a recent study, a reduction of serum ferritin was observed in 67% of subjects; whereas 33% of the patients randomized to DFP arm were considered unresponsive defined by stabilization or increase of serum ferritin. There are also few published prospective trials comparing the effects of DFP and DFO on LIC. Olivieri and Brittenham (52) reported significant increase in LIC in patients treated with DFP ($p<0.01$), while no significant change of LIC was observed in patients treated with DFO. In a randomized one-year study by Maggio et.al (53), no significant change between the initial and final LIC in both, DFP and DFO treated patients, was reported. Further, Pennell et al. (54) did not detect significant changes in liver iron in patients treated with a higher dose of DFP (100 mg/kg/day) and those treated with standard DFO regimen after a study period of one year. Aydinok et.al (55) noted that a decrease in LIC was achieved in 42% of patients only who were randomized to the DFP (75 mg/kg/day) monotherapy and the overall decrease in LIC was not significant while a significant decrease in LIC was observed in DFO reference group.

Combination therapy of DFP and DFO: It was first reported in 1998 that the combination therapy with DFP and DFO resulted in urinary iron excretion at least equivalent to the sum of iron excretion observed when the two drugs are given on separate days (56). According to the "shuttle hypothesis" described by Giardina and Grady (57) simultaneous administration of DFO and DFP resulted in shuttling of iron from DFP to DFO and clinical studies of combination therapy indicated additive or even synergistic effects on total iron excretion. The basis of this effect is that DFP eas-

ily enters cells and subsequently able to transfer the intracellularly chelated iron to the stronger iron chelator, DFO in plasma. The clinical studies in which DFP has been given at a daily dose of 75 mg/kg in combination with DFO (40-50 mg/kg twice weekly) have further shown that the mean serum ferritin levels decreased consistently during the study period in all trials (55,56,58,59,60,61,62 ,63,64,65). A few uncontrolled clinical studies also demonstrated a decrease of the mean liver iron concentration (LIC) following six months or yearly combination therapy with DFP and DFO in comparison to pre-study values (64,66). In a recent prospective randomized study was reported that all patients except one randomized to the combination treatment showed a decrease in LIC and the change in liver iron after one year was significant. Furthermore, the combination regimen was found superior in reducing liver iron if compared to the DFP monotherapy and was as effective as DFO standard therapy in reducing liver iron (54).

Deferiprone and cardiac iron: The cardioprotective effect of iron chelation therapy is a critical feature for evaluating the efficacy because iron induced heart disease remains a cause of death in thalassemia. In a recent prospective randomized trial, Pennell et.al (54) compared higher doses of DFP (mean 92 mg/kg/d) to standard DFO in the patients with thalassemia major. The decrease in cardiac iron was significantly higher to DFP than to DFO. In addition there was a greater increase in LVEF in DFP group compared with the DFO group. Taken together the results of this trial with other retrospective series of Anderson et.al (67) and survival data of Borgna-Pignatti et.al (68), DFP probably better in vivo at myocardial iron than DFO.

Adverse effects: Nausea and other gastrointestinal disturbances are met mainly at the first few weeks & months of the therapy and generally resolved by reducing dose, taking drug with meals and temporary supportive (anti-emetic) therapy. After resolving the symptoms, the dose slowly increases to the target levels. Arthralgia resolves after temporary discontinuation of the drug or reduction of the dose, at a median time of 12 days. Transient fluctuating increase in liver enzyme levels is generally normalized without any intervention. Neutropenia (5%) and agranulocytosis (0.5%) are the most serious adverse effects of DFP therapy and close monitoring (7-10 daily control of WBC & ANC) is required. The reintroduction of DFP after an initial episode of agranulocytosis is not recommended. Plasma zinc levels would also fall and zinc supplementation is needed (49).

Deferasirox (DFX); is a new class oral chelator which is administered once daily dosing permits circulating drug in plasma at 24 hours. It is formulated as dispersible tablet which helps to use easily. Phase III clinical trial confirmed non-inferiority of DFX at doses of 20-30 mg/kg per day compared with DFO and optimal ratio of DFO to DFX is 2 to 1. The maintenance dose of the drug to prevent further iron accumulation, in most but not all patients was 20 mg/kg whereas 30 mg/kg reduced existing body iron stores (69). Transfusional iron loading had a dramatic effect on the ability of DFX doses to maintain or reduce hepatic iron. In that point of view, 20 mg/kg DFX is able to maintain or reduce LIC in the patients receiving transfusional iron intake of 0.3-0.5 mg/kg/d (7-14 ml/kg/month packed RBC) whereas 30 mg/kg may be required in higher blood consumption (70). Based on the Phase III study results of DFX, the United States Food and Drug Administration (FDA) approved the drug for transfusional iron overload for patients older than 2 years of age as first line therapy in November 2005.

We look forward to a feature where the quality of life of our patients is improved with the advances in iron chelation regimen which presents a life to them without complications due to iron overload.

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Management of the vaso-occlusive crises of sickle cell disease on the basis of the underlying pathophysiology

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Acute, dreadful and often fatal vaso-occlusive crises and the associated chronic morbidity and bad quality of life are the main feature of Sickle Cell Disease (SCD). All these result from a substitution of a valine for glutamic acid at position 6 of the β-chain of the human adult hemoglobin ($\alpha_2\beta_2^s$. i.e. HbS) caused by a G to T transversion in the respective triplet of the β-chain structural gene. Because of this molecular change, on delivering the oxygen they carry, the HbS molecules polymerize through the formation of intermolecular weak bonds and produce rod like structures which distort the red cells in a way which makes them resemble a sickle. As sickled cells cannot pass easily through the microvasculature the blood flow is blocked, tissue oxygenation is significantly decreased and pain and damage occurs.

Based on this information, management of the sickle cell disease over the past years aimed at hydrating the patients, offer them more oxygen, analgesics, and confort. However, recent studies have clearly shown that the vaso-occlusive event is more complex than a simple obstruction of the microcirculation by the sickled red cells (a), and that its pathophysiology involves several other mechanisms, such as (b) the interaction of the circulating white cells and platelets with the endothelium, and (c) the function of the underlying vascular muscles. Understanding these issues has opened new issues to the approach of the patient and has already resulted or promise to alleviate the impact of the disease, decrease the frequency and severity of painful crises and improve quality of life. The present communication will review this information, first outlining the pathophysiology of the event, then propose potential solutions and, finally, report, if any, the respective clinical results.

The sicking process

This depends on the following factors:

1. Oxygenation of red cells. As oxy-HbS delivers easily its oxygen to the tissues and de-oxyHbS readily polymerizes any steps towards maintaining a high oxygen tension until red cells pass through the microvessels is of potential therapeutic interest. Oxygen delivery is increased when the temperature rises, when the blood pH is lowered (Bohr effect) and when the atmospheric pressure goes down; there is no doubt that avoiding these conditions may prevent the crises. Increasing oxygen affinity might have a therapeutic effect; examples of agents with this property include **tucaresol** and **3,5-dibromosalicyl-fumarate.** Although low efficacy and side effects do not allow clinical application, there is no doubt that the approach deserves further exploration.

2. Intracellular Hb S concentration. Polymer formation is easier when HbS molecules are in close contact. As a consequence, any steps towards "diluting" the intracellular hemoglobin or decreasing the chances of molecular contact might be beneficial and, in fact, have been explored in various ways which are summarized further below:

(a) ***Increasing the intracellular water***. Earlier attempts used DDAVP, a synthetic peptide with anti-diuretic properties, which causes water retention thus decreasing plasma osmolality and introducing water into the red cells. However, this approach had its own risks and was not further pur-

sued. Instead, over the last years, the use of various agents which prevent cellular potassium and water loss have reached the level of clinical trials. Of these, most promising appears to be **magnesium pidolate**, which improves the function of the Mg^{++} activated K^+ pump and **clotrimazole**, **miconazole** and **charybdotoxine**, all potent antifungal agents, which inhibits the function of the Ca^{++} activated K^+ retaining pump.

(b) ***Decreasing the intracellular HbS concentration***. Considering that patients with SCD have less crises when they carry also one or more a-thalassemia genes, a situation where the cellular MCHC decreases, has led to exploring whether a similar improvement could be obtained by making patients iron deficient, hence lowering the MCHC, by frequent blood lettings. However, despite a positive published report, this approach was not pursued.

(c) ***Decreasing the chances of contact and polymerization*** by dispersing molecules of a non-sickling hemoglobin among the HbS molecules. Patients with sickle cell/β^{++}-thalassemia (hence producing relatively high amounts of HbA) fare much better than those who carry β^0-thalassemic genes; a better quality of life is also the rule for the patients who have the potential to complement their red cell hemoglobin with varying amounts of fetal hemoglobin through enhanced function of their γ-genes resulting from γ-promoter mutations, silencer deletions 3' to the γ-genes and other genetic changes, compared to patients who are lacking this property. Within this context, the possibility to increase HbF synthesis by various exogenous agents has led to intensive research over the recent years. Induction of HbF synthesis can be achieved (a) by inhibiting methylation of gene sequences promoting γ-chain synthesis, (b) by inhibiting histone de-acetylation, or (c) by enhancing promoting sequences 5' to the $^A\gamma$ globin gene. Examples of agents which have these properties include 5'-azacytidine, decitabine and butyric acid respectively; the latter agent may act also by inhibiting histone de-acetylation. At this time, excluding 5'-azacytidine which is a potent but potentially cancerogenic medication, there are several reports of efficacy in patients with sickle cell disease (and/or thalassemia), which show a clear response in terms of HbF increase and, some of them, in terms of preventing vaso-occlusive crises. Of note, that the increase of HbF in the hemolysate does not reflect the true increase in γ-chain synthesis, because red cells with a certain amount of HbF survive better in the circulation and increase the percentage or amount of HbF.

(d) Putting in cycle a population of red cell precursors which maintain the "program" for γ-chain synthesis but are not active in the adult bone marrow unless "recruited" in specific conditions. Acutely stressed erythropoiesis following myeloablative therapy, where the whole population of normally proliferating erythroid precursors is acutely removed is a typical example of this situation and hydroxyurea is the agent which has shown a striking capacity to produce this effect. Obviously,the emerging HbF supplemented red cells have less tendency to sickle and survive better in the circulation. Increasing HbF by hydroxyurea was first reported in 1972 in the animals; results were then confirmed in several sporadic reports in patients with sickle cell disease and were finally concluded in a large double blind series in the US where the systematic administration of the drug in a large number of patients resulted in preventing vaso-occlusive crises or increasing the crisis-free interval, diminishing the incidence of acute chest syndrome, and decreasing the need for blood transfusions. Concomitant studies and a large number of reports which followed confirmed the HbF increasing property of hydroxyurea and established its good tolerance and absence of major side effects in both adult and pediatric SCD patients. Of note, that several subsequent studies showed that the benefit of hydroxyurea derives not only from the HbF increase but also by other interactions which will be presented further below.

B. Cellular adhesion

Participation of white cells, platelets, reticulocytes and endothelial cells

The time required for the HbS cells to pass through the microvasculature and release their oxygen is shorter that than that required for the

completion of the sickling process; therefore, unless this passage were retarded the vaso-occlusion cannot occur. In fact, studies carried out over the past years have confirmed the occurrence of this phenomenon and related it with the abnormal adhesion of the circulating white cells and platelets as well as young red cells onto the endothelia lining the microcapillaries. Why is this abnormal is not clear; the general concept is that this is associated with a continuous mild "inflammation" which underlies sickle cell disease and results in increased expression of various adhesion molecules (integrins, VCAM-1, etc) on the surface of the endothelial cells and enhanced participation of other molecules such as vWF, fibrinogen and thrombospondin; as the latter attract not only an increasing number of leucocytes and platelets which are also "activated" but also numerous young red cells which express high numbers of CD36 molecules on their surface, the blood flow is definitely retarded and sickling occurs.

Preventing or reversing this situation can be achieved by **monoclonal antibodies** blocking integrin $a_v\beta_3$ and glycoprotein Ib/III, **sulfasalazine** inhibiting the expression of the nuclear factor kB (NF-kB, promotes synthesis of several pro-adhesion molecules) etc., and, indirectly, by **hydroxyurea** which lowers the number of circulating reticulocytes, white blood cells and platelets. **Pluronium F-68** and other "lubricant" agents have also been tried.

C. Vascular tone

The function of the underlying muscular sheath

Vasoconstriction is definitely contributing in the retardation of the blood flow and appears to result from depletion of the major vasodilating agent, nitrogen monoxide (NO). The crucial role of this factor has been recognized only recently but has now become the major target of our approach to SCD. Nitrogen Monoxide is produced in the endothelial cells by the conversion of arginine to ornithine by NO-synthase. NO is a gas molecule; it therefore diffuses rapidly through the cellular membranes and causes prompt relaxation of the vascular smooth muscle cells by binding to the heme moiety of the soluble guanylate cyclase which converts GTP to cGMP; the latter activates cGMP-dependent protein kinases which cause intracellular sequestration of Ca^{++} ions and vasodilatation. This mechanism is severely damaged in SCD, where the endothelial NO fails to reach the smooth muscle in the appropriate concentration because it is neutralized by immediate binding to the oxy-hemoglobin molecules which are found in the plasma of the patients as a result of their slight but continuous intravascular hemolysis. In addition, the decreased availability of NO may enhance platelet activation (which is normally inhibited by the gas) and favor coagulation, while the arginase released from the lysed red cells lowers the concentration of plasma L-arginine and deprives the endothelia from the substrate which is needed for the synthesis of NO. As expected, the experimental evidence supporting these mechanisms has rapidly led to various interventions aiming to increase the concentration of free NO (non-hemoglobin bound) in the plasma and thus achieve the much desired vasodilatation. Such interventions include the administration of **sildanefil**, administration of **sodium nitroprusside** or **nitroglycerine**, inhalations of **nitric oxide**, and infusions of **arginine.**

Conclusions

The pathophysiology of sickle cell disease is very complex; in consequence, any steps towards its better understanding may prove valuable for designing novel medications or approaches to palliate its severity.

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Prevention and molecular genetic diagnosis of hemoglobinopathies

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Abstract

The hemoglobinopathies are the commonest monogenic diseases in humans. It has been estimated that 3-4% of the world's population (180-240 million) carry a gene for the important hemoglobin disorders, such as thalassemias (thal), hemoglobins (Hb) S, C and E, the majority of which live in south-east Asia, where the thalassemias are most common, and in Africa with its very high frequency of sickling disorders (WHO 1983,1985,1987,1989). It has been estimated that ~365 000 infants are born each year with major hemoglobin disorders. Because of major demographic changes in the pattern of disease in many of the developing countries, they will pose an increasingly serious health problem in the new millennium. Hence, the great challenge for the future is how to translate our knowledge of the molecular pathology of these diseases into their better control and treatment. Here, I have tried to summarize the present understanding of the prevention (screening and counseling) and molecular genetic diagnosis of hemoglobinopathies.

Over the last 20 years comprehensive control programs have succeeded in limiting the numbers of new births and prolonging life in affected individuals. Such programs have been successful in a minority of countries and have little global impact. Their experience has provided a valuable baseline for future planning for its control in the larger mainland population of the Middle East, Indian subcontinent and south-east Asia.

Avoiding rather than preventing genetic diseases like hemoglobinopathies is the right way in controlling these diseases. They can be avoided by identification of the carriers in the community and providing them with appropriate genetic counseling. There are several different options available to them. They might wish to avoid marrying a similarly affected person. Alternatively, if two carriers marry they could avoid having children altogether, adopt, have children in the hope that they will be fortunate enough not to have a severely affected child, or undergo prenatal diagnosis with termination of those pregnancies in which the fetus has received the abnormal globin gene from both of them.

Screening

Screening must be preceded by an estimation of the gene frequency for hemoglobinopathies in the particular population. It is very important to obtain an adequate overall spectrum of its frequency, and to develop screening programs if they are likely to be cost-effective. The WHO Working Group recommended that in developed countries carrier screening, counseling and prenatal diagnosis services should be provided as part of the general health-care program, whatever the frequency of the disorders. Screening programs in less developed countries is more complicated. If the birth rate of affected infants exceeds 0.1/1000 a screening program should be developed.

Who should be screened? Genetic screening may be retrospective or prospective. Retrospective screening, that is, testing and giving advice to a couple who have already had an affected child, is ineffective and has relatively little influence on the birth rate of new cases of thalassemias. Prospective screening can either be done in the antenatal clinic or at an earlier stage. The most advanced

population screening programs have been developed in Montreal, Sardinia, Greece and Cyprus. The most effective way to develop a national screening program is through schools on a voluntary basis. The screening program in Sardinia has been remarkable successful. It has involved a major public education program via the mass media. Similar public education program has been established in Cyprus. Meetings between pediatricians, obstetricians, family planning associations, nurses and social workers appear to have been an important part of these educational programs. Similarly, the use of educational booklets, have been made available.

Methods for screening. The best screening method for thalassemias is the measurement of the red-cell indices, that is the mean cell volume (MCV) and mean cell hemoglobin (MCH). Of course, the measurement has to be made by electronic counters. It is now generally accepted that blood samples with an MCH bellow 27 pg or an MCV bellow 76-77 fl should be investigated further. However, coinheritance of α-thal allele and the presence of mild β-thal allele might escaped detection of β-thal carriers.

An alternative approach to the initial screening is a single-tube osmotic-fragility screen as a simple approach to carrying out thalassemias screening in large populations developed by Silvestroni and Bianco (1959) and evaluated and modified by Kattamis et al (1981). Based on our 30 years experience, the determination of red cell indices together with the osmotic fragility test (Kattamis et al. 1981) detect almost all the carriers of β-thalassemia. Even so, samples with reduced MCH and MCV values should be studied further. The confirmatory analysis is the determination of the level of Hb A_2. What ever method is used, levels of Hb A_2 above 3.5% are considered as a cut-of figure for a carrier of β-thalassemia. Of course, levels of Hb A_2 of 3.2-3.5% are found in carriers of mild β-thal or in carriers with β-thal interacting with α-thal and they should be further investigated. In individuals with normal Hb A_2 levels and thalassemic red cell indices in whom iron deficiency anemia has been excluded, α-thal or normal Hb A_2 β-thal should be excluded. This can be accomplished by biosynthetic and/or DNA analysis.

Counseling

Before screening is undertaken adequate facilities should be available for transmitting the results and for detailed counseling. A counseling program means that national centers with experts in the field should be organized. Normally these experts are pediatricians and/or hematologists. These centers, one or more depending of the size of the country, should take care for the entire life of their patients. The counseling program should also have hospital and the community counselor and nurse. With the help of simple pamphlets and publications they must explain to the carriers the nature of the disease, the consequences of having children with another carrier. Similar information must be given to women who have been found to be carriers in antenatal screening programs.

A population-based screening program can be successful only if it is backed up by adequate education and counseling. Where these programs are successful the uptake of prenatal diagnosis is over 90%, as was the case with Sardinia (Cao and Rosatelli, 1993) and a province in Iran (Ghanei et al. 1997).

Concerning the cost-benefit consideration it should be pointed out that in view of the increasing number of patients with thalassemias who will survive to require treatment in the future, the results of most cost analyses have strongly supported the use of prenatal diagnosis. Not only do they reduce the number of affected children born, but they also increase the availability of treatment for those who already exist.

The options for couples at risk for having thalassemic children are rather limited. They are: avoid pregnancy, adoption, risk having an affected child, prenatal diagnosis with termination if fetus is affected, pre-implantation diagnosis, and use of egg or sperm donor with normal globin genotype.

Prenatal diagnosis

Following the development of a safe approach to fetal blood sampling (Kan et al, 1974), studies of hemoglobin patterns during fetal development combined with the development of methods for in vitro estimation of globin chain synthesis led to the first attempts at prenatal diagnosis of thalassemias (Kan et al. 1975). This method was used successfully for the prenatal diagnosis of α and β thalassemias and sickle cell disease in many

countries until the middle of 1980 when it was replaced by fetal DNA analysis.

Prenatal diagnosis by direct mutation identification was developed using DNA from amniotic fluid cells in the second trimester, however it was soon replaced by chorionic villus sampling (CVS) late in the first trimester. Even so, methods of DNA analysis, in particular Southern blotting combined with RFLPs or the hybridization with oligonucleotide probes, were complex and time consuming. The development of methods based on PCR made the prenatal diagnosis to be carried out rapidly, simply and cheaply. The remaining of my presentation will summarized the diagnostic strategy of prenatal diagnosis currently in use, also in our laboratory.

Fetal blood sampling and analysis of fetal globin production. The first approach to fetal blood sampling was aspiration of blood from the placenta under ultrasound guidance (Kan et al. 1974a). Although this method was reasonably successful, it yielded a sample consisting of a mixture of fetal and maternal cells, and additional treatments were necessary to enrich the sample with fetal cells. Later on, direct fetal blood sampling by fetoscopy (Rodeck 1980) and cordocentesis yielded pure fetal blood samples, which are incubated with ^{3}H-Leu and the in vitro synthesized globin chains separated by CM-cellulose chromatography. For the diagnosis of β-thal, the ratio of β- to γ- chain production is determined. In a normal fetus β-chain synthesis is approximately 10% of that of γ-chains, giving a β/γ ratio of app 0.1. A fetus with β-thal trait synthesizes about half the normal amount of β globin, giving a $\tilde{\beta}\gamma$ ratio of app 0.06, while a fetus with thal major, depending of the type (β^{+} - or β^{0} - thal) will have $\tilde{\beta}\gamma$ ratio bellow 0.025 or 0. Other important hemoglobinopathies can be diagnosed by this technique, such as Hb Bart's hydrops fetalis syndrome, Hb H disease and structural Hb variants. The CM-cellulose chromatography method gives very reproducible results. However, it is very slow and expensive. It was later on replaced by HPLC, IEF and PAGE in the presence of Triton X-100 and urea. Using these methods, during the period from 1974 to 1989 more than 20 centers performed over 14,000 prenatal diagnosis (Alter 1990, Loukopoulos et al. 1990), of which the majority of cases were for β-thalassemia.

Prenatal diagnosis by DNA analysis. DNA analysis was applied to prenatal diagnosis in the late 1970 and early 1980, first using amniotic fluid cell DNA and, later, chorionic villus DNA. However, fetal blood sampling remained throughout the 1980s because of the complex and sophisticated nature of the DNA analysis techniques. It was not until the introduction of the PCR that many centers switched to DNA analysis. For DNA analysis, first amniocentesis, and later CVS, was used for obtaining fetal DNA. Over the recent years CVS has become the method of choice for prenatal diagnosis, with amniocentesis as a backup for women who present too late for this procedure.

Many different methods of DNA analysis have been used for prenatal diagnosis of hemoglobinopathies. They are: RFLP linkage analysis, Deletion mapping, Restriction-enzyme analysis, Allele-specific hybridization and PCR methods. The RFLP linkage analysis was the first DNA based method to be widely exploited for prenatal diagnosis. One of the first RFLPs to be reported, was Hpa I polymorphism 3'to the β-globin gene, observed to be strongly associated with the sickle cell (βS) gene in West Africans (Kan and Dozy 1978b). It was found to exist in linkage disequilibrium with 70% of Afro-American sickle cell genes and thus homozygous inheritance could be excluded in 70% pregnancies at risk for sickle-cell anemia (Kan and Dozy 1978a). The first RFLP to be found in linkage disequilibrium to a β-thal gene was a Bam HI polymorphism associated with CD39 C→T mutation (Kan et al. 1980). Another useful RFLP for the prenatal diagnosis of β thal in Mediterranean populations is Ava II site (Wainscoat et al. 1984a). RFLP linkage analysis by a family study can also be used for prenatal diagnosis. The idea is to study the parents and any previously born children or other relatives using several restriction enzymes and appropriate gene probes to see if an RFLP can be found on the chromosome carrying the mutant gene in each parent. If so, and if the fetus is homozygous for the particular RFLP, then it must also be homozygous for the particular mutation.

Deletion mapping is the second DNA method that has been and is in use for prenatal diagnosis. Large gene deletions can be diagnosed either by the disappearance of a particular restriction-enzyme fragment on a Southern blot, or by the appearance of a new fragment containing one or both of the brake point ends of the deletion. The

disorders that were detected in this way for prenatal diagnosis included the α^0 thal, the Indian form of β^0 thal due to a 619 bp deletion, the δβ thal and Hb Lepore (Alter 1984).

Restriction-enzyme analysis was the first direct method for the detection of point mutation to be used for prenatal diagnosis, and again the sickle-cell gene was the first target. Several groups observed that mutation in the β globin gene responsible for Hb S alters the DNA sequence which is normally recognized and cleaved by three different restriction enzymes: Dde I, Mnl I and Mst II (Gever at al. 1981; Chang and Kan 1982; Orkin et al. 1982c). However, although this approach is the most useful method for prenatal diagnosis of sickle-cell anemia it is of little value for thalassemia. Only a few rare β-thal mutations create or abolish restriction enzyme sites. The need for a general method for the direct detection of all the β-thal mutations led to the development of the technique known as allele-specific oligonucleotide (ASO) hybridization. This method became very useful after the discovery of PCR. In the original description of PCR it was shown that sickle-cell mutation can be detected in amplified DNA by using radioactively labeled oligonucleotide probes (Saiki et al. 1985). Soon after the description of the PCR method the same group simplified the procedure by using thermostable DNA polymerase, and by varying out the reaction in thermal cyclers (Saiki et al. 1988). However this application of PCR was restricted mainly to experienced diagnostic laboratories, and it was not until the development of non-radioactive PCR techniques that prenatal diagnosis programs began to be established in new laboratories in developing countries.

A simpler method for the identification of β-thal mutaions based on the amplification refractory mutation system (ARMS) was developed by Newton et al. (1989). This utilizes the principle of allele-specific priming of the PCR, in which a primer will only permit amplification to take place when it perfectly matches the target DNA sequence at the 3' terminal nucleotide. ARMS is currently the main approach for prenatal diagnosis of β-thal, Hb E β thal and the sickling disorders (Model et al. 1997).

Recently, reverse dot blotting has been developed (Maggio et al. 1993). In this method probes for the specific mutations are fixed to a nylon filter and then hybridized together in one reaction to labeled, amplified genomic DNA. This permits a large number of mutations to be screened in a single hybridization step. A kit for the detection of several β thal mutations is now commercially available.

Finally, gene deletions can be detected by amplification across the brake points, a technique known as Gap PCR. For the small number of β-thal deletions of under 1 kb in size, the primer pairs spans both brake point ends and generates two products, the smaller fragment arising from the deletion allele.

In conclusion, it should be stressed that if adequate screening and counseling programs are established the majority of families referred for prenatal diagnosis will have already been investigated for their hemoglobin genotype. To provide a comprehensive service, at least one central laboratory in each country should be able to sequence globin genes for those rare cases in which there is evidence of β-thal in the parents, but mutations can not be identified.

The overall results of prenatal diagnosis programs have been discussed by Modell and Kuliev (1998). The most remarkable results have been obtained in Cyprus and Sardinia, where the frequency of births of affected babies has dropped dramatically over the period since screening and prenatal diagnosis was introduced.

What are the recent developments in prenatal diagnosis of thalassemias? The ability to amplify DNA from a single fetal cell has led to the development of alternative methods for prenatal diagnosis, i.e. the use of nested PCR. In short, in the first reaction a pair of primers is used to amplify a short DNA region encompassing the mutation, after which a small aliquot of the product is re-amplified with an internal set of nested primers in a second round of PCR. Mutations can be detected by ASO dot blotting, heteroduplex analysis or restriction-enzyme digestion. This technique has stimulated research directed at prenatal diagnosis by the analysis of fetal cells in maternal blood, an attractive, non-invasive approach to prenatal diagnosis, and also to preimplantation diagnosis. However, it is too early to use these methods on a wider scale. There are still many issues to be addressed but there is no doubt that screening and prenatal diagnosis for thalassemias and sickle cell anemia are now well established for control of these inherited diseases.

Non-Transplant treatment approach in multiple myeloma

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Multiple myeloma has been recognized since the middle of the 19th century. The list of agents used in the treatment of myeloma is very long but the success rate has only approached the desirable level very recently. About 150 years ago when myeloma was initially described in London, patients were being treated with Dower's powder, camphor, julep, tincture of camphor, acetate of ammonia, cupping, blood removal(!), leeches, steel and quinine. 100 years later new drugs ie urethane and stilbamidine was added to the list by Nils Alwall from the University of Lund. When 40 years ago urethane was tested against placebo it was found to exert no benefit. Melphalan was not used until 1958 when Blokhin (USSR) and in 1962 when Bergsagel (MD Anderson) reported the best results so far (1). Since then Melphalan has been the standard drug in myeloma. When the Oxford myeloma trialists group finished the meta analysis on melphalan (35 years of expertise, 20 trials on 4930 patients) revealing superior response (60% vs 53.2%, $p<0.00001$) but a statistically insignificant reduction in death rate (1.5%) and no impact on survival with combination therapies compared to melphalan +prednisolone(MP). It is important to mention that the design of most of these trials do not meet the criteria required for current trials. Since melphalan is toxic to hematopoietic stem cells and threatens mobilisation, transplant groups have favored combination chemotherapies including VAD (vincristine,adriamycin,dexamethasone), VBMCP (vincristine, BCNU, melphalan, cyclophosphamide, prednisone), VBAP/D(vincristine, BCNU, Adriamycin, predisolone /dexamethasone), ABCM (Adriamycin, BCNU,Cyclophosphamide, methotrexate). A comparison of ABCM vs Melphalan alone, reported by MRC and thus not included in the meta analysis, showed a beneficiary outcome with ABCM. When this outcome was compared with those of the MP in the meta analysis the advantage dissappeared. Therefore it was decided to accept combination therapies and MP roughly equivalent(2,3). Another important aspect of melphalan is the excretion. It is hydrolysed and excreted via the kidneys so there is concern that bone marrow suppression may develop when full doses are used. However, it has been shown that the extent of drug accumulation is variable in each individual and cannot be predicted from the degree of renal impairment. Manufacturers' data recommend that initial doses of melphalan should be reduced by 50% if the glomerular filtration rate (GFR) is <40–50 ml/min and titrated against bone marrow toxicity in subsequent courses. The second most widely used regimen is VAD and was described by Alexanian in 1992. It gained its popularity by lacking stem cell toxicity and nephrotoxicity. VAD and MP have never been tested in the same trial and carry many differences: iv vs oral, mega dose vs standard dose steroids, toxicity profiles etc. The major advantage of VAD comes from dexamethasone, Vincristine alone is not active against myeloma and VAD has not been compared to Dex alone either.

I. Evaluation of response to therapy

Evaluation of tumour response is based on changes in serum levels of M-protein and/or urinary light chain excretion. In addition, a clinical response requires that no new myeloma-related organ or tissue damage occurs. Current CR definitions in myeloma rely mainly on the detection

of monoclonal protein and random bone marrow examination. However, focal lesions recognized on magnetic resonance imaging (MRI) and harboring viable monoclonal plasma cells can persist in clinical CR; their resolution with further therapy (MRI-CR) lags 2 years behind the onset of clinical CR and, when achieved, has favorable survival implications. The presence of such myeloma cells in focal lesions has only recently been appreciated as a potential source of disease recurrence.

The criteria defined by an International Working Group *(Blade et al, 1998)* and used for a long time has been revisited and new International response criteria has been published recently (Durie 2006).

II. Standard dose combination therapies

During the recent years there has been many publications on comparison of combination chemotherapies with autologous transplants. In these studies MP(GIMMO), VAD (MAG), VMCP/VBAP (IFM, SWOG), C-VAMP (MRC), VBMCP/VBAD (PETHEMA) therapies have been compared to ASCT.Three of these trials showed improved survival with ASCT(Attal, Child and Palumbo) and is the reason why ASCT has become a standard in myeloma. However two additional trials which share the same combination treatment(VBMCP) as the control arm (Standard dose therapy:SDT)and the same conditioning regimen have found no benefit from ASCT on survival. One of these is the PETHEMA trial where only the responding patients were randomized. The complete remission (CR) rate was significantly higher with HDT (30% vs 11%; P = .002). However, progression-free survival (PFS) was not significantly different between HDT and conventional therapy (median, 42 vs 33 months), and overall survival (OS) was similar in both groups (median, 61 vs 66 months). The other is the SWOG S9321 trial, in which all patients following an initial combination of VAD were randomized to ASCT or SDT. Extended courses of VMCP up to 12 months increased the CR rate from 5 % (after four courses of VAD) to 15 %. The HDT and SDT yielded comparable response rates, PFS and OS durations(PFS: 17% and 16%, OS: 37% and 42%, respectively). The CR following ASCT was 17%, a result less than the response obtained in most of the ASCT trials. On the contrary the CR in the control arm was better than most of the previous trials which may be explained with the duration and composition of the VBMCP combination. Using VBMCP/VBAP as frontline therapy, the PETHEMA had obtained a CR of 11%. There has never been a prospective comparison of VAD vs VMCP vs VAMP vs C-VAMP. With these regimens CR ranges between 10-25%. The disadvantage is 30-35% catheter infections, 5-8% risk of thrombosis in addition to the risks of cardiotoxicity, neuropathy, mucositis and alopecia.

III. Treatment prior to high dose therapy

The key questions here are: when to start, with which combination therapy, for how long and which patients are candidates for intensive treatment. The generally accepted approach is to delay treatment in smoldering (asymptomatic) myeloma until signs of tissue damage ie anemia, hypercalcemia, bone lesions, renal dysfunction develops. It is needless to point to the need for a careful, frequent follow up with sensitive tools including ie immunofixation(IF), FLC(Free Light Chain) and MRI during this quiescent phase. Once the decision to treat is made, the second challenge rises about the eligibility for a clinical trial, and intensive therapies. Usually there is no upper age limit for ASCT. It is important to avoid melphalan which is a strong myelosupressive prior to ASCT.

As summarized above there is no data to suggest one of these combinations to be superior. Novel agent containing or oral combinations have been designed to overcome intravenous line related complications: Thalidomide-Dexamethasone (TD), Idarubicin-Dexamethasone (Z-Dex), Cyclophosphamide-Thalidomide-Dexamethasone (CTD). These new regimens have been compared to VAD : rapid infusion VAD vs VAD, TD vs VAD, Z-Dex vs VAD. Rapid infusion VAD was shown to be as effective as VAD in newly diagnosed patients by HOVON (Segeren et al 1999). The activity of rapid infusion in relapsing/refractory patients have not been tested against VAD. Z-Dex has been found to be an effective regimen but the CR is not indicative of a substitution for VAD: 58% vs 74% (p=0.075) (Cook 2004). Since high dose Dex(HDD) is the most potent component of VAD regimen it has been used as a sigle agent and found to induce 62 % CR, similar to the CR obtained with VAD(74%, p=0.25) when given before ASCT. (Kumar 2004). HDD alone is widely used in the USA for initial therapy prior to HDT.

TD has been compared with Dex alone prospectively or with VAD retrospectively. Both reports

agree on the superiority of TD. TD vs VAD (CR: 76% vs 52%, p<0.001, Cavo 2005), TD vs Dex: 63% vs 41% (p<0.002)(Rajkumar 2005). It is noteworthy to mention that the increased response rate with TD is balanced with an augmented risk of thrombosis and neuropathy. DVT (15 and 17% vs 3%, without propylaxis) risk is more frequent during the first months, increases with age and warrants prophylactic anticoagulation. Nevertheless this oral combination doesnot require hospitalisation and compromise harvest of stem cells. There is cumulating evidence that better CR achieved with TD also increased the success rate of ASCT. However, the impact on cure rate and the long term outcome when induction is not followed by ASCT is not known yet. As will be mentioned later in this review there are doubts about its impact on posttransplant relapse and OS as well. The authors(Rajkumar et al) caution the use of this combination in elderly patients and suggest to initiate treatment in patients with low tumor burden with Dex alone and assess the response within 1-2 months, add Thalidomide if required. They recommend to reserve the combination for patients with advanced disease ie painful bone lesions, impending spinal cord compression or those with poor prognostic features (JCO editorial). Nevertheless thalidomide promises to be a very effective agent in the management of refractory or patients with renal failure. The pharmacokinetics of thalidomide seem to be unaltered in patients with renal dysfunction. The clearance of thalidomide is increased during dialysis; however, it appears not necessary to give a supplementary dose. Less than 1% of thalidomide is excreted unchanged in the urine and it does not appear to be hepatically or renally metabolised to any large extent, appearing to undergo non-enzymatic hydrolysis in plasma to form multiple degradation products. Manufacturers do not recommend dosage reduction.

Another frontline combined treatment approach with Thalidomide is the TAD regimen administered in the HOVON/ 50/GMMG-HD3-Trial A first group of 406 patients (of 1050 included) were evaluable for the comparison of VAD vs. TAD and response after 1st HDT and were presented at the ASH meeting in 2005 by Goldschmidt. A trend for a higher toxicity was observed in the TAD- compared with the VAD-arm (drop out: 15% vs. 8%, p= 0.10). Low molecular weight heparin was effective in the prevention of DVT during TAD-treatment (8% vs. 4% p= 0.15). TAD induced a significantly higher response rate (80% vs 63%,p<0.001), but this effect was completely offset by HDM(91% vs 88%,p=0.4). Since patients were given only three courses of induction preASCT, CR rates between TAD vs VAD before and after ASCT were: 7% vs 3%, 19% vs 13%,p:n.s. The authors concluded that results on EFS/PFS are necessary before thalidomide containing regimens can be defined as standard for induction treatment before HDT.

Impact of inclusion of thalidomide to the induction and maintenance steps of a intensive treatment protocol have been evaluated in a recent publication by Barlogie et al. In an intensive treatment protocol, patients were randomized to Thalidomide (+) vs no Thalidomide during primary remission-induction therapy, between the two transplantations, with consolidation therapy, and as maintenance treatment. The thalidomide treated patients had a significantly higher rates of both CR (62 percent vs. 43 percent) and five-year EFS (56 percent vs. 44 percent). However, OS curves in the two groups were nearly identical, owing in part to the poorer outcome after relapse in the thalidomide group. In particular, thalidomide-treated patients had a lower rate of response to salvage therapy and shorter OS after relapse than the control patients.

Most debilitating was the incidence of peripheral neuropathy (grade of more than 2) which was more common in the thalidomide group than in the control group (27 percent vs. 17 percent, P<0.001) and among patients at least 65 years old than among younger patients (29 percent vs. 20 percent, P=0.02). Peripheral neuropathy improved to less than grade 2 within three to four months after a dose reduction or cessation of thalidomide in nearly 90 percent of affected patients. Severe constipation, neutropenia were also more common in the thalidomide group. Treatment after relapse included further thalidomide or thalidomide (75 percent of the thalidomide group and 83 percent of the control group), other agents and further high-dose therapy (7 percent and 2 percent, respectively). Although survival after relapse was longer (2.7 vs. 1.1 years, P=0.001) among patients initially assigned to receive no thalidomide than among those assigned to receive thalidomide it is important to mention that the majority of the control group received thalidomide following relapse. Based on these findings Barlogie et al cautions about the impact of CR on survival and the higher

rate of failure to respond to salvage therapy in the thalidomide group, especially with respect to the salvage potential of new drugs in patients who had received thalidomide as initial treatment. A meta analysis on four SWOG Phase III trials revelaed no impact of response to frontline therapy on OS and PFS (Durie et al 2004). However it is worth to mention that there are also reports showing achieving CR (after HDT as well as CR not completed by ASCT) appears to be a good prognostic factor for remission duration and overall survival (OS) (*Lahuerta et al, 2000; Davies et al, 2001*, Kyle 2006), The ECOG E 9486 study showed the prognostic role of CR obtained by combination therapy alone(VBMCP+/-INF). The median duration of survival from the date of objective response was 5.1 years for those who achieved a CR and 3.3 years for those with a partial response (P < .0001). The median postresponse survival was 6.6 years in the 21 patients in CR with nonclonal disease and 4.4 years in the 11 patients in CR who had persistent clonal disease. Barlogie draws attention to the study on comparison of bortezomib with dexamethasone as second-line therapy for myeloma: response rates to bortezomib were superior to those observed with dexamethasone regardless of the type of first-line therapy, with the exception of prior thalidomide treatment. We do not know if this finding is a consequence of the clinical features or prior thalidomide exposure.

There are recent data about the role of Bortezomib in newly diagnosed patients prior to ASCT. Richardson *et al.*[16] reported a response rate of 30%, with 11% complete remission (similar to that previously reported in refractory patients), Dispenzieri *et al.*, using the same dose and schedule observed a higher response rate (PR:73%). These differences may be due to the number of cycles administered (median 2 vs 5, respectively). The addition of HDD was associated with a higher overall response rate (≥ PR 80-90%, with 18% CR or nearCR). Similar results were obtained with the PAD regimen (bortezomib, adriamycin, and dexamethasone) (89% response rate, with 16% CR or near CR) and the VTD scheme (bortezomib, thalidomide and dexamethasone) (92% response rate with 19% CR). These results show that the vast majority of newly diagnosed MM patients will respond to bortezomib-based regimens and around one in five will achieve CR, resulting in a picture similar to that observed after ASCT. All these studies, showed that stem cell mobilization was not hampered with exposure to Bortezomib. Moreover, the use of high dose melphalan after these bortezomib-based induction regimens was associated with an intensification in the CR rate. Thus, in the PAD study the 16% CR or near CR prior to transplantation increased to 54% after Mel200; in the DT-PACE study the percentage of CR increased from 16% to 58%, and in the VTD from 19% to 31%. These data strongly support the complementary value of this sequential strategy (i.e., novel drugs combinations upfront, followed by ASCT)(J.San Miguel and J.Blade 2006).

Another novel agent, lenalidomide was combined with HDD and was associated with higher response rates compared to Dex alone with almost no severe neurotoxic effects in newly diagnosed patients. However, severe neutropenia occurred in 12.0 to 16.5 percent of patients who received lenalidomide(Rajkumar et al 2005). This drug has very recently received approval from FDA for use in refractory patients and is not commercially available in Europe yet.

As can be seen from these results the landscape of myeloma treatment has changed substantially over the past few years. New agents have moved and keep on moving rapidly from the bench to the bedside. They are important additions to the treatments now available for myeloma. Currently, the main clinical challenges are determining the optimal dose of new agents, whether they should be given sequentially or in combination, and how they should be integrated with old therapies.

Two treatment strategies are being explored. The first aims to eradicate the tumor with the use of a combination of all, or most of, the available agents and high-dose therapy with ASCT. Whether such an approach can modify the course of myeloma is uncertain, as reflected in the study by Barlogie et al. About 15 % of myeloma patients are <60 an additional 15% are 60-65 and only 2% are <40 years of age. There is a cost for a 50% CR rate: considerable toxicity and the need to extend treatment which endangers the quality of life.

The second approach is to reserve the use of new agents for the sequential treatment of relapses as a means of controlling the growth or regrowth of tumor, thereby converting myeloma into an indolent disease. The results of the studies using these novel agents following posttransplant

relapse or during maintenance will give an answer to this question.

Genomic and proteomic studies incorporated into trials of these agents will help to identify molecular mechanisms of drug sensitivity and resistance, and they will aid in the design of treatment for individual patients. It is important to note that the message of the study by Barlogie et al. is one of hope. Through biologic and clinical research in myeloma, we are uncovering effective new treatments that promise long-term benefit to a substantial proportion of patients with a cancer for which palliation was a reasonable goal even as recently as a decade ago(Cavo and Baccarani, 2006).

IV. Initial chemotherapy where HDT is not planned

Standard first-line treatment for multiple myeloma (MM) patients ineligible for ASCT is MP. However, CRs are rare. The aim of therapy in these patients (usually older and less fit) is to achieve a response with minimal treatment-related toxicity. An oral regimen is therefore preferable. Thus either melphalan or cyclophosphamide with or without prednisolone may be used. Doses should be modified according to nadir cell counts, bearing in mind that antimyeloma activity is achievable only with doses that cause some degree of fall in counts. Cyclophosphamide causes less cytopenia and is recommended for patients with neutrophil counts below $1{\cdot}0 \times 10^9$/l or platelet counts below 75×10^9/l. Duration of therapy is to continue to maximum response plus 3 months. Randomised trials have shown no advantage of continuing chemotherapy further.

The effects of thalidomide in combination with MP compared to MP alone has been published by Palumbo et al recently(2006). Patients treated with thalidomide had higher response rates and longer EFS than patients who were not. Combined CR or PR rates were 76·0% for MPT and 47·6% for MP alone , and the near-CR or CR rates were 27·9% and 7·2%, respectively. 2-year EFS rates were 54% for MPT and 27% for MP (hazard ratio [HR] for MPT 0·51, p=0·0006). 3-year survival rates were 80% for MPT and 64% for MP (HR for MPT 0·68, p=0·19). Rates of grade 3 or 4 adverse events were 48% in MPT patients and 25% in MP patients (p=0·0002). Introduction of enoxaparin prophylaxis reduced rate of thromboembolism from 20% to 3% (p=0·005). Similar studies are currently active in Nordic countries, Europe and Turkey.

To potentiate the response in elderly newly diagnosed myeloma patients another novel agent, Bortezomib has been added to the MP protocol. A phase 1/2 trial in 60 untreated MM patients aged at least 65 years (half older than 75 years) has been designed to determine dosing, safety, and efficacy of bortezomib plus MP (VMP) and has been published very recently. VMP response rate was 89%, including 32% immunofixation-negative CRs, of whom half of the IF^- CR patients analyzed achieved immunophenotypic remission (no detectable plasma cells at 10^{-4} to 10^{-5} sensitivity). VMP appeared to overcome the poor prognosis conferred by retinoblastoma gene deletion and IgH translocations. Results compare favorably with a historical control data for MP—notably, response rate (89% versus 42%), EFS at 16 months (83% versus 51%), and survival at 16 months (90% versus 62%). Side effects were predictable and manageable; principal toxicities were hematologic, gastrointestinal, and peripheral neuropathy and were more evident during early cycles and in patients aged 75 years or more. In conclusion, in elderly patients ineligible for transplantation, the combination of bortezomib plus MP appeared to be significantly superior to MP, producing very high CR rates, including immunophenotypic CRs, even in patients with poor prognostic features. Based on these findings a multicentric trial (VISTA) comparing MP vs VMP has been inititated and approaching closure.

The long term outcome following novel agent+ MP regimens is uncertain. To give ASCT a chance, a group of elderly patients aged who are not candidates for standard dose of 140-200 mg/sq.m Melphalan were given two courses of 100 mg/sq.m Melphalan with stem cell rescue and compared to a control group who received MP alone. NearCR was 6% after MP and 25% after MEL100 (P = .0002). At 3 years, MEL100 increased EFS from 16% to 37% and OS from 62% to 77% (P < .001). Similar results were observed in patients aged 65 to 70: nCR was 8% after MP and 25% after MEL100 (P = .05); at 3 years, MEL100 improved EFS from 18% to 31% (P = .01) and OS from 58% to 73% (P = .01). Patients aged 65 to 70 had a median OS of 37.2 months (MP) versus 58 months (MEL100). Intermediate-dose melphalan improves response rate, EFS, and OS in myeloma patients, specifically in those aged 65 to 70. Thus ASCT is still an option for a selected group of patients.

To address the role of ASCT and addition of thalidomide, a prospective study was initiated by IFM 99-06. The preliminary findings presented at ASH2005 by Facon et al showed a benefit from MPT. CR rates were 2% vs 15% vs17% following MP vs MPT vs Mel100. Similarly EFS and OS were significantly better with MPT: 17.2 vs 29.5 vs 19 months and 30.3 vs >55 vs 38.6 months. The long term results of this trial will be interesting and is not published yet.

A recent published report has evaluated the role of thalidomide-dexamethasone-liposomal doxorubicin (ThaDD)-for untreated patients older than 65 years. Offidani and colleagues report that ThaDD yielded 36-month EFS and OS rates of 57 % and 74 %, respectively. Toxicities were manageable. These short term results are:34% CR, 7% nCR, an ORR of 98%. Three year projected TTP, EFS and OS were all significantly higher in those patients achieving a response of at least VGPR versus those who did not. These are comparable with the success achieved with ASCT. As mentioned above the impact on cure and long term OS and EFS remains to be seen.

Barlogie is cautious about use of novel agents in frontline therapies and draws attention to myeloma which evolves from MGUS, has a lower CR rate withut any adverse consequences on survival. Also, within their experience, despite similar CR rates of 40% after MEL treatment, one third of patients with cytogenetic abnormalities had a short median survival of only 2 to 3 years, compared with 7 or more years in the remainder. Thus, although high CR rates may translate into extended survival for MEL treatment of patients with good-risk myeloma, this may not hold true following treatment with the newer nongenotoxic agents. Barlogie suggests: before being indiscriminately considered as primary therapy for all patients with myeloma, new combination regimens should demonstrate survival benefits in patients with notoriously high-risk disease. This will prevent compromising 10-year survival expectations approaching 50% in good-risk disease with high-dose MEL. Metaphase cytogenetics and molecular genetic studies should be routinely performed to guide patient selection into clinical trials for high-risk patients. Barlogie et al have published the only analysis on prognostic factors in thalidomide treated patients: This reports the phase 2 trial of *thalidomide* (200 mg/d; 200 mg increment every 2 weeks to 800 mg) for 169 patients with advanced myeloma (abnormal cytogenetics: 67%; prior autotransplant: 76%) extending the earlier results in 84 patients. A 25% myeloma protein reduction was obtained in 37% of patients (50% reduction in 30% of patients; near-CR or CR in 14%) and was more frequent with low plasma cell labeling index (PCLI) (below 0.5%) and normal cytogenetics. Two-year EFS and OS rates were 20% ± 6% and 48% ± 6%, respectively, and these were superior with normal cytogenetics, PCLI of less than 0.5%, and β_2-microglobulin of 3 mg/L. Response rates were higher and survival was longer especially in high-risk patients given more than 42 g thalidomide in 3 months (median cumulative dose) (landmark analysis); this supports a need for high doses of thalidomide in high risk myeloma. A subgroup of patients in the APEX tral were analyzed for presence of cytogenetic abnormalities. Bortezomib was found to be active regardless of 13 q deletion. A recent publication (Chang et al 2006) about Bortezomib treated 65 patients have revealed no difference in OS and clinical response with cytogenetic abnormalities(cIg-FISH): with or without 13q deletion (77% versus 50%), t(4;14) (67% versus 56%), t(11;4) (33% versus 62%), or CKS1B amplification (67% versus 57%). These reports need to be confirmed by others to be able to make a statement about the activity of novel agents in high risk myeloma.

V. Patients relapsing or refractory to initial therapy

There is a lack of evidence from randomised controlled trials on the optimum approach to treating primary refractory disease. However, it is clear that patients refractory to one regimen may respond well to another regimen. Patients refractory to alkylating agents may respond to VAD-type regimens *(Barlogie et al, 1984)*. Attempts to circumvent resistance with cyclosporin or PSC 341 have resulted with increased toxicity but insufficient antimyeloma activity (Sonneveld et al 2001). Younger patients who fail to respond to VAD as primary therapy prior to planned stem cell transplantation (SCT) may still respond to high-dose melphalan (HDM) *(Rajkumar et al, 1999; Vesole et al, 1999)* and these patients must be given the option of HDT; *Alexanian et al (2004)* reported a response rate of 69% in 89 patients with primary resistant myeloma following HDT. There is a group of patients who do not reach CR with MP a *non-progressive* PR,which may be result with a survival that is equivalent to patients who achieve a stable response with MP.

Thalidomide alone *(Singhal et al, 1999; Juliusson et al, 2000; Barlogie et al, 2001)*, in combination with dexamethasone *(Dimopoulos et al, 2001; Palumbo et al, 2001, 2004b)* or with dexamethasone plus cyclophosphamide *(Dimopoulos et al, 2004)* has been approved for this indication and is increasingly being used. Thalidomide as a single agent has been shown to produce responses in at least 30% of relapsed/refractory patients *(Singhal et al, 1999; Juliusson et al, 2000; Barlogie et al, 2001). Glasmacher et al (2005a,b)* have reviewed 42 phase II trials of single agent thalidomide in relapsed or refractory disease. In most of these studies, the target dose was 800 mg daily but the median tolerated dose was 400 mg. There was clear and convincing evidence of efficacy with a 29% PR/CR rate in 1629 patients. There was no clear dose–response relationship. Neuropathy occurred in approximately 30% patients, but VTE appears not to be increased when thalidomide is used alone. Combination of thalidomide with HDD or with HDD and cyclophosphamide has achieved higher response rates (60%)in relapsed/refractory patients. *(Dimopoulos et al, 2001; Palumbo et al, 2001) (Kropff et al, 2003; Dimopoulos et al, 2004; Garcia-Sanz et al, 2004).* However incorporation of HDD increased the frequency of DVT as well. Most patients who respond to thalidomide have a decline in their M-protein after 3 weeks.Thus, to start with thalidomide alone and to add HDD only in patients not responding after 3–4 weeks can be a strategy*(Waage et al, 2004).*

The thalidomide analogues lenalidomide [Revimid and Actimid ™, Celgene, USA] appear to have activity similar to that of the parent compound *(Raje et al, 2004; Schey et al, 2004)* and combinations of these drugs with chemotherapy are currently being evaluated. It is early to comment on the frequency of side effects but only very few incidences of neuropathy or DVT have been observed with lenalidomide. Dimopoulos and Weber have presented during ASH 2005, the results of two parallel trials comparing Revlimid plus HDD vs Placebo+HDD in refractory/relapsing patients. The CR rates were, similar in both studies,: 8% vs 1% and the overall response rates were higher among the Revlimid treated patients: 53 or 51% vs 16 or 19%.

In younger patients, more intensive combinations, e.g. etoposide, methylprednisolone, cytarabine and cisplatin (ESHAP) *(D'Sa et al, 2004)* or dexamethasone, cyclophosphamide, etoposide, and cisplatin (DCEP) *(Lazzarino et al, 2001]* may both achieve a response and mobilise stem cells to continue with ASCT.

Bortezomib is a novel dipeptide boronic acid which has a 26S proteasome inhibitory activity whicah has also been approved for relapsing or refractory myeloma as a second line agent. It is antiproliferative, pro-apoptotic effects (via NF-kB blockage), downregulates the expression of adhesion molecules, inhibits angiogenesis, inhibits effectors involved in DNA repair, and blocks the unfolded protein response, resulting in accumulation of improperly folded proteins and subsequent endoplasmic reticulum stress and cell death. Two phase 2 studies, SUMMIT and CREST, were designed to evaluate activity in relapsed/refractory MM patients. Patients were treated with bortezomib 1.3 mg/m^2 on days 1, 4, 8 and 11 every 3 weeks; Dexamethasone was allowed in patients with suboptimal responses to bortezomib alone. The overall response rate was 35%, including 10% CR or near CR with an OS of 17 months. The randomized CREST study, comparing two dosages of bortezomib (1.3 vs 1.0 mg/m^2) showed that a reduced dose was able to produce responses in up to one third of the patients with a trend towards a lower toxicity. Jagannath et al. reported an improvement in response by adding dexamethasone in patients who showed suboptimal response to bortezomib from 18% to 33% of patients with suboptimal response included in the SUMMIT and CREST trials, respectively. A subsequent randomized phase 3 trial (APEX) including 669 patients with relapsed MM has shown that bortezomib is more effective than high-dose dexamethasone as demonstrated by a significant improvement in response rate (43% vs 18%), median time to progression (6.2 vs 3.4 months) and 1-year survival rate (80% vs 67%, respectively) (updated at ASH 2005). It is important to note that the response is usually very quick (1 or 2 cycles), independent of previous therapy. There is further proof about effectivity on extremedullary plasmacytomas. Although these results are encouraging, acquired resistance has already been observed. In vitro synergy of bortezomib with other agents, justifies combination therapy. Two pilot studies have shown that bortezomib in combination with melphalan or pegylated liposomal doxorubicin or cyclophosphamide plus dexamethasone produces a response rate of 50% to 76% in refractory MM, including a substantial number of CRs (6 to 30%).

Bortezomib has also been combined with thalidomide. Zangari et al have reported (ASH 2005)in 79 refractory patients (67% prior thalidomide, 95% prior ASCT) the results of this combination. Thalidomide was started at 50 mg/day and increased in a phase-1 fashion up to a maximum dose of 200 mg. Bortezomib was started at 1.0 mg/m^2 and also increased to a maximum of 1.3 mg/m^2. The response rates for patients in the study was 52% (CR + PR), with 17% achieving a very good partial remission (VGPR) or better. Patients who had not been previously exposed to thalidomide and who received the higher bortezomib dose, had superior survival. Toxicity was primarily hematologic, and the incidence of grade 3 or 4 peripheral neuropathy was quite low. A combination of thalidomide and bortezomib with adriamycin and dexamethasone is also being explored; the CR+PR rate is about 55% and toxicities are manageable.These response rates are clearly superior to those obtained with bortezomib alone. The most common side effects of bortezomib, used alone in refractory patients, were gastrointestinal symptoms, fatigue, and anorexia, although these were mostly grade 1-2. Thrombocytopenia grade 3-4, due to a reversible blockage in platelet release, was found in 30% of cases, while anemia and neutropenia are uncommon (<10%). The most troublesome side effect is painful/sensory peripheral neuropathy (37%, with only 9% grade 3), although this resolved or improved in two-thirds of patients after completion or discontinuation of therapy. Clinicians should be aware that an early reduction of the dose as soon as peripheral neuropathy emerges, according to well established guidelines, helps to avoid more severe symptoms and the need for interruption of treatment. So far the reported side effects with the combination therapies in newly diagnosed patients are similar to those previously reported in refractory treated patients. Therefore, overall, the toxicity profile of bortezomib is now well defined and most complications are predictable and manageable(J San Miguel 2006). There are two potential properties of Bortezomib which may be important in the treatment of myeloma: activity against plasmacytomas and osteoblast inducing activity (Heider U.et al 2006, Zangari M. et al 2005, Laura R. et al 2006)

The HDT and ASCT may be considered in patients who have not had a prior stem cell transplant *(Fermand et al, 1998).* A second HDT can also be an effective strategy in selected patients who relapse after an initial autograft: those with a low β_2-microglobulin at salvage, one prior transplant, and late relapse (>12 months) *(Tricot et al, 1995; Mehta et al, 1998).* Patients with at least one favourable variable had a projected survival at 18 months of 79%, compared with 38% for patients with no favourable variable *(Tricot et al, 1995).* There have as yet been no reported studies comparing a second autograft with other relapse strategies. There is insufficient evidence to recommend stem cell allografting as a salvage procedure.

Arsenic trioxide has been used in myeloma but with limited success *(Hussein et al, 2004; Rousselot et al, 2004,* Wu et al, in press 2006).

Steroids alone may be useful in patients at second or later relapse or in patients at second or later relapse in for whom chemotherapy is otherwise contraindicated, e.g. due to pancytopenia *(Alexanian et al, 1986).* Weekly oral or i.v. cyclophosphamide remains a useful regimen for patients with cytopenia.

Double hemi-body irradiation is useful palliative therapy in patients with widespread bone pain and in those refractory to chemotherapy and steroids *(Singer et al, 1989; Miszczyk & Sasiadek, 2001).* Caution is required as it can cause significant myelosuppression.

VI. Role of maintenance therapy

The role of anti-myeloma maintenance therapy following the achievement of plateau phase is unclear either after chemotherapy alone or after stem cell transplantation. A number of studies have examined the therapeutic role of IFN maintenance therapy following induction chemotherapy. A meta-analysis has evaluated individual patient data on 1543 patients in 12 trials where patients were randomised to receive IFN after induction therapy and in a further 2469 patients in 12 trials where patients were randomised to receive IFN in the induction phase *(Myeloma Trialists' Collaborative Group, 2001).* Many of those given IFN in induction continued with IFN as maintenance. In patients who received IFN only as maintenance, PFS was again significantly improved (P = 0·00001), with a prolongation of about 6 months in median PFS and 7 months in median OS. If all 4066 patents from all 24 trials were analysed together, the gain in median OS for IFN-treated patients was only 4 months. Similar results were obtained in the meta-analysis of published data on IFN trials *(Fritz & Ludwig,*

2000). Median PFS and OS were prolonged by 4 and 7 months, respectively. If studies where IFN was given as induction were included, the gain in OS was only 3·1 months. A preliminary report on a US Intergroup study showed a lack of benefit for IFN maintenance after both conventional and HDT *(Crowley et al, 2004).* Overall, the data do not show significantly better response or survival in any particular patient group. Dosages of IFN have varied, but any benefit for doses >3 MU/m^2 s.c. three times per week has not been shown. There are no data on duration of therapy.

Several newer agents are now being studied as maintenance therapies in plateau phase following initial chemotherapy or stem cell transplantation, including thalidomide *(Feyler et al, 2003; Goldschmidt et al, 2003; Sahebi et al, 2003; Attal et al, 2004)*, lenalidomide (Revimid, Celgene, USA) and bortezomib. Their role in this setting remains unclear until further follow-up accrues, although preliminary data suggest remission may be prolonged *(Attal et al, 2004).* The dose of thalidomide that is tolerable for maintenance therapy remains to be determined. A randomized trial of maintenance treatment with thalidomide and pamidronate was conducted by IFM and published recently. Two months after high dose therapy, 597 patients under the age of 65 years were randomly assigned to receive no maintenance (arm A), pamidronate (arm B), or pamidronate plus thalidomide (arm C). A CR or VGPR was achieved by 55% of patients in arm A, 57% in arm B, and 67% in arm C (P=0.03). The 3-year post-randomization probability of EFS and the 4-year post-diagnosis probability of OS were all better in arm C (P<0.009, P<0.04). Maintenance treatment with pamidronate did not decrease the incidence of bone events. Patients who received thalidomide after transplantation had improved median survival (65.5 months) compared with patients who did not receive thalidomide (44.5 months; P = .09). When they were separated according to reasons for thalidomide use, patients who received thalidomide as maintenance had improved OS compared with patients who received thalidomide as salvage (65 months vs. 54 months; P = .05).

The data presented by Barlogie in 2006 shows that relapses in the thalidomide group appeared to be more drug-resistant than relapses in the control group. Superior response rates have been reported for TD as compared with HDD alone for induction therapy in patients with multiple myeloma. Since many patients in these trials received HDT after induction therapy with TD, the long-term benefit of this combination cannot be ascertained. Reserving thalidomide for maintenance therapy after transplantation, as was done in the pilot and larger trials conducted by the IFM, has several advantages: resistance may be avoided; the risk of DVT can be reduced, since this risk is highest during induction therapy, when the burden of tumor is high; and the incidence of neurotoxic effects should be reduced with the later introduction of thalidomide at lower doses (50 to 100 mg) during maintenance therapy. High rates of CR approaching the rates observed with ASCT have recently been found in trials of thalidomide combined with standard treatment with MP. Similarly, combinations of bortezomib and dexamethasone,plus pegylated doxorubicin or thalidomide, have shown promise. Little is known, however, about the durability of the responses induced by these treatments, especially after the discontinuation of the drugs. Although ASCT has considerable transplant related complications the low mortality and infrequent chronic adverse effects have to be balanced against the potential of irreversible and incapacitating chronic adverse effects of the newer agents.

As almost all patients with myeloma will relapse, the overall management strategy should include plans when and how to treat relapse, when it occurs. As generally accepted, the criteria to initiate treatment during relapse is the same as the initial treatment. It is not recommended to start treatment when patients are in a non-progressive plateau that may last for years. When signs of tissue damage becomes evident the therapeutic objectives are to achieve disease control, ameliorate symptoms, improve quality of life and prolong survival. While early relapse carries a poor prognosis and is likely to respond poorly to most therapy, patients whose disease relapses or progresses after a long plateau phase are likely to respond well to further treatment; survival from relapse/progression may be longer than the duration of initial remission.

Due to the limitation in the size of the manuscript, specific aspects of treatment ie approach to bone disease, anemia,neurological problems, renal failure, infections, plasmacytomas and plasma cell leukemia could not be reviewed. Readers may refer to a recent guideline prepared in 2005, and published in 2006 (Smith A. et al 2006).

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Myeloma bone disease, current treatment approaches

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Multiple myeloma (MM) is a monoclonal B cell malignancy, arising from a late stage differentiated B cell, sharing many characteristics with immunoglobulin (Ig)-secreting plasma cells. A striking feature of this disease is the accumulation of plasma cells with a low proliferate index and an extended life span, in the bone marrow, in close contact with stromal cells.

The bone marrow (BM) microenvironment plays a crucial role in the pathogenesis of MM. Malignant cells as well as their interaction with the microenvironment are of equal importance in the pathogenesis of MM, includes two important areas. The first requires a multi-step transformation process that implies the sequential generation of primary Ig translocations, chromosomal instability as well as secondary translocation. The second area of MM pathogenesis may have important implications for treatment is the interaction between the malignant cell and the BM microenvironment (1).

A central role in the biology of MM plays the complex interactive network between the tumor cells and the BM microenvironment, in which stromal fibroblasts, osteoclasts and endothelial cells represent important cellular components. The major role of the microenvironment is to support MM cell proliferation and survival. Myeloma cells interact with different cell types in the BM stroma leading to different events, which are directly linked to the pathogenesis of this malignant disease. These include prevention of apoptosis, stimulation of proliferation, acquisition of drug resistance in the tumor cells and induction of osteolysis and angiogenesis.

Nature of the Bone Disease

Multiple myeloma is characterized by an unique form of destructive bone disease, which occurs in the majority of patients. The progressive bone destruction is responsible for the most prominent and distressing clinical features of this disease, intractable bone pain, spontaneous fractures or following trivial injury, and hypercalcemia with its attendant symptoms and signs. The extent of the bone disease is an important factor in the prognosis of a patient with myeloma.

The bone lesions in myeloma occur in several patterns. Most patients have multiple discrete lytic bone lesions occurring at the site of deposits or nests of myeloma cells. Occasionally, patients develop single osteolytic lesions that are associated with solitary plasmacytomas. Some patients have diffuse bone loss (osteopenia) due to the dissemination of the myeloma cells throughout the axial skeleton. Rarely, patients with myeloma have an increase in the formation of new bone around myeloma cells rather than lytic lesions or bone loss, a rare condition known as osteosclerotic myeloma.

Pathophysiology of Bone Lesions

Osteolysis in myeloma is due to an increase activity of osteoclasts, the only cells known to have the capacity to resorb mineralized bone. This is the only cellular mechanism for bone destruction which is clearly evident in myeloma. Recent evidence suggests that the frequency of osteolytic bone disease may be correlated with the pattern of infiltration of the marrow by myeloma cells, the highest frequency of lytic lesions being in seen

in patients with nodular or diffuse, rather than insterstitial involvement.

Hypercalcemia is the most common in MM patients and always is associated with markedly increased bone resorption and frequently with impaired renal function due to direct effects of the disease on renal function.

The myeloma bone disease is associated with a multitude of cytokines that are capable of influencing bone resorption and bone formation. These include Receptor Activator of NF-κB (RANK), Macrophage Inflammatory Protein 1 alpha (MIP1α), RANK Ligand (RANKL), osteoprotegerin (OPG), Dkk1, interleukin-6 (IL-6), PTH-rP, and a number of others (2).

RANK plays an important role in MM-induced osteolysis. RANK is predominantly expressed on osteoclast precursors and mature osteoclasts whereas its cognate ligand (RANKL) is expressed by stromal cells and osteoblasts. In vivo the biological activities of RANKL are blocked by OPG. Myeloma cells seem to trigger osteoclastogenesis by disrupting the balance between RANKL and its natural inhibitor OPG. These cells can increase RANKL and decrease OPG production by stromal cells. New data provide evidence that the balance between a soluble form of RANKL and OPG may play a critical role in regulating osteoclast formation and bone disease in MM. The change in the RANKL/OPG ratio in favor of RANKL is responsible for the exaggerated osteoclast formation and activity seen in patients (2, 3).

Osteoprotegerin is a member of the tumor necrosis factor (TNF) receptor super family group of molecules. OPG is a very important molecule that does not only regulate bone resorption but may also function as a paracrine survival factor of MM cells by inhibiting apoptosis in the tumor cells which is induced by an another member of this family, TRAL (TNF-related apotosis-inducing ligand). Interactions between OPG and TRAIL also could be important in the bone loss associated with MM bone disease.

One such critical mediator identified recently is RANK Ligand (RANKL) in the myeloma bone microenvironment. RANKL is essential for the differentiation, function, and survival of osteoclasts, which play a key role in establishment and propagation of skeletal disease in patients with multiple myeloma or bone metastases as well as many other skeletal diseases. It exists principally as a membrane-bound molecule, although a soluble form (sRANKL) can be produced as well. RANKL is expressed by tumor cells and is overexpressed by osteoblasts and bone marrow stromal cells.

RANK.Fc and OPG.Fc, synthetic chimeric antagonists of RANKL/RANK interactions, each blockades osteolytic lesions in preclinical mouse models of multiple myeloma. Restoring the balance between OPG and RANKL by using RANKL inhibitors, OPG, or RANKL-Fc not only inhibits bone destruction but may decrease tumor burden in patients with MM as well (2).

Myeloma cells secrete factors that cause upregulation of RANKL by stromal osteoblasts. These cells stimulate the proliferation and survival of MM cells, by direct cell-to-cell contact. The osteoblasts also produce osteopontin, a molecule that enhances MM cell growth in concert with IL-6.

MM cells produce a very important chemokine, i.e. MIP-1α that is involved in MM bone disease since it can directly activate osteoclast precursor cells. MIP-1α is overexpressed in bone marrow of myeloma patients compared with other hematological neoplasms and has been implicated in myeloma-induced osteolysis. This chemokine, which is known to be increased in MM bone marrow plasma, increases bone resorption in vivo, and the bone destruction associated with MM in a preclinical model was abrogated by neutralizing antibodies to this factor. The bone lesions, as well as the tumor burden, are completely inhibited by antibodies to MIP1α.

Some authors introduce a hypothesis that there is a direct relationship between MIP1α and RANKL, each one enhancing the production of the other (2, 3). Such a cycle could explain the beneficial effects of either MIP-1α antibodies or inhibitors of RANKL on bone resorption in preclinical models of MM bone disease. Neutralizing anti-MIP-1α antibodies and MIP-1α antisense block osteolytic lesions and myeloma tumor progression in preclinical mouse models of multiple myeloma.

The myeloma bone disease and tumor growth are interdependent. The experiments confirmed the vital role of osteoclasts on maintaining the disease process (2). Osteoblasts, in contrast, had diverse effects on myeloma cells. The increased osteoblast activity may help control tumor growth even in patients with advanced myeloma. In these

patients, myeloma cells reduce osteoblasts activity, either via induction of osteoblast apoptosis or by inhibition of their differentiation (3), as part of mechanisms by which myeloma cells alert the bone marrow microenvironment for their advantage.

In vitro studies showed that myeloma cells induce apoptosis in osteoblast through direct physical contact and via production of soluble factors (3). MM cells from patients with bone disease produce the Wnt signaling inhibitor, dickkopf-1 (DKK1), that inhibits osteoblast differentiation. Dkk1 may be regulated by proteasome inhibitors and its expression can decrease by proteasome inhibitors. Some authors suggest that bortezomib therapy is associated with increased markers of bone formation (4).

The mechanisms by which osteoblasts interfere with myeloma cell growth are still unclear. Osteoblasts secrete osteonectin and high level of OPG, which indirectly impedes myeloma growth in vivo thought inhibition of osteoclast differentiation.

The data showed that the increased osteoblast activity via exogenous cytotherapy and/or endogenous approaches, such as a treatment with bone anabolic agents will benefit patients with myeloma for various reasons: it will increase bone formation, and relieve skeletal complication; it may help control of myeloma progression, particularly when combined with specific inhibitors of osteoclasts activity.

One important but still unsolved issue in the treatment of MM bone disease is the relationship between bone lesions and tumor burden. With few exceptions, when bone destruction is inhibited in the preclinical models, tumor burden decreases concomitantly. This finding is seen with RANK. Fc, MIP-1α antibodies, in most situations with bisphosphonates, and with OPG.

Management of Myeloma Bone Disease

Drug therapy

Bisphosphonates, which inhibit osteoclastic bone resorption mainly by inducing osteoclast apoptosis, have become the standard anti-osteolytic therapy for myeloma-induced bone disease. These drugs are small inorganic molecules that bind to hydroxyapatite on the surface of damaged bones and osteoclasts are inhibited and destroyed. Bisphosphonates therefore have several beneficial effects, including: preventing further bone damage; reducing bone pain and the need for painkillers; correcting and preventing hypercalcemia; reducing the need for radiotherapy; reducing pathologic fractures due to myeloma; improving quality of life.

Bisphosphonates might be classified into two major groups according to their different modes of action. One group comprises those that resemble closely pyrophosphate, such as etidronate, tiludronate and clodronate. The second group comprises nitrogen (N)-containing compounds and these have the ability to inhibit the mevalonate pathway.

The N-containing bisphosphonates have a different molecular mechanism of action from that of the older compounds etidronate and clodronate. These drugs inhibit an enzyme in the mevalonate pathway of cholesterol biosynthesis. The major enzyme targeted appears to be farnesyl diphosphate synthase, and the capacity of these compounds to inhibit this enzyme apparently correlates very closely with their potency and efficacy as antiresorbtion agents. The most potent of the antiresorptive bisphosphonates are zoledronic acid (ZOL) and risedronate.

There are other in vitro effects of the bisphosphonates that could be linked to their capacity to cause tumor cell apoptosis, including decreased release of IL-6 by osteoclasts, myeloma cells, and osteoblasts; effects on cell proliferation; effects on tumor cell apoptosis; and effects on angiogenesis.

The final target of bisphosponates action is the osteoclast. This action can be divided into three levels: tissue level - the main effect of the bisphosponates is a decrease in bone turnover, which is secondary to the inhibition of bone resorbtion. This effect is due to a decrease in the number of osteoclasts actively destroying bone; cellular level - inhibition of osteoclast adhesion and osteoclast recruitment; shortening of the life span of osteoclast and inhibition of osteoclast activity; molecular level - the action is on the cellular binding site, which induces a cellular transduction mechanism. Bisphosphonates can decrease the acid production of various cells and also inhibit lysosomal enzymes in vitro (5). The bisphosponates act also through the other cells. The likely candidates are the osteoblasts and the macrophages, which release many cytokines that are able to modulate the osteoclasts that are influenced by the bisphosphonates. The

bisphosphonates can also inhibit the adhesion of tumor cells.

The more recent approval of the third generation bisphosphonate ZOL for use in bone disease is showing a marked reduction in skeletal-related events in myeloma patients. This molecule can induce apoptosis in osteoclasts but has also some direct effects on osteoblast-like cells. ZOL can increase the production of OPG protein while it reduces membrane RANKL protein expression in primary human osteoblast-like cells. ZOL also has direct antiproliferative and pro-apoptotic activity in vitro on bone marrow stromal cells. This agent is more potent than pamidronate (PAM), and this can be used by infusion over a shorter period of time (but not less than 15 minutes).

Bisphosphonates are generally very well tolerated. The most common side effects are fever, vein irritation, general aches and pains, kidney dysfunction, and osteonecrosis of the jaws (ONJ). ONJ is a complication of bisphophonate treatment, associated with the time of exposure to this treatment in patients with MM. The risk appears to be higher with ZOL than with PAM (6). Other side effects are generally rare.

Recommendation of bisphosphonates is for all myeloma-related bone disease. This includes patients with MM of bone and/or other situations in which active bone destruction is occurring. PAM, ZOL, and clodronate are all beneficial in MM. Third generation bisphosphonates (e.g. ZOL and ibandronate), which appear to be more than 100 times more potent than second generation aminobisphosphonates, are in clinical practice. Intravenous ZOL 6 mg is equivalent in efficacy to PAM 90 mg monthly and may be used in preference to PAM. The choice of therapy will depend on patient and physician preference. The doses and frequencies should not be exceeded and infusion times should not be shorter than those recommended by the manufacturers. Other bisphosphonates such as aledronate and risedronate are available but have not been specifically evaluated in myeloma. The newer bisphosphonate ibandronate has not been shown to be effective in clinical studies, possibly because the doses studied were not optimal. Research in this area is intensive and is likely to lead to the eventual introduction of new bisphosphonates or related drugs that are safe, can be given to patients orally and lead to powerful and beneficial effects on morbidity and survival as well as mortality.

Radiation therapy

The most common indication for radiotherapy is painful lesion. Radiation therapy should be used sparingly for acute problems such as spinal cord compression, severe refractory pain, and treatment or prevention of pathologic fracture.

Surgery

All patients required from a corset. The indication of surgical intervention is progressive spinal canal impingement and cord compression (radioresistant); progressive kyphotic spinal deformity with element disruption and progressive shear deformity. Surgical procedure includes vertebroplasty or endoscope spinal decompression and stabilization. A new surgical treatment approach is kyphoplasty. This procedure involves the injection of liquid cement using the balloon technique in an attempt to provide acute pain relief and improvement in the structural integrity of collapsed vertebrae or other damaged bones.

Novel biologically based therapies

In patients with MM, even when conventional chemotherapeutic regimens reduce tumor burden, the underlying bone disease may remain refractory to treatment. There is therefore a compelling need for the development of novel biologically based therapies that may concurrently improve both the bone lesions and reduce the tumor burden in myeloma

One other treatment that deserves attention is the use of bortezomib and other proteasome inhibitors for bone formation. This new drugs are powerful stimulators of bone formation and osteoblast differentiation. Proteasome inhibitors cause MM cell apoptosis by their inhibitory effects of NF-αB, inhibit bone resorption, again by their inhibitory effects on NF-αB, and enhance bone formation by reversing the effects of MM cells to enhance Dkk expression (4, 7).

When tumor or myeloma cells invade bone, they secrete growth factors that stimulate RANKL production, promoting increased bone resorption. Two agents that block RANKL effects are RANK. Fc and OPG.Fc, both inhibit osteoclast activation, and may be more effective anti-osteolytic agents

in myeloma because they also effectively prevent osteoclast formation.

As a part of a large program targeting the RANK/RANKL/OPG pathway was developed a new agent, Denosumab, targeting the receptor activator of nuclear factor-kappaB ligand, for the potential treatment of diseases associated with bone loss, such as osteoporosis and bone metastases. The antibody is currently undergoing phase III clinical trials.

Denosumab (formerly known as AMG 162) is a fully human monoclonal antibody (IgG2) that binds to RANKL with high affinity and specificity and blocks the interaction of RANKL with RANK, mimicking the endogenous effects of osteoprotegerin. Preclinical models have demonstrated that inhibiting RANKL leads to significant improvements in cortical and trabecular bone density, volume and strength. Denosumab is currently being studied for its potential in a broad range of bone loss conditions including osteoporosis, treatment-induced bone loss, bone metastases, multiple myeloma and rheumatoid arthritis

A single s.c. dose of denosumab given to patients with multiple myeloma is well tolerated and reduced bone resorption for at least 84 days (8). The pharmacokinetics of denosumab also was assessed. Following a single s.c.dose, levels of urinary and serum N-telopeptide decreased within 1 day, and this decrease lasted through 84 days at the higher denosumab doses.

A comprehensive clinical program evaluating denosumab is ongoing including: a 11 phase 3 trials investigating the frequency of SREs in advanced solid tumors and a phase 2 studies for the treatment of patients with multiple myeloma. The most frequent adverse events reported for denosumab-treated patients were nausea, vomiting, asthenia (weakness), and diarrhea. No binding or neutralizing antibodies were detected.

By targeting RANKL, denosumab works differently from bisphosphonates because it inhibits osteoclasts at all stages of development and activity. As the only investigational RANKL inhibitor in late-stage development, denosumab represents a potential new way to treat bone disease.

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Primary amyloidosis as of 2006: Diagnosis problems and treatment options

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Amyloidosis is not a single disease but a term for disease that share a common feature: the extracelular deposition of pathologic insoluble fibrillar proteins in organs and tissues (6).

Amyloid is an extracelular deposit mainly constituted of autologous proteins that produces a diagnostic apple-green birefringence when viewed in polarized light after Congo red staining, suggesting an organized structure. Electron microscope analysis reveled that amyloid is composed of rigid unbranche aggregated fibrils of indefinite lenght, 7,5-10 nm wide. Each fibril consist of units (filaments) that are mainly composed of proteins arranged in an antiparallel, cross B-pleated sheet configuration with strands perpendicular to long axis of the filament. The peculiar structural architecture of amyloid is considered responsible for typical staining properties. All amyloid deposits contain two components : a major (85-90%) fibrillar components (specific) and a nonfibrillar component. The non fibrilar components include the "P" (pentagonal) component, apolipoprotein E and the heparan sulfate proteoglican. "P" component (5-10% of the total deposited protein) is derived from circulating serum amyloid P (SAP) component, has a specific binding motif for the common conformation of amyloid fibrils and makes amyloid fibril resistant to proteolysis. Amyloidosis are diseases of "protein misfolding or misconformation" (16).

Hystorical, the amyloidosis were classified according to clinical or pathologic feature of the associated disease. Secundary amyloidosis accompanied chronic inflamatory process. Familial amyloidosis was recognaised by distinctive clinical manifestation within kindreads. All other types, except the type occuring in association with myeloma, were termed "primary" (idiopatic). The development of methods for dissolving and fractionating amyloid fibriles extracted from tissues permited the identification of 24 differant proteins as amyloid precursore to date (11). Classification now is based on the chemical nature of fibrillar component of deposits (Table 1).

A major development was the recognition that amyloid fibrils in "primary" amyloidosis are fragments of immunoglobulin light chains and now this type is named "Light chain amyloidosis" (AL amyloidosis). There are both localized and systemic forms of AL amyloidosis.

Primary systemic amyloidosis or systemic AL amyloidosis is both a disorder of protein conformation and a clonal plasma cell disorders (6). Multiple organ disease results from extracellular deposition of monoclonal immunoglobulin light chain fragments in an abnormal insoluble fibrilar form. Accumulation of amyloid progressively distrupts the normal tissue structure and ultimately leads to organ failure, frequently including the kidneys, heart, liver and peripheral nervous system (12) AL amyloidosis also involve the soft tisues, joints, endocrine organs and vasculature. The natural history of AL amyloidosis is that it is progressive and fatal within 2 year in about 80% of patients (2). Until recentrly systemic AL amyloidosis was viewed as untreatable disease. It is a rare disorder (8 cases per one million persons/year in USA). AL amyloidosis is one fifth as common as multiple myeloma but has an incidence similar to that of chronic myelogenous leukemia and Hodg-

Table 1. The principal types of amyloidoses by the chemical nature of fibrillar precursor proteins (8).

Precursor Protein	*Abbreviation*	*Clinical syndrome*
Immunoglobulin protein	AL	Light chain amyloid
	AH	Heavy chain amyloid
	AL	Myeloma or macroglobulinemia (this is not secondary amyloid)
	AL	Localized bladder and bronchus
Amyloid A protein	AA	Secondary to infection, renal cell cancer and familial in familial Mediterranean fever (FMF)
Transthyretin	ATTR	Native transthyretin (TTR) in senile systemic amyloidosis, mutant in familial amyloidosis
Fibrinogen Aα	AFib	Hereditary renal amyloid
Apolipoprotein A	A Apo I	Cardiomyopathy neuropathy
β 2 microglobulin	Aβ2 M	Dialysis amyloid

kin's lymphoma. AL amyloidosis appear to be more common in men. At diagnosis, 60% patients were between 50 and 70 years old and only 10% were less than 50 years (12).

AL amyloidosis may be associated in 10-15% of cases with myeloma or other B cell malignancy (BW, LMN). It is rare for AL amyloidosis to progress to overt myeloma probably because of the short survival of patients with AL amyloidosis.

Amyloidogenic clone and the amyloidogenic immunoglobuline light chain. In Al amyloidosis the protein precursor is synthesized by a neoplazic B clone. The clone is composed of post germinal center cells circulating in the peripheral blood together with bone marrow lymphoid, lymphoplasmocytoid cells and plasma cells. Simple morphological counting of plasma cells underestimates the amyloidogenic cell mass (15). The bone marrow clone is small with minimal proliferative activity. The procentage of bone marrow plasma cells remains stable over time. The monoclonal protein concentration is very low with lambda isotype predominance. Because of extravascular deposition of monoclonal protein, measurement of monoclonal protein produced by plasma cells is complex. The fibril precursor protein for amyloid is usually a monoclonal free immunoglobulin light chain or rarely a fragment of an immunoglobulin heavy chain. AL amyloid fibrils are derived from the N-terminal region of monoclonal immunoglobulin light chains and consist of the whole or part of the variable (VL) domain. Intact light chains may rarely be faund and the molecular weight therefore varies between about 8.000 and 30.000 Da. The variable region of immunoglobulin light chains is globular and rich in α β strands and disproportionately made in plasma cells. The effects of primary structure on the conformation of immunoglobulin light chains appears to be the basis for amyloid. Uncommon amino acid substitutions in critical residues may affect the stability and interactive properties of misfolded or partially folded light chains. Such intermediate forms may be prone to self-assemble aberrantly. Over time, this filaments either form thicker unit or grow by extension as soluble light chain monomer or stack one upon another. Once the process has started, "seeding" may also play an important facilitating role, so that amyloid deposition, may progress exponentially as expansion of the amyloid tamplate "captures" further precursor molecules. The levels of monoclonal protein and the plasma cell infiltrates in the marrow do not increase over time as is the case in multiple myeloma.

The clones that cause AL amyloidosis are distinctly different from myeloma clones with respect to their repertoire of immunoglobulin light chain variable region germline (Ig VL) genes but similat in that their immunoglobulin gene are highly mutated (antigen driven or post germinal center clones) and surprinsingly similar to myeloma clones with respect to their cytogenetics. Aneuploidy is commoun, with trisomies of chromosomes 7, 9, 11, 15 and 18 seen in a significant fraction of cases. Immunoglobulin heavy chain translocations of chromosome 14, including t(4;14) and the anormality of chromosome 13(13q deletion) are common.

Nearly half of all AL clones employ one of the three immunoglobulin λ VL genes the 2a2 (λ II),

the 3r (λ III) and 6a (λ VI) germline donors. Patients with clone derived from the 6aVλ germline gene were significantly more likely to have dominant renal involvement, whereas those with clones derived from the 2a2 and 3rVλ genes were more likely to have cardiac and multisystemic disease. Patients with Vk clones were more likely to have dominant hepatic involvement (1).

Diagnosis and differential diagnosis. Diagnosis of amyloidosis is based on a clinical suspicion and established by a tissue biopsie. The initial simptoms are frequently fatigue and weight loss, but the diagnosis is rarely until symptoms or signs referable to a particular organ appear (6). AL amyloidosis has a widest spectrum of organ involvement. A patient's symptoms reflect the organs most prominently involved, although hystologic examination will revel some degree of amyloid deposition in virtually evry organ system except the central nervous system. The organs most commonly involved ar the kidney and the heart, either individually or together. There are **seven critical clinical syndromes** commonly associated with amyloidosis that shoud trigger screening: a) infiltrative cardiomyopathy manifesting a spectrum from fatigue to overt congestive failure; b) albuminuria with or without renal insufficiency; c) peripheral or autonomic neuropathy; d) unexplained hepatomegaly; e) carpal tunnel syndrome; f) enlargement of the tongue; g) weight loss associated with intestinal symptoms of pseudo-obstruction or malabsorbtion (9). Diagnosis of AL must be considered when any one of this syndromes is seen. Occasionally, patients are recognized because of their monoclonal protein and are diagnosed as atypical multiple myeloma because they have a light chain present but less shan 10% bone marrow plasma cells (8).

Initial investigation should confirm the **diagnosis of amyloidosis on tissue biopsy** and this should be followed by investigation to establish the **type of amyloid** present and the **extent of organ involvement**.

The diagnosis of amyloidosis requires a tissue biopsie that demonstrates apple-green birefringence when stained with Congo red and wiewed under polarizing microscope. Biopsy of affected organ is usually diagnostic but less invasive alternative are possible with lower risk. Fine needle aspiration of the abdominal fat is a simple procedure that is positive for amyloid deposits in >70% of patient. Other sampling sites include rectal biopsy, salivary glands, stomach and bone marrow. The bone marrow demonstrates amyloid deposits in half of patients if the biopsy specimen contains blood wessels. With Congo red studies of fat and bone marrow the diagnosis is established in 90% of patients. Immunohistochemical staining of the biopsied amyloid deposite with kappa and lambda antisera is helpful only in 50% of cases.

Because the **various types of amyloidosis** require different approaches to treatment, only determining **that a patient has amyloidosis is not adequate**. The clinical pictures of different types of amyloidosis often are similar and distinguishing between AL ans other types of amyloidosis may be impossible clinically. **Evidence of clonality of bone marrow plasma cells and monoclonal light chains in the serum and urine supports the diagnosis of AL**.To screen for AL amyloidosis immunofixation electrophoresis of serum and urine is necessary. The urine must be evaluated because one forth of patients with AL fail to demonstrate a monoclonal light chain in the serum. When the serum and urine are studied by immunofixation electrophoresis nearly 90% of AL patient will have a detectable monoclonal light chain. Even more sensitive is the nephelometric assay for serum free light chains (FLC)(FREELITE) which can detect circulating free light chain with > 10 – fold sensitivity than immunofixation electrophoresis. These antisera recognize epitopes for FLC but do not detect light chains associated with an intact immunoglobulin molecule (3). This is a quantitative test. The free light chain k/λ ratio and serum immunofixation identified 99% of patients with AL amyloidosis. Bone marrow aspirate and biopsy show only a small increase in percentage of plasma cells. 60% of patients have fewer than 10% plasma cell in the bone marrow (median number: 7%). Light chain immunofenotyping of marrow cells, even in absence of increased numbers of plasma cells, reveals the distorsion in the ratio of k to λ reflecting the L-chain type of the amyloid precursor. Immunohistochemical staining for k and λ chains executed on citospin preparation can detected the monoclonal plasma cells in cases with < 5% plasma cells.

Any patient with amyloidosis who lacks a clonal plasma cells disorders with free light chains in serum and urine should be evaluated for the presence of **localized, secondary, senile or familial amyloidosis**. **Localized AL amyloidosis** is most often identified in the upper respiratory, urogenital

and gastrointestinal tracts, the skin and the orbit. The amyloidogenic light chains are produced by a subtile focal infiltrate of clonal lymphoplasmocytoid cells in proximity to the amyloid deposits. This tip of amyloid is frequently nodular in character. The AL nature of localized amyloid can often be confirmed immunohistochemically or by sequencing the fibril protein. Monoclonal immunoglobulin cannot be detected in the serum or urine of patients with localized AL amyloidosis. The phenotip of hereditary systemic amyloidosis associated with certain apolipoprotein A1 variant can mimic localized larynged Al amyloidosis. Treatment is generally confirmed to local surgical intervention according to symptoms. **Secundary amyloidosis** (AA) can involves organs in a fashion indistinguishable from AL amyloidosis. AA is a consequences of long standing uncontrolled systemic inflammation. The most common clinical manifestation of AA is nephrotic range proteinuria. AA amyloid is readly detected immunohistochemical. **Senile cardiac systemic amyloidosis** occurs with high frequency in man over the age of 80. The amyloid deposition is formed from normal transthyretin. **Hereditary systemic amyloidosis** are autosomal dominant disorder caused by mutation in the genes for transthyretin, fibrinogen Aα-chain, lysozym or apolipoprotein I. The clinical features may be indistinguishable from AL amyloidosis. Hereditary transthyretin and fibrinogen Aα-chain amyloidosis are much more common then previously thought. 31 of 34 patients in whon hereditary amyloidosis was misdiagnosed as AL amyloidosis in a British series of 350 cases had amyloid of either variant transthyretin (13 patients) or fibrinogen Aα-chain type (18 patients) (14). Hereditary transthyretin amyloidosis presented with polyneuropathy and/or amyloid cardiomyopathy. Feature suggestive of variant fibrinogen Aα-chain amyloidosis are exclusive renal presentation compled with a distinctive appearance on renal biopsy. The diagnostic of hereditary amyloidosis has implication for prognostic, genetic counseling and treatment wich may include liver transplantation to correct the underlying metabolic defect. This form of "surgical gene therapy" has been successful in familial amyloid polyneuropathy associated with variant forms of transthyretin and in amyloidosis due to Glu 526 Val variant of fibrinogen Aα-chain. In hereditary amyloidosis caused by variants of lysosim, apolipoprotein AI a progressive renal impairment develop slowly. Hereditary forms of amyloidosis may coexist with MGUS. Immunohistochemical staining with the use of commercial antiserum against transthyretin, lysozime, apoilipoprotein AI an fibrinogen are specific. In cases of doubt DNA analysis and amyloid fibril sequencing may be necessary. Mass spectroscopic analysis of extracted protein has been used successfully as a diagnostic tool. Once a diagnosis of AL amyloidosis has been made, investigation are required to evaluate the **extent and severity of organ involvement**. There are a consensus for defining organ involvement and treatment response in AL amyloidosis reported in 2005 (10).

Prognosis is generally poor if AL amyloidosis is untreated. The median survival is 1-2 years. Symptomatic or substantial echocardiographic evidence of cardiac amyloid is associated with a median survival of only 6 months and is the most important clinical predictor of survival. All the patients being assesed for amyloid need echocardiography (also Doppler) including measures of diastolic performance, ejection fraction and mitral deceleration time. Chemical markers of myocite injury: [the troponins and amino terminal pro-brain natriuretic peptide (NT-pro BNP)] have been shown to be useful screening and prognostic marker of AL cardiac involvement (8). B2 microglobulin concentration in serum and presence of circulating plasma cells are independent predictor of survival (9).

A large whole body amyloid load on SAP scintigraphy, autonomic neuropathy, liver involvement, leak of supression of underlying clonal disease by chemotherapy, associated multiple myeloma are all associated with a poor prognosis.

The actual treatment options

The major objective of the therapy is to suppress rapidly the production of the amyloidogenic free light chains (FLC) by reducing the clonal plasma cell population. This creates the condition for the prevalence of the amyloid clearance from its deposits and thus spares the target organs and tissues from the additional damages provoqued by the continuation of the amyloid deposition. A reduction by 50-70% of the serum FLC associates with a stabilization or even a regression of the amyloid deposits. Changes in the amyloid load are positively correlated with changes in serum FLC concentration and, for this, their measurement are used for monitoring the evolution under the treatment (3). The actual emerging principle of the first line therapy is high-dose Melphalan (Melph) followed by peripheral blood autologous stem cell transplantation (ASCT) (2, 4, 8). Data of several

Table 2. Risk – adapted approach for stem cell transplantation for primary amyloidosis (8).

• Good risk	
(all criteria met)	
any age	
1 or 2 organs involved	200 mg/m^2 if ≤ 60 y
No cardiac involvement	140 mg/m^2 if 61-70 y
ClCr ≥ 51 mL/min	100 mg/m^2 if 71 y
• Intermediate risk	
(either criteria)	
≤ 71 y age	
1 or 2 organs involved	140 mg if ≤ 60 y
Asymptomatic/compensated	100 mg if 61-70 y
cardiac disease	
ClCr < 51 mL/min	
• Poor risk/ineligible	
(either criteria)	Standard therapy or
3 organs involved	clinical trials
Advanced cardiac disease	

studies shown in previously untreated AL amyloidosis response rates of near 60%, with 31% complete hematologic response and 34% organ responses. A complete hematologic response is associated with high rate of clinical responses (70%), amelioration of organ disfunction, improvement of the quality of life and long-term overall survival (50% at 5 years) (8). The existence of the amyloid deposits do not impair the stem cell mobilization and the recovery after the stem cell infusion.

Unfortunately only 1/3 of patients are suitable for ASCT. The procedure is charged by a significant mortality, varying from 14 to 39% of cases. There are retained some unexpected deaths (during the stem cell mobilization or even in the time of the graft infusion) and a lot of previsible risk factors which contribute to a fatal issue (advanced age, bad performance status, >2 organs involved, symptomatic amyloidic cardiomyiopathy and autonomic neuropathy, severe renal failure, high creatininemia, hyperbilirubinemia, history of G.1. bleeding due to amyloid). The absolute contraindications are: advanced congestive heart failure, echocardiography ejection fraction <30%, ≥3 organs involved, bilirubin levels >3.0 mg/dL. To minimise the risks of the treatment-related mortality (TRM) is recommended a careful selection of the patients plus some adjustements of the procedure: mobilization only with G-CSF (6 μg/kg every 12 h x 5 days), the use of heparin during leukapheresis, collection in the fifth day at 4 h interval after the last dose of G-CSF, and risk adapted doses of Melph. for conditioning (Table 2) (8).

Complete hematologic response rates, the key of the improvement of organ dysfunction, appear to be dose-related: 55% - for Melph 200 mg/m^2 versus 35% for the lower doses. The median time to achieve a hematologic or organ response was 3,8 months but in general the amelioration of organ disfunction is more slow, requiring about 1 year. The benefit for the complete response is the long-term survival which is consistently prolonged in comparison with the conventional chemotherapy. The utility of the maintenance therapy or of other transplant procedures (double autotransplant or allogeneic-myeloablative or nonmyeloablative SCT) is not yet established.

The conventional cytoreductive therapy is tailored after the models employed in multiple myeloma. It is currently reserved to the patients not eligible for ASCT or who relapse after this procedure. It is agreed that it works too slow for reduce efficiently the FLC. This cannot prevents the fatal issue for a large number of patients. The most employed regimens are Melphalan + Prednison or Dexamethasone (MP, M-DEX), High-dose Dexamethasone (HDD), Vincristine + Adriamycin + Dexamethasone (VAD).

MP is the only chemotherapy regimen that has been evaluated in randomized controled clinical trials (13). The response, assessed by organ function and paraprotein levels, was seen in 28% of patients, with a median time to response of 1 year. The responders survive 6 times longer than the nonresponders (89 mo vs 14 mo). Patients with nephrotic syndrome had a better outcome than those with cardiomyopathy or peripheral neuropathy. The abnormal high values of the serum creatinin (>3 mg/dL), bilirubin or alkaline phosphatase (>4 times the normal) reduce deeply the responses. Melphalan has adverse effects on the hematopoietic bone marrow, expressed by cytopenias, impairment of the medullary stocks of stem cells and in long term survivors, by the risk for developing myelodysplasia and secondary leukemia.

The combination M-DEX in the form of i.v. intermediate dose Melph (25 mg/m2) plus oral DEX, produces hematologic responses in 67% of cases, in a median time of 4,5 months with a TRM of 4%. One third of these, attained the complete remission and 48% the functional improvement of the target organs. The responses were maintained after

a median time of 24 months. This model was used to the patients resistant or contraindicated to VAD therapy, as a reserve first-line therapy. The M-DEX regimen may deplete stem cell reserve. It may be prudent to harvest stem cell before its from patients who might subsequently benefit from ASCT.

HDD (40 mg on days 1-4, 9-12, 17-20 every 5 weeks) for 3 cycles folowed by maintenance DEX (40 mg x 4 days/mo) and α Interferon., for 1 year collects 24% complete responses, a median progression-free survival of 27 months and an overall survival of 31 months (2). The fluid overload was the main incident requiring dose reduction. HDD remains an indication for the patients in whom other regimens may not be feasible due to expected toxicity or in those who are refractory to other theraphy.

VAD is a controversial type of therapy. Firstly it realises a rapid decrease in tumour burden and a good response rate (63%) and a projected median survival of 50 months. Secondly it does not deplete the stem cell reserve and it can be also employed in first-line with the purpose of preparing the conditions for a ASCT with a reduced risks. Thirdly its side effects target organs affected in amyloidosis (the heart and the nervous system), imposing a special tactic for its prescription (2,8).

A lot of investigational non cytostatic approaches in the treatment of the AL amyloidosis has been developed. Thalidomide is efficient in refractory/ relapsed disease. In monotherapy, the response rates near 30% are registered. In combination with Dexamethasone the respone rates raise to 60%. Commonly side effects are somnolence, constipation, peripheral neuropathy and the propensity to venous thromboembolism. The intolerance is noteworthy and only 35% of patients tolerate the full dose and a long-term treatment. Further information is necessary. Other attractive attempts are under studies: Revlimid (an analogue of Thalidomide), Bortezomib (the proteasome inhibitor), Etanercept (anti TNFα), 4'–iodo-4'deoxydoxorubicin (Idox) (antiamyloid activity) amyloid-reactive humanized antibodies, anti-SAP compounds.

In 2006, AL amyloidosis is a treatable disease and a substantial proportion of patients naw attain a long survival. There are several reasons for this improvement: early diagnosis, better treatment selection, more efective regimens and improved supportive therapy. New and sensitive biomarkers of cardiac dysfunction (BNP, troponins) and for monitoring the clone (by serial FLC quantification) are likely to improve patient selection, therapy management and posibly the outcome (8).

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Hodgkin lymphoma in the pediatric population

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In the Unites States, 800-900 cases of Hodgkin lymphoma (HL) are diagnosed each year in patients younger than 20 years old. Hodgkin lymphoma is rare in children under 5 years of age. In children younger than age 10, it is more common in boys than girls. Among adolescents and young adults, boys and girls are equally affected.

Clinical presentation

Most patients present with indolent, painless lymphadenopathy in the lower cervical and supraclavicular regions. Mediastinal involvement is seen in 60% of patients. Inguinal lymph nodes are affected in less than 5% of cases. Up to 25% of the patients have "B" symptoms. Bone marrow involvement is seen in less than 2% of patients.

Pathology

World Health Organization Classification:

1) Classical Hodgkin lymphoma
   a) Nodular sclerosis (70%)
   b) Mixed cellularity (16%)
   c) Lymphocyte-rich (7%)
   d) Lymphocyte-depleted (less than 2%)
   e) Not otherwise specified (6%)

These percentages reflect the National Cancer Institute's Surveillance, Epidemiology, and End Results data. Mixed cellularity Hodgkin lymphoma (MCHL) is more common in the prepubertal child, accounting for 30-35% of HL. MCHL is most frequently associated with the presence of Epstein-Barr virus (EBV) in tumor cells.

2) Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL): NLPHL occur most commonly in children with a male: female ratio of 5:1. Most patients have low stage disease with peripheral adenopathy. Patients with ALPS have an increased risk for this disease, pointing to a mutation of FAS in at least some individuals at risk. Children with NLPHL do well with chemotherapy alone. Sixty-eight children with low stage NLPHL received COPP-ABV on CCG05942 with EFS and OS of 100% regardless of randomization to RT or no further therapy. In addition, recent studies in children with stage I NLPHL suggest that observation alone may be appropriate after complete resection.

The risk for childhood HL in the United States is greater with increased number of siblings and with reduced parental socioeconomic status and educational achievement. These social factors may enhance risk of early exposure to viral infection. In contrast, adult onset HL is associated with smaller number of siblings and high parental socioeconomic status and education. These factors are associated with later exposure to virus. Day-care or nursery school in early childhood (with presumed early viral exposure) reduces the subsequent risk for young adult HL. Outside of the United States and Western Europe, the higher prevalence of EBV-associated childhood HL is likely to be related to socioeconomic factors that enhance risk for viral exposure in early childhood and correlate with a higher overall incidence of childhood HL.

In cases associated with EBV, the virus is localized to the Reed-Sternberg cell, EBV latent gene products are expressed, and the EBV infection is clonal.

EBV latent membrane protein 1 expression in Hodgkin lymphoma:

HIV-associated lymphocyte-depleted: 100%

Mixed cellularity: 75%

Lymphocyte-rich: 40-45%

Nodular sclerosis: 10-40%
NLPHL: rare

The precise role of EBV in the pathogenesis is not clear. Infected Hodgkin and Reed-Sternberg cells express high levels of LMP1, a member of the tumor necrosis factor receptor superfamily. LMP1 expression is associated with activation of NFKB, up-regulation of cellular bcl-2, IL-10 and major histocompatibility complex class I proteins.

Prognostic factors in pediatric Hodgkin lymphoma:

Advanced stage (stage IIIB, IV)

Bulky mediastinal adenopathy

Nodular sclerosis subtype

B symptomatology: Unexplained fever with temperature over 38°C orally, unexplained weight loss of 10% within 6 months preceding diagnosis, and drenching night sweats

Anemia

Leukocytosis

Hypoalbuminemia

Prognostic indexes using a combination of these factors have not been validated in large prospective trials.

The predictive value of prognostic factors is decreased by effective therapy.

Diagnosis and staging:

Physical examination with measurement of lymph nodes

Lymph node biopsy
Complete blood count
Erythrocyte sedimentation rate
Serum ferritin level
Liver and renal profiles, albumin level

Bilateral bone marrow biopsies (in advanced stage)

Chest XR with measurement of mediastinal mass to thoracic cavity ratio

CT scan of neck, chest, abdomen and pelvis

Whole-body positron-emission tomography (PET) scan

Echocardiogram
Pulmonary function studies
Sperm banking

Treatment:

Involved filed radiation therapy (IFRT) (less than 25Gy) combined with chemotherapy improves the event-free survival rate compared with chemotherapy alone in advanced Hodgkin lymphoma (Children's Cancer Study Group 5942 and Pediatric Oncology group study 8725). However, the overall survival rates of these two treatment options are similar.

Multivariate Model predictive of Local Failure

Variable	*Hazard ratio*	*P*
Male sex	2.89	.034
Bulky mediastinal disease	2.52	.043
Hemoglobin <11g/dl	3.185	.018

Risk categories of Hodgkin lymphoma in pediatric patients

Categories	*Clinical features*	*5-year EFS*	*5-year OS*
Favorable	Stage I and II, non-bulky disease, no "B" symptoms	≥95%	≥99%
Intermediate	Stage I-IIA or B, bulky or extranodal disease, stage IIIA	80-94%	90-98%
High	Stage IIIB and IV	68-91%	88-94%

It is not necessary to prolong therapy after complete remission is achieved.

The gonadotoxic and leukemogenic potentials of alkylating agents led to attempts to modify chemotherapy. Mechlorethamine and procarbazine have now been replaced with less toxic drugs.

Hematopoietic stem cell transplantation (HSCT) can play an important role in the management of patients for whom frontline therapy fails to produce a response.

Therapy for primary disease:

The current treatment approach is risk-adapted, combined-modality therapy. In ongoing clinical trials, therapy is determined on the basis of tumor response at the end of second or third cycle of chemotherapy. Patients with 60% or greater reduction in tumor burden are considered rapid early responders (RERs), and patients with less reduction in tumor burden are considered slow early responders (SERs). The dose of subsequent radiotherapy, subsequent chemotherapy, or the combination of these is determined by the response.

In the current North American pediatric trial, low risk patients (Stage I and IIA without bulky disease) receive three cycles of dose- and time-intensive chemotherapy. After disease evaluation, those with complete response stop treatment.

Regimen	*Cycle*	*Dox*	*Bleo*	*VB*	*Dtc*	*VP*	*CY*	*M*	*Vc*	*Pcz*	*Prd*	*Mtx*
ABVD/MOPP	4,6,8	50	20	12	750			12	3	1400	560	
ABVE	2,4	50	20			625			2.8		280	
ABVE-PC	3,5	60	15			375	800		2.8		560	
BEACOPPe	4,8	35	10			600	1200		1.4	700	560	
COPP/ABV	4,6	35	10	6			600		1.4	700	560	
VEPA	6	50		12		400					560	
VAMP	4	50		12							560	40
EBVP	4		10	12		500					280	
OEPA	2	80				500			4.5		840	
OPPA	2	80							4.5	1400	840	
COPP	2,4						1000		3	1400	560	

Dox, doxorubicin; Bleo, bleomycin, Vlb, vinblastina; Dtc, dacarbazine; VP, etoposide; CY, cyclophosphamide; M, mustargen; Vcr, vincristine; Prd, prednisone; Mtx, methotrexate.

Those with partial response receive IFRT. For intermediate-risk Hodgkin lymphoma, RERs are randomly assigned to receive or not receive IFRT. All SERs receive radiotherapy, and half of them are treated with augmented chemotherapy.

Recurrent or relapsed Hodgkin lymphoma:

1) Re-induction with non-cross resistant agents: cisplatin, cytosine arabinoside, ifosfamide, gemcitabine, vinorelbine, rituximab, anti-CD30 monoclonal antibody
2) BEAM: carmustine (BCNU), etoposide, cytosine arabinoside, melphalan
3) Autologous stem cell rescue
4) Local radiotherapy to sites of previous disease involvement

Allogeneic HSCT is reserved for patients with incomplete response after re-induction chemotherapy or patients with bone marrow involvement. Reduced-intensity preparative regimens with fludarabine with melphalan or cyclophosphamide are being tested.

Late effect	*Etiology*	*Modification in treatment*
Hypoplasia	Full dose radiation	Chemotherapy alone or low-dose RT
Secondary leukemia	MOPP, alkylating agents	Limit dose or use of MOPP
Secondary solid tumor	Full dose radiation	Chemotherapy alone or low-dose RT
Myocardial dysfunction	Doxorubicin, mediastinal RT	Limit doxorubicin, limit mediastinal RT
Cardiac ischemia	Full dose radiation	Chemotherapy alone or low-dose RT
Pulmonary toxicity	Bleomycin, RT	Reduce bleomycin dose
Gonadal toxicity	Alkylating agents	Eliminate procarbazine. Replace nitrogen mustard with cyclophosphamide.
Ovarian failure	Pelvic radiation	Oophoropexy

Long term side effects:

Overall survival for pediatric HL is 90%. The risk of death due to disease almost equaled at 20 years from diagnosis by the risk of death due to other causes, particularly the long-term consequences of therapy.

1) Late effects of radiotherapy:
   a) Atherosclerotic heart disease
   b) Thyroid dysfunction
   c) Soft-tissue hypoplasia and retardation of bone growth: Lower sitting height, "pear shaped" body habitus after mantle irradiation.
   d) Second malignant neoplasms (SMN): Risk was increased 18.5 fold in the LESG cohort of children diagnosed with HL before age 16 years. Cumulative incidence of SMN increased form 10.6% at 10 years to 26.35 at 30 years. Breast cancer was most common. Other cancers included thyroid, lung, bone tumors, colorectal, gastric and skin cancers. The risk of SMN after low dose radiation is not yet well described.
2) Late effects of chemotherapy:
   a) Sterility from alkylating agents
   b) Secondary leukemia form alikylating agents and etoposide
   c) Pulmonary fibrosis from bleomycin
   d) Cardiomyopathy from anthracyclines

Treatment of indolent lymphomas: From watch and wait to high dose therapy

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Indolent B-cell non-Hodgkin's lymphomas (IBC-NHL) represent a heterogenous disease group, including follicular lymphomas grades 1 and 2 (FL1/2), which account for approximately 20% of all NHL worldwide, small lymphocytic lymphoma (SLL), lymphoplasmacytic lymphoma (LPL; 1%), mantle cell lymphoma (MCL; 6%), and marginal zone lymphomas, either splenic (SMZL; 1%), nodal (NMZL, 2%) or extranodal MALT (8%) (1,2). With the exception of FL1/2, the rarity of these types of IBC-NHL and their long natural history have prevented the design of randomized trials, so that evidence-based treatment approaches are lacking. As a result various treatment strategies ranging from "watch and wait" policies or oral alkylating agent monotherapy to more aggressive combination chemotherapy (CT), chemoimmunotherapy or even CT followed by high dose therapy and autologous stem cell transplantation (ASCT) have been used in previously untreated patients with IBC-NHL.

This review is focused to the first-line treatment of nodal and splenic (but not primary extranodal) IBC-NHL. Data regarding relapsed and refractory patients will be briefly presented in relation to various investigational approaches.

Follicular Lymphomas, Grades 1 and 2

Ann Arbor Stages I and II

Approximately 1/4 of the total patient population with FL1/2 have localized disease, when evaluated with conventional staging. Involved field radiotherapy (IF-RT) may produce long-term disease free survival in 40-50% of the patients (2-5). Recently Positron Emission Tomography (PET) scan has been incorporated in the staging of lymphomas and may upstage a subgroup of patients with FL1/2. Furthermore, many patients with "localized" disease have molecular evidence of bcl-2 or immunoglobulin heavy chain gene rearrangements in the blood and/or bone marrow. It is not known whether these novel techniques will modify the current standard of care, i.e. IF-RT. Although the administration of the anti-CD20 monoclonal antibody rituximab in the eradication of residual disease appears reasonable, it cannot as yet be based on published data.

Ann Arbor stages III and IV

Low-Intensity Appoaches

A "watch and wait" policy can be applied in patients with asymptomatic, non-bulky FL1/2 (6). Systemic treatment should be instituted, when patients develop constitutional symptoms or symptoms related to tumor burden.

Low-intensity treatment approaches for advanced FL include oral alkylating agents, such as intermittent chlorambucil (possibly combined with monthly rituximab infusions), alkylator-based combination chemotherapy combined with rituximab (R-CVP) or monotherapy with rituximab. These options can be safely selected for the elderly patients or those who cannot tolerate more intensive CT, although younger patients can also be treated with such approaches.

Rituximab, when given as first-line monotherapy, produces RR of approximately 70% while maintenance therapy with 4 bimonthly infusions prolongs the PFS over rituximab induction alone (median 36 vs 19 months) (7). It is not clear wheth-

er in this setting rituximab maintenance is superior to rituximab induction followed only by "on demand" retreatment upon progression (8). Given the favorable toxicity profile of rituximab and the potential of durable responses, it can be considered as front-line therapy at least in patients not eligible for aggressive CT.

High-Intensity Approaches

In contrast to the low-intensity approaches, many centers prefer to administer chemoimmunotherapy with rituximab combined with anthracycline-based CT (CHOP or similar regimens, MCP: mitoxantrone, chlorambucil and prednisone, etc) or CT combining anthracyclines and purine analogues (FND, FCM etc), especially in younger patients.

Several randomized trials have now convincingly demonstrated that the addition of rituximab to conventional CT produces not only superior PFS rates (9-12), but also OS or lymphoma-specific survival benefits as well (9-11). Thus the addition of rituximab to the first-line CT regimen is strongly recommended. It should be however underlined that although these trials have established the role of rituximab in untreated, advanced FL, they do not provide any guidance as to which CT regimen is the most effective.

The role of rituximab maintenance in previously untreated patients with FL1/2 after the 1st remission achieved by rituximab-based chemoimmunotherapy has not been established yet in a randomized fashion. In contrast maintenance therapy with rituximab prongs PFS and OS in patients with relapsed/refactory disease, who have responded to salvage chemoimmunotherapy (13,14).

A recent meta-analysis suggested that interferon-alpha (IFN-α) incorporated in the initial CT regimen and/or given as maintenance to responding patients may result in superior PFS rates and a modest increase in 10-year OS, in the order of 6-8% (15). However the individual randomized trials included in this analysis were performed prior to the introduction of rituximab, so that the benefit of IFN-α in the era of rituximab remains uncertain.

The value of ASCT as a consolidation approach in patients with FL in 1st remission has been tested in 3 randomized trials (16-18). Two of them demonstrated significant prolongation of PFS, while the third (17) revealed an OS benefit despite similar PFS! Firm conclusions on OS cannot be drawn yet, but two trials demonstrated a significant increase of MDS/ANLL in ASCT-treated patients with a TBI containing regimen. Furthermore, rituximab was not used in anyone of these trials. Thus, ASCT is still an experimental approach in the first-line treatment of FL. In contrast, it is clearly indicated as consolidation in patients with relapsed/refractory disease, who have responded to salvage therapy.

Investigational Approaches

Radioimmunotherapy with ^{131}I-Tositumomab (Bexxar) or ^{90}Y Ibritumomab tiuxetan (Zevalin), which can be administered on an outpatient basis, has been approved for the treatment of relapsed/refractory disease (19). In this setting response rates (RR) are in the order of 70-80% with approximately 30% complete remissions (CR). As first-line therapy, a single one week course of Bexxar produced a RR of 95% with 75% CRs in 76 previously untreated stage III/IV FL patients. Among CRs, 80% were extended at the molecular level as well, while the 5-year PFS was 59% and 5-year OS 89% (20). Radioimmunotherapy at myeloablative doses with stem cell support has produced very promising results in relapsed/refractory disease. Recently bortezomib, a proteasome inhibitor, was shown to induce responses in patients with relapsed/refractory FL (reviewed in ref. 19).

Mantle Cell Lymphoma

MCL cannot be strictly considered as an IBC-NHL, since median survival is short, usually in the range of 2 to 4 years. The preferred treatment approach is highly depended on patient's age. Clinical stage is advanced (III/IV) in 85-90% of patients with MCL.

CHOP or similar regimens are given by most centers, while others also use fludarabine-based regimens. The addition of rituximab to CHOP moderately prolonged PFS but had no effect on OS in a recent study (21). Impressive preliminary results have been reported with the combination of rituximab and the hyperCVAD regimen (22). However hyperCVAD is associated with considerable acute toxicity and non-negligible rates of toxic death and secondary myelodysplasia/acute non lymphoblastic leukemia. Furthermore there is no plateau in PFS curves there is no randomized evidence for the superiority of hyperCVAD over other approaches.

The use of ASCT as consolidation in MCL patients after conventional CT was evaluated in

a recent randomized trial: Patients younger than 65 years old, who responded to conventional CT (CHOP or CHOP-like in 74% of patients, R-CHOP in 26%) were randomized to 2 additional cycles of CT plus IFN-α maintenance or to Dexa-BEAM, stem cell mobilization and high dose therapy including total body irradiation followed by ASCT. Although PFS was significantly prolonged in the ASCT arm, no clear OS benefit was evident at 3 years. More importantly, the PFS curve did not reach a plateau, a fact that suggests that neither ASCT is not curative in MCL (23). Manipulations that may potentiate the efficacy of ASCT-based approaches include *in vivo* purging with rituximab prior to consolidation with high-dose therapy or the administration of high-dose radioimmunotherapy (24,25). Allogeneic SCT – either myeloablative or based on reduced intensity conditioning regimens - are the only potentially curable approaches and deserve further evaluation.

In contrast to these high-intensity approaches, some patients with MCL may achieve relatively durable remissions with chlorambucil monotherapy (26). Elderly asymptomatic patients without features of histologic aggressiveness (non-blastoid MCL) may be treated in this way. The combination of rituximab with chlorambucil also appears reasonable. Rituximab monotherapy may produce RR of 25-30% in both untreated and relapsed/refractory MCL patients, but CRs are very rare (~2%) and the median PFS is in the range of 6-12 months. Maintenance rituximab does not appear to improve these results (27). In the rare "splenic form" of MCL, splenectomy may be a reasonable first-line approach, delaying the administration of CT (28). Novel agents, as the proteasome inhibitor bortezomib and temsirolimus a rapamycin kinase inhibitor that regulates cyclin-D1 translation are effective in relapsed/refractory patients and require further evaluation (19,29-32), while radioimmunotherapy with Bexxar or Zevalin has also produced promising results (19).

Splenic Marginal Zone Lymphoma

The "watch and wait" approach may be applied in patients with SMZL and asymptomatic splenomegaly or non significant cytopenias (33). When treatment is needed, splenectomy has traditionally been a reasonable option. However recent data suggest that rituximab monotherapy may substitute splenectomy by producing durable responses and survival (34). Various CT regimens, including CHOP or fludarabine-based ones as well as oral alkylating agents have been used, mainly in splenectomy failures or in patients not eligible for splenectomy (33). The selection of the CT regimen is at present arbitrary. The goal of treatment is to achieve a good response, but not necessarily a CR. The subgroup of patients with SMZL and hepatitis C virus infection achieve long-lasting partial remissions of excellent quality with interferon-a and/or ribavirine without CT (35).

Small Lymphocytic, Lymphoplasmacytic, and Nodal Marginal Zone Lymphoma

Specific data for these subtypes of IBC-NHL, particularly for NMZL, are lacking. We favor treatment of these patients with monthly intermittent chlorambucil for 1-2 years. High-dose chlorambucil produced a RR of 72% with 30% CRs in a recent randomized trial, with 5-year PFS rates of 20-30%. The addition of epirubicine or the administration of IFN-a maintenance did not improve these results (36). Rituximab produces responses similar to that observed in FL and can be considered as monotherapy in these patients (37). As many as 70% of previously untreated patients respond to induction plus maintenance rituximab, with a median PFS of approximately 2.5 years.

Combination CT (CVP, CHOP) is usually deserved for relapsed disease while purine analogues may also have a role in the treatment of these patients, although clear data are not yet available.

Conclusion

Evidence-based approaches are emerging for FL and are likely to be available in the near future for MCL, but are difficult to be established for SLL, LPL and marginal zone lymphomas. The introduction of rituximab has revolutionized the treatment of IBC-NHL. The combination of rituximab with various chemotherapy approaches is the standard of care for previously untreated patients with advanced stage FL1/2, who have indications for treatment initiation. ASCT is indicated as salvage therapy in FL but there are no data to justify its use in previously untreated patients with IBC-NHL outside clinical trials. Newer approaches including radioimmunotherapy, bortezomib and temsirolimus may improve the outcome of patients with IBC-NHL, especially those with MCL. For the time being, only allogeneic SCT can cure a fraction of relapsed/refractory patients at the expense of a high rate of early mortality.

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Allogeneic transplantation in non-Hodgkin's lymphomas

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Both indolent and aggressive NHL are very sensitive to chemotherapy, with overall response rates over 70-90% (1,2). However, indolent B-cell malignancies are incurable with chemotherapy and relapse is imminent with relapse free period ranging from 15 months to 4 years (3), with each subsequent chemotherapy resulting in shorter duration of response. In aggressive NHL, such as diffuse large B cell lymphoma, a fraction of the patients can be cured with initial chemotherapy. However, until recently, despite trials with multiple chemotherapy regimens, the cure rate has not improved over CHOP regimen and stayed around 40-45 % (2). Although addition of rituximab has improved the survival, large number of patients will still relapse (4,5). High dose chemotherapy with autologous stem cell support has been shown to be superior to standard chemotherapy for both indolent and aggressive NHL (6,7). However, it has been recognized early on that patients with chemorefractory disease do not do well with autologous BMT (8,9) and most subsequent trials have excluded such patients. Philip T, et al reported in 1987 that 3 year disease free survival for primary chemorefractory, chemorefractory relapse and chemosensitive relapse were 0%, 14% and 36% respectively. Even with patients with chemosensitive relapse, a large fraction will relapse after autologous BMT. For patients with primary chemorefractory disease, chemorefractory relapse, and those who relapse after autologous BMT, treatment options are limited. The lack of curative options for such patients, and lack of general progress in the conventional treatments have resulted in allogeneic transplant being more intensively studied in NHL. The curative potential of the allogeneic stem cell transplantation resides both in the efficacy of the pretransplant conditioning regimens supported by tumor-free stem cell grafts and in putative graft-versus-lymphoma effect.

A number of studies have been published comparing allografting to autologous transplants for various lymphomas (Table 1). Hosing et al have reported on 112 patients undergoing transplantation for refractory or relapsed follicular lymphoma between 1991 and 2000 (10). The patients were preferentially selected for autologous BMT if they had chemosensitive disease and less than 10% marrow involvement. Forty-three percent of the patients in the allogeneic group had resistant disease. Despite the fact that patients in the autologous group were more likely to have chemosensitive disease and to be in remission, the overall survival was 34% and disease free survival was 17% at a median follow-up of 71 months. This contrasted to an OS and DFS of 49% and 45% respectively in the allogeneic transplant group. Treatment related mortality was high in allogeneic transplant group with 15 of 44 (34%) patients dying in the first 100 days, compared to 6% in autologous group. Statistical analysis showed a significant advantage in DFS for allotransplant, but OS was of borderline significance (P = .05). Of note, no late relapses were observed in the allogeneic group whereas relapses as late as eight years out were noted in the autologous group. In a similar analysis, investigators from the International Bone Marrow Transplant Registry (IBMTR) reported on 904 patients with follicular NHL undergoing allogeneic or autologous transplant from 1990 to 1999 (11). As in the previously series, allogeneic patients were more likely to have advanced disease, bone marrow involvement, poor performance status, or chemo-resistant disease.

Table 1. Comparisons of allogeneic and autologous transplants

Reference	*Study Dates*	*N*		*NHL type/grade*		*Results/Comments*
Hosing[10]	1991-2000	Allo	44	Indolent	112	Long term results favors allografts
		Auto	68			
Van Beisen[11]	1990-1999	Allo	176	Folicular	904	Low relapse, high TRM
		Auto	597			
		PAuto	131			
Peniket[12]	1982-1998	Allo	1018	Low	231	Low relapse, high TRM
		Auto	3054	Intermediate	147	
				High	255	
				Lymphoblastic	314	
				Burkitt	71	
Bierman[13]	1985-1998	Allo	891	Dü_ük	762	No GVL demonstrated in comparison to syngeneic
		Auto	2018	Orta	1779	transplants. Number of patients low in syngeneic
		PAuto	376	Yüksek	669	group.
		Syn	89			
Schimmer[14]	1986-1997	A	44	Indolent	32%	Low relapse, high TRM
		0	385	Aggressive	68%	

Abbreviations: Allo, allogeneic; Auto, autologous; PAuto, purged autologous; Syn, syngeneic; TRM, transplant related mortality; GVL, graft-versus-lymphoma.

Treatment-related mortality (TRM) was higher with allografts, with 24% TRM at one year and 30% at five years, but the relapse rate of 19% at one year, with few relapses thereafter, compared favorably to one and five year relapse rates of 36% and 58% after unpurged autologous and 25% and 43% relapse rates after purged autologous transplant, respectively. The early TRM but lower late relapse risk in allogeneic patients resulted in almost identical survival times in comparison to autotransplant. An EBMT registry study of 1185 allogeneic transplants for lymphoma between 1982 and 1998 compared results to autografts by first examining variables associated with outcome for specific NHL subtypes and then performing a 1:3 matched analysis (12). In this study all lymphoma subtypes were included although the long duration of time over which patients were included raises questions as to the accuracy of disease classification, since lymphoma classification evolved significantly during the period in question. With striking similarity to the previously mentioned studies, relapse rates were lower with allografting (Burkitt's lymphoma was the one clear exception to this rule) but survival was not, due to higher mortality rates with allografting.

It should be noted that these are mostly retrospective analysis that include patients transplanted over long periods of time and not entirely representative of modern transplant techniques. For example, majority of the patients were transplanted using bone marrow grafts in the study reported by Peniket *et al* (12). Nowadays, however, majority of grafts are peripheral blood stem cell grafts. Additionally, improvements in supportive care practices in recent years have also improved outcome after allogeneic transplants. In the study reported by Van Besien et al, TRM has decreased and DFS has improved during successive 3-year periods. Finally, above reports are confounded by heterogenous treatment practices and patient selection criteria. Most importantly, allogeneic patients included in the series typically have more advanced and/or refractory disease and are more heavily pre-treated than autologous transplant patients, and are by definition higher risk group. Despite their shortcomings, these reports do shed some light on the benefits and limitations of transplantation for NHL.

Lower relapse rates after allogeneic transplant generally have been attributed to a graft-versus-tumor effect analogous to that observed in patients with chronic myelogenous leukemia. This belief has been challenged by a large retrospective investigation. Bierman et al. analyzed registry data from the IBMTR and the EBMT and compared relapse rates after syngeneic, autologous, and allogeneic transplants (13). Patients who received purged autografts and T-cell depleted allografts were included also in order to further study the role of tumor cell contamination and GVL effects on relapse, respectively. The major findings of this study were that in all histologies the relapse rates were similar after syngeneic and allogeneic

transplants, and that T-cell depletion was not associated with a higher relapse rate than T-cell replete grafts. The most important implication of this study is that enthusiasm for reduced intensity regimens, which rely primarily upon a GVL effect, may be unwarranted. On the contrary, as described below, some of the recent results observed with reduced intensity regimens are the strongest evidence of a GVL effect.

Growing recognition in the past decade of the immunotherapeutic potential of allografts has led to a reconsideration of the necessity of high-dose myeloablative conditioning regimens traditionally administered prior to transplantation. Several clinical trials presented in the late 1990s established the ability to achieve donor engraftment following less-than-ablative conditioning regimens, rise to the new sub-field of nonmyeloablative transplantation (15-18). In many respects, nonmyeloablative transplantation is an ideal therapy for selected patients with NHL. Indolent and mantle cell lymphomas appear susceptible to GVT effects and primarily affect an older age population not usually eligible to receive traditional ablative therapies (19). The growth rate of such tumors may allow sufficient time for GVL effects to occur. Patients with aggressive lymphomas, in contrast, may be less likely to benefit from a reduced intensity approach if their disease is not in remission at the time of transplant or is kinetically active. In either case, a reduction in the treatment-related toxicity attributed to high-dose conditioning regimens in lymphoma patients could be expected to decrease treatment-related mortality. In a large retrospective review, the EBMT reported results of reduced intensity allografting in 188 patients between 1996 and 2000 (20). Included in this series were 52 patients with indolent lymphomas, 62 patients with typical aggressive histologies, and 22 patients with mantle cell lymphoma. Of note, almost half of the patients had undergone prior high dose therapy and autologous transplant, a group that is traditionally considered to be at risk for very high rates of TRM if a second transplant is performed. The majority of patients were treated with a fludarabine based conditioning regimen and over a third of the patients also received alemtuzamab (Campath) that results in a partial in vivo depletion of transplanted T-lymphocytes. Estimated overall survival at two years was 50%. By multivariate analysis only chemosensitivity was associated with improved survival. The rate of progression of disease was particularly high for patients with aggressive histologies (78% at 2 years) and mantle cell NHL (100% at 2 years). Treatment related mortality for patients with a previous transplant was 30% at one year. The poor results in aggressive NHL and mantle cell lymphoma (MCL) may reflect the inability of less intensive regimens to control such diseases for' an adequate time period for GVL effects to develop; however, GVL effect might have been abrogated by in vivo T cell depletion by Campath.

In contrast to the poor results of MCL in the latter investigation, two separate studies have shown a more potent GVL effect with T-cell replete grafts in patients with MCL. In a study of 18 patients with relapsed MCL, including five who had relapsed after autologous transplant, Khouri et al. described a current event-free survival of 82% at three years (19). Most impressively, no patient developed greater than grade 2 GVHD, suggesting that a GVL effect could occur without GVHD. In another study, among a larger series of patients with different lymphoma histologies, Hertzberg et al reported that 8 out of 9 patients with MCL have remained disease free after transplant (21). As it seems very unlikely that any patient in these two series was cured by the preparative regimen, the long-term disease-free survival provides indirect but strong evidence for a GVL effect.

Reduced intensity allogeneic transplant also has recently been shown to be potentially curative in patients with chronic lymphocytic lymphoma (ClL) and, by inference, small lymphocytic lymphoma (SLL). In a study of the Cooperative German Transplant Study Group, 62% of 30 patients had progression-free survival at two years (22). The CR rate increased from 25% at one year to 66% at two years, suggesting an ongoing GVL effect that can clearly not be explained by the preparative regimen. Six of 12 patients who had CR were refractory to fludarabine and none of the patients who achieved at least a partial remission (PR) died of progressive CLL. These results provide great hope for patients with fludarabine refractory disease and provide support for further investigation of allogeneic transplant in patients with recurrent poor prognosis disease.

Despite the impressive data for. reduced-intensity transplant in patients with low-grade and mantle cell lymphoma, the available information is not as encouraging for patients with more aggressive histologies with active disease. In the EBMT study discussed above, 78% of patients showed

disease progression at two years. Similarly, Kusumi et al. reported results of reduced intensity conditioning transplants in 112 patients in Japan (23). The progression free survival rate among 24 patients with chemorefractory aggressive NHL was 30% at 3 years, compared to 64% in patients with chemorefractory indolent NHL.

More encouraging preliminary results were presented recently in patients who relapsed after a prior autologous transplant and then achieved a good remission prior to reduced-intensity allogeneic transplant. In a series of 20 patients, including nine patients with diffuse large cell lymphoma, all 20 patients achieved a CR after allogeneic transplant and only one patient died (non-relapse mortality) (24). The low TRM stands in marked contrast with the unacceptably high rates of TRM reported with standard conditioning, which suggests that relapse after autologous transplant is not necessarily fatal.

A limited number of reports in the past have suggested that recurrent lymphoma following allogeneic transplantation may be treated in the same fashion as other hematologic malignancies, with attempts at inducing graft-versus-tumor effects by withdrawing immunosuppression and/or administering donor lymphocytes. Such approaches have met with mixed success and may be dependent on factors such as disease burden and histologic classification. Since GVL effects are somewhat time-dependent, it has been suggested that aggressive lymphomas may be less susceptible to such maneuvers based solely on a kinetic basis. Adjunctive treatments capable of controlling post-transplant disease, without causing undue toxicity or interference with the development of donor-derived immune function, therefore might be expected to improve the odds of successfully inducing GVL effects. In the EBMT registry series of reduced intensity transplantation reported by Robinson et al, the rate of response to DLI was encouraging and was not limited to patients with indolent lymphoma (20).

In our institution, patients with comorbidity or advanced age who would be considered poor candidates for allogeneic transplantation and patients with indolent low-grade lymphoma regardless of age or organ function are offered an allogeneic stem cell transplant with reduced intensity conditioning regimen. The conditioning regimen includes busulfan 0.8 mg/kg IV every 6 hours for eight doses, fludarabine 30 mg/m2 daily for 6 days, and ATG 10 mg/kg for 4 days. Initially, ATG was given to all patients. It was later on dropped for patients receiving MSD transplants. Younger patients with aggressive NHL are offered allogeneic BMT with myeloablative conditioning. The conditioning regimen is BEAM for most patients. The results of allogeneic BMT in 28 patients with various NHL have been published (25). In that study, DFS was 82 % for patients with chemosensitive disease, and 42% for chemorefractory disease. Additionally, in patients who relapsed after a prior autograft, disease free survival was 55%.

Ten patients had persistent or recurrent disease following allograft and received further therapy that was tailored to the patients prior history and clinical situation. Seven patients were treated with the hope of inducing a GVL effect by withdrawing immunosuppression and, in six cases, administering donor lymphocytes (DLl). Additional therapies administered at the time of recurrence to some patients also included rituximab, interferon and radiotherapy to isolated sites of disease. Four patients responded to this strategy, all of whom developed GVHD. Two of these patients, with aggressive histologies, remain in complete remission at 46 and 58 months post-transplant. One patient died of disease progression and one died in continued remission at 37 months post-transplant of sequelae of chronic GVHD.

In summary, allogeneic stem cell transplantation appears to be a potentially curative procedure for several previously incurable lymphomas including relapsed mantle cell lymphoma and various small cell lymphomas. Reduced intensity transplants are highly effective in small cell and mantle cell lymphomas, and appear to be feasible and effective in patients that have exhausted other treatments including autologous transplant. Moreover, reduced intensity transplants have allowed an older generation a chance for a cure that was not previously available. However, reduced intensity transplants have not yet proved effective in intermediate grade lymphomas with active disease. Further investigation of reduced intensity transplants are required for patients with intermediate grade lymphomas that have achieved CR or near CR but are likely to relapse.

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A hard task, performing transplantation in a developing country

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For more than 30 years stem cell transplantation (SCT) has been performing as a standard and routine therapy for malignant and non-malignant congenital or acquired disorders of the hematopoietic system (1-3). The indications for stem cell transplantation have been changed during the last decade, especially in acute leukemia, lymphoma and chronic myeloid leukemia. Treatment depends primarily on prognostic risk factors of the disease (4, 5). Donors for SCT could be related and unrelated (6) and most of the centers are using stem cells from marrow, peripheral blood and cord blood (7). Recently reduced intensity conditioning has been introduced to decrease immediate posttransplant toxicity (8). By these preparative regimens stem cell transplantation has further expanded to patients with older age and with co-morbidities (9). The problems in transitional countries are limited recourses for allogeneic transplantation. In such circumstances it is mandatory to use transplants rationally and to avoid therapy in patients who could be treated with less toxic therapy with similar efficacy. The examples are the changes in treatment approach with stem cell transplantation in the near past or currently, i.e. transient use of autologous transplantation for breast cancer, decline of allogeneic transplantation for CML (10) and increasing demand of autologous transplantation in lymphoma and myeloma (11, 12).

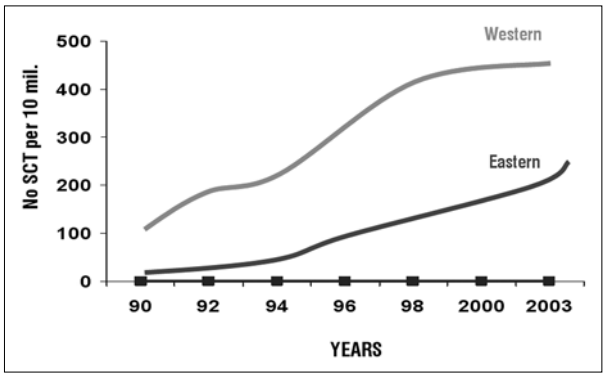

Figure 1. Total transplantation per 10 millions in Western and Eastern Europe

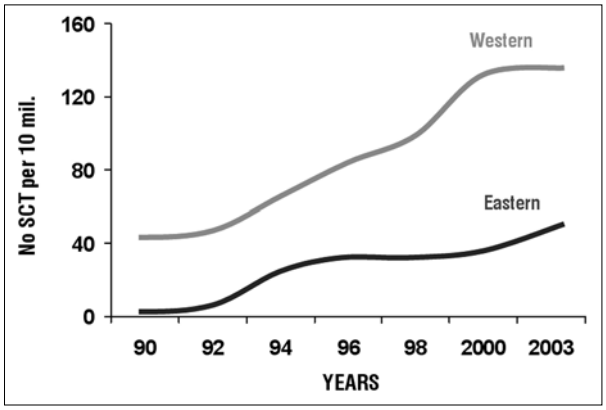

Figure 2. Allogeneic transplantation per 10 millions in Western and Eastern Europe

Stem cell transplantation in transitional countries

The rates of stem cell transplantation in transitional countries reporting their data in EBMTG Registry (13) is illustrated in figure 1. All these countries geographically are coming from Eastern Europe. We compare the rates with the western European countries. The rates of total transplants (Fig. 1.) are higher in western countries and were always 30% to 50% higher than in Eastern Europe. The total transplant rate is still increasing in eastern countries, while in Western Europe

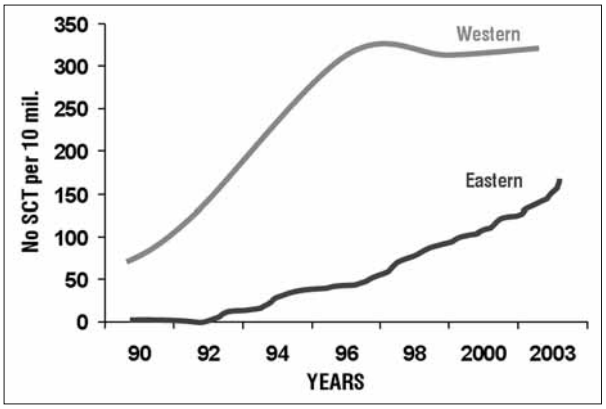

Figure 3. Autologous transplantation per 10 millions in Western and Eastern Europe

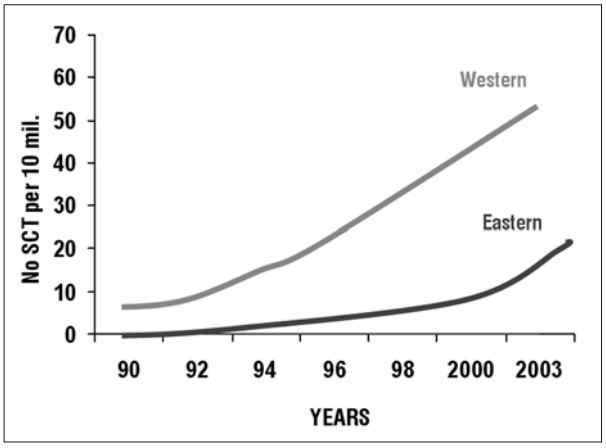

Figure 4. Allogeneic transplantation per 10 millions from unrelated donor in western and eastern Europe

a "plateau" was reached in 1998. When we looked autologous transplants (Fig.2.) the similar difference between eastern and western European countries is also found. The plateau in western country was reached in 1997, probably because of decreasing or stopping to perform autologous transplant in breast cancer. The rates of allogeneic transplants (Fig. 3.) are very similar to autologous transplantation, except the plateau in Western Europe is reached in 2000. The curves of allogeneic transplants (Fig. 4.) from unrelated matched donor clearly showed even more striking difference in transplant rates. There is a delay in MUD transplant programs for 5 to 6 years in Eastern Europe compared to Western Europe.

Trend for increasing transplants rates is presented in transitional as well as in well developed countries. When we compare the number of related and unrelated transplants per year the incidence of unrelated transplants reach about

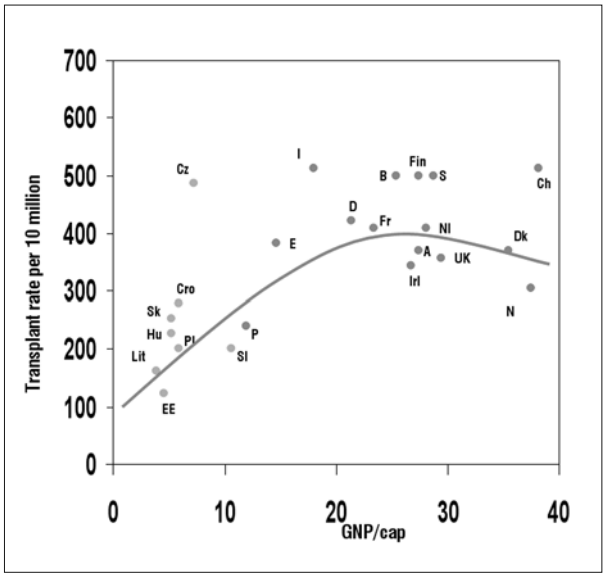

Figure 5. Correlation between transplant rates and gross national income *per capita* in selected eastern and western European countries. Individual points reflect participating countries. Regression curve is based on all European countries (data points restricted to selected countries; Table 1). Blue – Eastern Europe; red – Western Europe; GNI/cap – gross national income *per capita*, in US$; A – Austria; B – Belgium; CH – Switzerland; CR – Croatia; CZ – Czech Republic; D – Germany; DK –Denmark; E – Spain; EE – Estonia; F – France; FIN – Finland; HU – Hungary; I – Italy; IRL – Ireland; Lit – Lithuania; N – Norway; NL – Netherlands; P – Portugal; PL – Poland; S – Sweden; SK – Slovakia; SL – Slovenia; UK – United Kingdom.

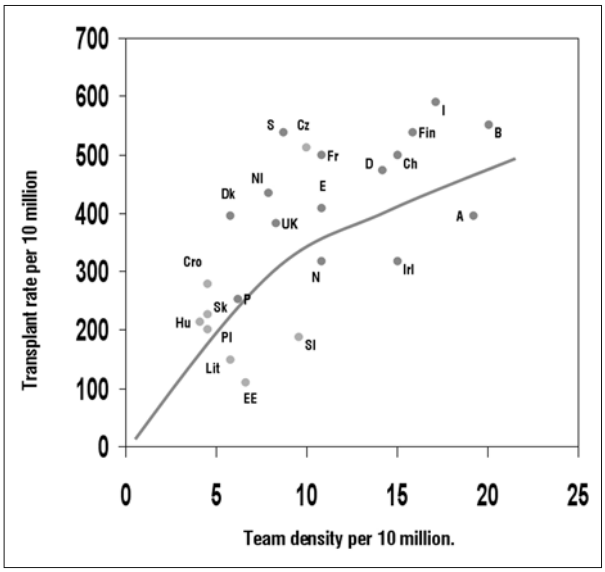

Figure 6. Correlation between transplant rates and team density in selected eastern and western European countries. Individual points reflect participating countries. Regression curve is based on all European countries (data points restricted to selected countries;
Table 1). blue – Eastern Europe; red – Western Europe; GNI/cap – gross national income *per capita*, in US$; A – Austria; B – Belgium; CH – Switzerland; CR – Croatia; CZ – Czech Republic; D – Germany; DK – Denmark; E – Spain; EE – Estonia; F – France; FIN – Finland; HU – Hungary; I – Italy; IRL – Ireland; Lit – Lithuania; N – Norway; NL – Netherlands; P – Portugal; PL – Poland; S – Sweden; SK – Slovakia; SL – Slovenia; UK – United Kingdom.

50% of related transplants in both Eastern and Western Europe.

It is also important to show the difference between Eastern and Western Europe concerning the transplant rates and grosses national income (GNI) per capita. GNI per capita on transplant rates were lower in eastern European countries, with the exception of Czech Republic where transplant rates paralleled the transplant rates of some western European countries despite the lower GNI per capita (Fig. 5.). There is not increment of transplant rates after the GNI per capita reached 20.000 or more. It is logical that transplant rates are substantially higher in the countries with higher team density (Fig. 6.). Team density was markedly lower in eastern compared to western European countries. There is weak correlation between team density and GNI per capita (Fig. 6.).

Discussion

As expected transplant rates and team densities in eastern European countries is lower then in western European countries. The trends for lower rates was obtained at the beginning of the EBMT activity survey in 1990 and remained lower despite a continuing increase in stem cell transplantation in the majority of countries during the observation period. This was the truth for all type of transplants. Stem cell transplantation from unrelated donors has begun to be an important part of the transplant activity in Eastern Europe in the most recent years. Economical power of some country is crucial for the transplant program. Bellow a certain level of economical power it is not possible to afford a substantial number of transplantation. This high tech medicine therapy is too expensive. On the other hand it is quite clear that the number of patients with the indications for stem cell transplantation will not increase with increasing GNI per capita.

Another very important observation from the data of EBMTG Registry is the correlation of transplant rates and team density (11). The fewer patients a team has to serve, the higher the likelihood that the individual patients will have the access to this procedure. There is a need to disseminate a given technology within the country for its optimal use. It is very difficult to define what is the optimal number of transplantation performed for one team and what is the optimal number of teams per country. A reasonable number seems to be one center per one to two million inhabitants. During the EBMT meeting there are some reports about the outcome of transplants in eastern European countries. Although this data is incomplete and the number of patients relatively small the outcome of transplants in patients with acute leukemia allografted or autografted in 1st CR is similar as in western European countries. But most of the centers from Eastern Europe reported in EBMTG for this survey are well experienced. The data from centers started recently with SCT is not included in the survey.

In conclusion it has to be stressed that the need for transplants or transplant teams in one country depends of the magnitude of the country (i.e. one center per two million inhabitants) and its economical power.

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Current regulations for hematopoietic stem cell transplantation in Turkey and EU

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Here, we will look at the concordance and differences between EU and Turkish Regulations on hematopoietic stem cell transplantation (HSCT). There are many similarities and significant concordance between Turkish and EU regulations on HSCT, which is legislated under a Law No:2238 on Transplantation of Organ and Tissues and Instruction on Bone Marrow Transplantation Centers and data Processing Ctrs. Implemented on Feb.2001. Though, there are some minor differences in the legislation and regulations, those will soon be corrected and parallel EU regulations will be implemented since Turkey is a candidate country for EU.

JACIE (The Joint Accreditation Committee of ISCT-EBMT) (www.jacie.org) standards, are based on the standards of the Foundation for the Accreditation of Cellular Therapy (FACT), is also setting and supervising the Standards for Haematopoietic Progenitor Cell Collection, Processing & Transplantation in most EU countries for HSCT which is supported by the European Commission under the Public Health Programme (2003-2008). JACIE has set the standards for HSCT in accordance with current EU directives on Human Tissues and Organs.

Below is a detailed table demonstrating the concordance and differences between EU and Turkish legislation on the Human Tissues and Cells which includes regulations also on HSCT.

TABLE OF CONCORDANCE BETWEEN EU and TURKISH LEGISLATION ON THE HUMAN TISSUES AND CELLS

EU Legislation	*Relevant Turkish Legislation*	*The main differences between TR and EU Legislation*
Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells	• LAW No: 2238 ON TRANSPLANTATION OF ORGAN AND TISSUE (OG No: 16655, 03 June 1979) • BY-LAW ON ORGAN AND TISSUES TRANSPLANTATION SERVICES (OG No: 24066, 01 June 2000) • BY-LAW ON CORD BLOOD BANKS (OG No: 25866, 05 July 2005) • BY-LAW ON CURATIVE CENTERS FOR THE PURPOSE OF ASSISTED REPRODUCTION (OG No: 19551, 21 August 1987) • INSTRUCTION ON BONE MARROW TRANSPLANTATION CENTERS AND DATA PROCESSING CENTERS (26 February 2001) • INSTRUCTION ON EYE BANK AND CORNEA TRANSPLANTATION CENTERS (26 February 2001) • INSTRUCTION ON HUMAN LEUCOCYTE ANTIGEN (HLA) TYPING LABORATORIES (26 February 2001) • CIRCULAR ON EMBRYONIC STEM CELLS RESEARCH (19 September 2005) • CIRCULAR ON NON-EMBRYONIC STEM CELLS RESEARCH (01 May 2006)	
Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells **Article 1** **Objective** This Directive lays down standards of quality and safety for human tissues and cells intended for human applications, in order to ensure a high level of protection of human health.	The Law on transplantation of organ and tissue Article 1 – Objective Donation, procurement, transplantation and preservation of organs and tissues for the purpose of diagnosis, treatment and scientific research, are subject to this law. The by-law on organ and tissue transplantation services Article 1 – Objective The objective of this by-law, is to authorise, operate and inspect the organ and tissue transplantation centers, organ and tissue procurement organisations and human leucocyte antigen (HLA) typing laboratories, which are necessary to perform the transplantation process for the patients, whose treatment are only possible by organ and tissue transplantation. Another objective of this by-law is to set up principles and procedures, which the related public bodies and authorities and private institutions should obey and to determine the principles that should be followed for the organ and tissue transplantation services.	

Article 2 **Scope** This Directive shall apply to the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells intended for human applications and of manufactured products derived from human tissues and cells intended for human applications. Where such manufactured products are covered by other directives, this Directive shall apply only to donation, procurement and testing. 2. This Directive shall not apply to: (a) tissues and cells used as an autologous graft within the same surgical procedure; (b) blood and blood components as defined by Directive 2002/98/EC; (c) organs or parts of organs if it is their function to be used for the same purpose as the entire organ in the human body.	The Law on transplantation of organ and tissue: **Article 2- Scope** Organ and tissue mentioned in this law mean all organs ,tissues and their components forming human organism. Auto-graft, procurement and transplantation of hair and skin, blood transfusion aren't subject to this Law, these are implemented by the provisions of other law, by-law and Regulation on Medical Deontology in force.	The Law on transplantation of organ and tissue: - includes both organs and tissues - excludes blood and blood products, cord blood, stem cells, auto-grafts, foetal tissues and reproductive cells . Legal regulations on cornea, blood and blood products, bone-marrow, cord blood banking, stem cells research and reproductive cells already exist in Turkey. But there are no regulations on other tissues, auto-grafts and foetal tissues There are two circulars on stem cell research in Turkey. The objective of the circular on non-embryonic stem cells research is to determine principles and procedures for clinical trial of non-embryonic stem cells research This circular applies to somatic stem cells research. Within the framework of this circular, a guideline has been issued regarding: - determination of standards of hospitals - determination of preconditions of trials and - determination of scientific competency of hospitals and scientists. Embryonic stem cells research is <u>prohibited</u> in Turkey by the circular on embryonic stem cells research. According to the by-law on curative centers for the purpose of assisted reproduction, the assisted reproduction methods can be applied provided that the partners meet the following provisions : - being married. - using the reproductive cells only belonging to them Donation of reproductive cells for allogeneic use is <u>prohibited</u> by the by-law on curative centers for the purpose of assisted reproduction.

Article 4 **Implementation** 1. Member States shall designate the competent authority or authorities responsible for implementing the requirements of this Directive. 2. This Directive shall not prevent a Member State from maintaining or introducing more stringent protective measures, provided that they comply with the provisions of the Treaty. In particular, a Member State may introduce requirements for voluntary unpaid donation, which include the prohibition or restriction of imports of human tissues and cells, to ensure a high level of health protection, provided that the conditions of the Treaty are met. 3. This Directive does not affect the decisions of the Member States prohibiting the donation, procurement, testing, processing, preservation, storage, distribution or use of any specific type of human tissues or cells or cells from any specified source, including where those decisions also concern imports of the same type of human tissues or cells. 4. In carrying out the activities covered by this Directive, the Commission may have recourse to technical and/or administrative assistance to the mutual benefit of the Commission and of the beneficiaries, relating to identification, preparation, management, monitoring, audit and control, as well as to support expenditure		The competent authority responsible for implementing the requirements of this Directive is the Ministry of Health.
Article 5 Supervision of human tissue and cell procurement 1. Member States shall ensure that tissue and cell procurement and testing are carried out by persons with appropriate training and experience and that they take place in conditions accredited, designated, authorised or licensed for that purpose by the competent authority or authorities. 2. The competent authority or authorities shall take all necessary measures to ensure that tissue and cell procurement complies with the requirements referred to in Article 28(b), (e) and (f). The tests required for donors shall be carried out by a qualified laboratory accredited, designated, authorised or licensed by the competent authority or authorities.		Procurement of human tissues and cells is carried out by persons who successfully completed a training programme as stated in the legislation. The tests reguired for donors are carried out by the laboratory licenced by the Ministry of Health.

Article 6 Accreditation, designation, authorisation or licensing of tissue establishments and tissue and cell preparation processes 1. Member States shall ensure that all tissue establishments where activities of testing, processing, preservation, storage or distribution of human tissues and cells intended for human applications are undertaken have been accredited, designated, authorised or licensed by a competent authority for the purpose of those activities. 2. The competent authority or authorities, having verified that the tissue establishment complies with the requirements referred to in Article 28(a), shall accredit, designate, authorise or license the tissue establishment and indicate which activities it may undertake and which conditions apply. designation, authorisation or licence for this activity. As Instructed by JACIE Haematopoietic Progenitor Cell and Therapeutic Cell Processing Standards D1.000 General D1.100 These Standards apply to the processing of marrow and/or peripheral blood cells by the collection facility and/or laboratory. D1.200 The Processing Facility must abide by all applicable National and/or European Union regulations and directives.	The by-law on organ and tissue transplantation services **Article 16**- Organ ve Tissue Transplant Centers These are transplant centers licensed by the Ministry of Health. The requirements and working principles and procedures for those organ and tissue transplant centers are determined by the Instructions to be prepared for every organ and tissue. These instructions are to be prepared by seperate Scientific Consultant Boards and put in force by the approval of the Minister. The subjects determined in these instructions are as follows : a) the qualifications of responsible person of transplant centers b) the qualifications of personnel of transplant centers c) the departments to be located in transplant centers d) the equipment in transplant centers **Article 17-** The organ and tissue transplant centres may be established by the public bodies and organisations as well as the natural persons and corporate bodies within the public and private hospitals as units. It is obligated to take Licence from the Ministry in order to have make centres operational. Turkish Health Ministry is the only principal authority governing licensing of BMT facilities, Tissue Establishment	As stated in the by-law, the tissue establismments are licensed by the Ministry of Health, provided they comply with standards of the legislation.

Article 10 Register of tissue establishments and reporting obligations 1. Tissue establishments shall keep a record of their activities, including the types and quantities of tissues and/or cells procured, tested, preserved, processed, stored and distributed, or otherwise disposed of, and on the origin and destination of the tissues and cells intended for human applications, in accordance with the requirements referred to in Article 28(f). They shall submit to the competent authority or authorities an annual report on these activities. This report shall be publicly accessible. 2. The competent authority or authorities shall establish and maintain a publicly accessible register of tissue establishments specifying the activities for which they have been accredited, designated, authorised or licensed. 3. Member States and the Commission shall establish a network linking the national tissue establishment registers.	By-law on organ and tissues transplantation services **Article 21- Reporting** Organ and tissue transplant centres are obliged to notify the Ministry of Health on the transplants and the patient observations every year by 31st January at the latest. These data shall be published by the Ministry as annual reports.	As determined in the by-law, the tissue establismsents submit to the Ministry of Health an annual report in accordance with the legislation. Because of the operational problems, this report is not publicly accessible. Every tissue establisment has data base relating to documentation for each donor and recipient The data relating to the retrieved tissue are registered at the tissue establisments. Tissue establismsents keep a record including the types, quantities, origin and destination of tissue and cells intended for human applications. However, notification covers only the number of transplants for bone marrow transplants.
Article 11 Notification of serious adverse events and reactions 1. Member States shall ensure that there is a system in place to report, investigate, register and transmit information about serious adverse events and reactions which may influence the quality and safety of tissues and cells and which may be attributed to the procurement, testing, processing, storage and distribution of tissues and cells, as well as any serious adverse reaction observed during or after clinical application which may be linked to the quality and safety of tissues and cells. 2. All persons or establishments using human tissues and cells regulated by this Directive shall report any relevant information to establishments engaged in the donation, procurement, testing, processing, storage and distribution of human tissues and cells in order to facilitate traceability and ensure quality and safety control. 3. The responsible person referred to in Article 17 shall ensure that the competent authority or authorities is or are notified of any serious adverse events and reactions referred to in paragraph 1 and is or are provided with a report analysing the cause and the ensuing outcome. 4. The procedure for notifying serious adverse events and reactions shall be established by the Commission, in accordance with the procedure referred to in Article 29(2). 5. Each tissue establishment shall ensure that an accurate, rapid and verifiable procedure is in place which will enable it to recall from distribution any product which may be related to an adverse event or reaction.		In Turkey, there isn't a nationwide system to report, investigate, register and transmit information about serious adverse event and reactions observed during or after clinical application. Data about serious advers reactions and events is kept by the tissue establismsents. The Ministry of Health isn't notified of all adverse events and reactions

Article 12 Principles governing tissue and cell donation 1. Member States shall endeavour to ensure voluntary and unpaid donations of tissues and cells. Donors may receive compensation, which is strictly limited to making good the expenses and inconveniences related to the donation. In that case, Member States define the condition under which compensation may be granted. Member States shall report to the Commission on these measures before 7 April 2006 and thereafter every three years. On the basis of these reports the Commission shall inform the European Parliament and the Council of any necessary further measures it intends to take at Community level. 2. Member States shall take all necessary measures to ensure that any promotion and publicity activities in support of the donation of human tissues and cells comply with guidelines or legislative provisions laid down by the Member States. Such guidelines or legislative provisions shall include appropriate restrictions or prohibitions on advertising the need for, or availability of, human tissues and cells with a view to offering or seeking financial gain or comparable advantage. Member States shall endeavour to ensure that the procurement of tissues and cells as such is carried out on a non-profit basis.	The Law on transplantation of organ and tissue **Article 3-** Procurement and purchasing of organs and tissues based on profit is prohibited.	As stated in the law, donation of organ and tissue is voluntary and un-paid
Article 13 Consent 1. The procurement of human tissues or cells shall be authorised only after all mandatory consent or authorisation requirements in force in the Member State concerned have been met. 2. Member States shall, in keeping with their national legislation, take all necessary measures to ensure that donors, their relatives or any persons granting authorisation on behalf of the donors are provided with all appropriate information referred to in the Annex.	The Law on transplantation of organ and tissue Organ and tissue procurement procedures and Research Obligation Article 7- The physicians responsible for the procurement of the organs and tissues are obliged to: a) inform the donor on the risks of the organ and tissue procurement as well as its medical, psychological and social consequences properly and in detail; b) inform the donor of tissue and organ on the therapeutic purposes for the recipient; c) refuse the donation of the persons who are not mentally adequate to decide for themselves; d) search and learn if the donor's spouse is informed of the donation in case the donor is married and to document the situation; e) refuse the donation of organs and tissues on monetary or profit basis or inhumane causes f) not to reveal the identities of the donor and the recipient exluding the conditions that the blood relationship, affinity or close personal relationships involved.	

Article 14 Data protection and confidentiality 1. Member States shall take all necessary measures to ensure that all data, including genetic information, collated within the scope of this Directive and to which third parties have access, have been rendered anonymous so that neither donors nor recipients remain identifiable.	The by-law on cord blood banking Data protection and confidentiality **Article 25-** The banks are obliged to store the identitiy and address information and HLA type information of the donors of cord blood seperately. The banks are also obliged to take the necessary security measurements for the data security.	
Article 16 Quality management 1. Member States shall take all necessary measures to ensure that each tissue establishment puts in place and updates a quality system based on the principles of good practice. 2. The Commission shall establish the Community standards and specifications referred to in Article 28(c) for activities relating to a quality system. 3. Tissue establishments shall take all necessary measures to ensure that the quality system includes at least the following documentation: — standard operating procedures, — guidelines, — training and reference manuals, — reporting forms, — donor records, — information on the final destination of tissues or cells. 4. Tissue establishments shall take all necessary measures to ensure that this documentation is available for inspection by the competent authority or authorities. 5. Tissue establishments shall keep the data necessary to ensure traceability in accordance with Article 8.		The Ministry of Health take necessary measures to ensure that each tissue establisment establishes a quality system based on the principles of good practice. Also, tissue establismenets try to take necessary measures for activities relating to the guality system. Documentation related the quality system in the tissue establisments covers most of those referred to in article 16 in the Directive 2004/23/EC and is available for inspection by the Ministry of Health

Article 17 Responsible person 1. Every tissue establishment shall designate a responsible person who shall at least fulfil the following conditions and have the following qualifications: (a) possession of a diploma, certificate or other evidence of formal qualifications in the field of medical or biological sciences awarded on completion of a university course of study or a course recognised as equivalent by the Member State concerned; (b) at least two years' practical experience in the relevant fields. 2. The person designated in paragraph 1 shall be responsible for: (a) ensuring that human tissues and cells intended for human applications in the establishment for which that person is responsible are procured, tested, processed, stored and distributed in accordance with this Directive and with the laws in force in the Member State; (b) providing information to the competent authority or authorities as required in Article 6; (c) implementing the requirements of Articles 7, 10, 11, 15, 16 and 18 to 24 within the tissue establishment. 3. Tissue establishments shall inform the competent authority or authorities of the name of the responsible person referred to in paragraph 1. Where the responsible person is permanently or temporarily replaced, the tissue establishment shall immediately inform the competent authority of the name of the new responsible person and the date on which the duties of that person commence. **Article 18** Personnel Personnel directly involved in activities relating to the procurement, processing, preservation, storage and distribution of tissues and cells in a tissue establishment shall be qualified to perform such tasks and shall be provided with the training referred to in Article 28(c).	Responsible person Instruction on bone marrow transplantation centers and data processing centers (Art 7/a) The responsible person of the tissue establisment, in case it is a BMT center, must be a haematology/oncology specialist who has at least two years practical experience in the relevant fields or an internal diseases /pediatry specialist who has five years practical experience in the relevant fields. JACIE requires minimum of 1 year experience and diplomate/cerification in JACIE acredited facilities. The by-law on cord blood banks (Art. 11) The haematology specialist physicians with at least 2 years of experience on cell processing and storing and who has a certificate in the relevant field shall be present in the banks as responsible persons	In TR legislation, the responsible person is defined by the related tissue instructions. Every tissue establisment designates a responsible person with necessary qualifications defined in the legislation. However, the conditions and qualifications vary according to the related tissue instruction. As defined in the Law, personel directly involved in activities relating to procurement, processing, preservation, storage and distribution and of human tissue and cells must have necesssary qualifications in accordance with the legislation. The qualifications and provisions are determined by the instructions issued for each organ and tissue.
Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells		Certain technical requirements, tests for tissue and cell required will be implemented soon in TR with additions to the by-law 24066, in accrodance with the EU Commission Directive 2006/17/EC

Article 1 Definitions For the purposes of this Directive, the following definitions defined …		Definitions stated in the directive aren't defined in TR legislation.
Article 2 Requirements for the procurement of human tissues and cells 1. With the exception of partner donation of reproductive cells for direct use, Member States shall ensure that the procurement of human tissues and cells is accredited, designated, authorised or licensed only when the requirements in paragraphs 2 to 12 are met. 2. Procurement of human tissues and cells shall be carried out by persons who have successfully completed a training programme specified by a clinical team specialising in the tissues and cells to be procured or a tissue establishment authorised for procurement.	Article 13- Other Health Staff The other health staff that will work for the bank should have certification training in relation to the operations that will be carried out in the unit and they should be certificated The instruction on bone marrow transplantation centers and data processing centers Article 7- The Personnel to work for the Bone Marrow Centres and their qualifications A nurse and a technician with at least 6 months of experience on hemapheresis should work at the bone marrow transplantation centers	As defined in the legislation, the persons responsible for procurement of tissues and cells need to have a certificate in the relevant field. Every tissue establisment has own standard operating procedures.(SOPs) Tissue establisments have been authorised to carry out procedures comply with the the requirements stated in the legislation at all stages involved in the reception, processing, labelling, storage, documentation, packaging and distribution of tissue and cells.
Article 3 Selection criteria for donors of tissues and cells The competent authority or authorities shall ensure that donors comply with the selection criteria set out in: (a) Annex I for donors of tissues and cells, except donors of reproductive cells; (b) Annex III for donors of reproductive cells		Selection criteria for donors is based on analysis the risks related application of the tissue /cells The risks are identified by physical examination, review of the medical history, biological testing, post-mortem examination, (for deceased donors) and any other appropriate investigation at the tissue establisments.
Article 4 Laboratory tests required for donors 1. The competent authority or authorities shall ensure that: (a) donors of tissues and cells, except donors of reproductive cells, undergo the biological tests set out in point 1 of Annex II; (b) the tests referred to in point (a) are carried out in compliance with the general requirements set out in point 2 of Annex II. 2. The competent authority or authorities shall ensure that: (a) donors of reproductive cells undergo the biological tests set out in points 1, 2 and 3 of Annex III; (b) the tests referred to in point (a) above are carried out in compliance with the general requirements set out in point 4 of Annex III.		The tests reguired for donors are carried out by the laboratory licenced by the Ministry of Health. The biological tests which donors must undergo are not defined in the TR legislation. However, at tissue establisments, the following biological tests are performed for all donors as a minimum requirement HIV 1 and 2 Hepatitis B Hepatitis C Depending on the donor's history and the characteristics of the tissue or cells donated , additional tests are also performed for donors

Stem cell transplantation for multiple myeloma

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The concept of dose intensification in multiple myeloma (MM) was introduced by McElwain and Powles in 1983(McElwain et al, Lancet 1983)[1]. At the initial publication, out of 8 patients treated with high dose melphalan (HDM) all had responded but the rate of bone marrow failure was high.

Barlogie et al in 1986 demonstrated that autologous bone marrow transplantation (ABMT) reduced myelosuppression and this strategy allowed more extensive use of high dose therapy (HDT)(Barlogie et al, Blood 1986)[2].

Following these works the clinical experience with high dose therapy supported by different sources of hematopoietic stem cells (SC) was accumulated and currently peripheral blood SCs (PBSC) are the SC of choice. Multiple studies showed that HDT is a useful salvage therapy for patients with primary refractory disease or for patients with chemosensitive relapse but not for those patients with refractory relapsed disease(Alexanian et al, Blood 1994)[3].

It became obvious that a rate of 30-50% of complete remissions (CR) could be achieved with this strategy in newly diagnosed patients(Harousseau et al, Semin Hematol 1997)([4], fact that was interpreted by most as a probable prolongation of remission and survival in comparisons with historic controls (Palumbo et al Blood 1999, Lenhoff et al Blood 2000) ([5,6]. However until 1996 no head to head comparison of conventional treatment with HDT in a randomized study was published.

As early as 1990, the Intergroup Francophone du Myelome initiated the first randomized trial designed to address this issue (Table 1). Table 1 summarizes the published experience with prospective randomized studies comparing conventional treatment with conventional treatment followed by autologous SC transplantation in previously untreated MM patients. The studies from France (Attal et al 1996)[7] and from UK (Child NEJM 2003)[8] show a significant increase of CR rate and prolonged event free (EFS) and overall survival (OS) in favor of the HDT group. However the MAG91 study from France (Fermand JCO 2005)[9] failed to show a significant advantage in any outcome marker for the high dose therapy group. Additionally PETHEMA clinical trial from Spain (Blade Blood 2005)[10], which ramdomized only patients who responded to initial treatment failed to show survival advantage of HDT over conventional treatment, although there was significantly more CR rate in the high dose group (30% vs 11%). More recently, in the US intergroup randomized trial, where high dose cyclophosphamide and VBMCP was given in the standard treatment group, the latter had similar outcomes comparing to the intensive treatment group (Barlogie et al, JCO 2006)[11](Table 1).

A couple of explanations can be given for the observed discrepancies between the randomized studies: a. the difference in the treatment given to the conventional treatment groups and b. the fact that many studies used a TBI containing high dose regimen, which has been shown inferior to randomized comparison to Mel200 alone (Moreau Blood 2002)[12]. However the fact that in PETHEMA study from Spain there is not a clear link between increase of the CR rate and survival extension deserves further investigation.

Table 1. Summarized results from randomized trials comparing conventional chemotherapy with high dose therapy in newly diagnosed myeloma patients.

Study	*No of pts*	*Age (years)*	*Conventional chemo (CC)*	*Induction chemo for HDT arm*	*High dose regimen*	*CR rate (%) CC vs HDT arm*	*Median EFS (months) CC vs HDT arm*	*Median OS (months) CC vs HDT arm*
IFM 90 (NEJM 1996) [7] France	200	≤65	VMCP-BVAP	VMCP-BVAP	Melphalan 140 + TBI 8Gy	5 vs 22*	18 vs 28*	44 vs 51*
MRC7 (NEJM 2003) [8] UK	401	≤65	Doxo, BCNU, CTX, Melphalan	CVAD	Melphalan 200	8 vs 44*	19 vs 31*	42 vs 54*
M97G (Blood 2004) [23] Italy	194	50-70	Melphalan+ Prednisone	VAD	Melphalan 100	6 vs 25*	16 vs 28*	42 vs 58*
MAG91 (JCO 2005) [9] France	190	55-65	VMCP	VAMP	Melphalan 200 or Melpahalan 140 + Busulfan	20 vs 36	19 vs 25	47.6 vs 47.8
PETHEMA (Blood 2005) [10] Spain	216	<65	VMCP-VBAD	VMCP-VBAD	Melphalan 200 or Melphalan 140 + TBI 12Gy	11 vs 30*	33 vs 42	66 vs 61
US Intergroup S9321 (JCO 2006) [11]	516	≤70	VAD, high dose CTX, VBMCP	VAD, high dose CTX	Melphalan 140 + TBI 12Gy	15 vs 17	21 vs 25	53 vs 58

*: statistically significant difference; CR: complete response; EFS: event free survival; OS: overall survival; CC: conventional chemotherapy; HDT: high dose therapy; CTX: cyclophosphamide; VMCP: vincristine, melphalan, cyclophosphamide, prednisone; VAMP: vincristine, doxorubicin, methylprednizolone; VBAD: vincristine, BCNU, doxorubicin, dexamethasone; BVAP: vincristine, BCNU, doxorubicin, prednisone; VBMCP: vincristine, BCNU, melphalan, cyclophosphamide, prednisone; TBI: total body irradiation

The issue of the maintenance post HDT is still under discussion. All the above mentioned clinical trials included IFNa containing maintenance treatment. Notwithstanding, only the US Intergroup trial (Barlogie JCO 2006)[11], included a 2nd randomization after induction with patients assigned to receive or not IFNa maintenance. This study showed equal OS and EFS between the groups who received or not IFNa. Moreover, patients who received this immunomodulatory agent had considerable toxicity and high rate of discontinuation. More recently the IFM 99 02 randomized study from France (Attal Blood 2006)[13] showed that maintenance with thalidomide post HDT was associated with significantly prolonged EFS and OS. This benefit was actually limited to those patients who had less then very good partial remission at the time of randomization (approximately 2 months after HDT).

Tandem autologous transplantation has also been explored for MM. The Group from Arkansas has the largest experience with tandem transplants in newly diagnosed myeloma patients followed by event free and overall survival of 43 and 68 months respectively (Barlogie Blood 1999)[14]. However, there is only a small number of randomized studies comparing single with double autologous transplants in myeloma. Only one study from France (IFM94) has been published as a full paper (Attal NEJM 2003)[15] showing statistically significant extension of EFS and OS in favor of the double transplant group. However these results have not yet been confirmed by other studies. Moreover in the subgroup analysis of IFM94, it seems that the benefit is limited only to those patients who did not achieve a very good partial remission after the 1st autologous transplant. Currently more mature data are needed in order to evaluate tandem transplants for MM.

The identification of prognostic factors for the outcome of HDT in MM may help select the patients who would be more probable to benefit from this treatment. The presence of high levels of beta2 microglobulin, cytogenetic abnormalities (especially in chromosome 13) have all been associated with poor prognosis even with tandem transplantations (Tricot BJH 2002)[16]. With the advance of molecular classification of MM we will soon be able to identify prognostic groups with poor prognosis even after HDT. For such patients new treatments should be explored.

Allogeneic SCT currently has still a limited role in the treatment of MM. Standard (myeloablative) conditioning regimens even in young patients is associated with very high treatment related mortality (TRM) mainly due to infection and graft versus host disease (GVHD). However there is evidence that one third of patients receiving allogeneic SCT early in the course of the disease remain disease free 6 years later (Corradini JCO 1999)[17]. Moreover, a small proportion of myeloma patients may be cured with allo SCT, in oppose to auto SCT where patients continue to relapse many years after transplant (Barlogie Blood 2004)[18]. Finally a graft versus myeloma effect has clearly been demonstrated after

donor lymphocyte infusions in patients relapsing after allogeneic SCT (Tricot Blood 1996)[19].

Allogeneic SCT with reduced intensity conditioning (RIC) regimens may take advantage of the graft versus myeloma effect, reducing at the same time the TRM. There are a couple of prospective trials evaluating the role of reduced intensity conditioning prior allo SCT (either with low dose TBI or fludarabine/melphalan containing regimens) in patients after autologous SCT with encouraging results (Maloney Blood 2003 and Kroger Blood 2004)[20,21]. However, a recently published prospective comparison of tandem autologous transplants with autologous followed by RIC allogeneic transplant from France, did not show any difference in survival (Garban Blood 2006)[22].

As a take home message for myeloma and stem cell transplantation in year 2006 one can argue that autologous stem cell transplant should still be considered superior in myeloma patients comparing to conventional treatment and that high dose melphalan is the conditioning regimen of choice. In the era of exciting new antimyeloma agents like thalidomide, bortezomib and lenalidomide, however, there are several crucial clinical trial questions that should be addressed (Barlogie JCO 2006)[11]: a. what is the respective role and timing of new agents in relationship to autotransplantations; b. is there a need for maximum cytoreduction before HDT; c. what is the duration of new agent-induced responses and what is the reversibility rate of bortezomib and thalidomide related polyneuropathy. A more systematic study of QOL issues in terms of the new toxicity profile of the new agents should be considered; d. is the achievement of CR a valid surrogate for survival; and e. which is the more correct planning and interpretation of new clinical trials in the current context of molecular classification of MM.

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Molecular pathology in Turkish children with selective vitamin B12 malabsorption

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Abstract

A total of 14 patients of 10 unrelated families among a group of 35 families with selective vitamin B12 (VB12) malabsorption who had been followed at Hacettepe İhsan Doğramacı Children's Hospital for the past 2-25 years were studied at the molecular level for the pathologies leading to selective VB12 malabsorption.

DNA studies of these patients revealed that abnormalities in 3 different genes, namely cubilin (CUBN), amnionless (AMN), and gastric intrinsic factor (GIF), were associated with selective VB12 malabsorption. Mutation analyses indicated that some of the mutations of the related genes were novel mutations; however, the study indicated that molecular pathology was heterogeneous among the Turkish patients and none of the 3 affected genes had unique mutations that could be assigned to the population studied.

Patients and Discussion

In 1960, for the first time in literature, Immerslund in Norway and Grasbeck in Finland reported, separately, a new syndrome in a group of patients with hereditary selective vitamin B12 (VB12) malabsorption and proteinuria (1,2). Later, it was shown that this disorder was present in other parts of the world. The largest number of patients was reported to be in Turkey and in Israel (3). In a study from Turkey, results of the Schilling test showed variations among patients indicating possible heterogeneity in molecular pathology (3). However molecular pathology remained to be unknown for many years.

It was shown that there are 3 different VB12 carrier proteins. One of them is haptocorrin, which is present in saliva and the upper gastro-intestinal system, where VB12 is bound to this carrier protein. Haptocorrin is sensitive to the low pH of gastric juice; therefore, cobalamin is freed from the complex and it is bound to the other carrier protein, gastric intrinsic factor (GIF). In the last step, in circulation, cobalamin is carried to the target organs by the third carrier protein, transcobalamin. Three-dimensional examination of transcobalamin has shown that there are 2 domains for cobalamin. It was suggested that other carrier proteins, like haptocorrin and GIF, are structured similarly with transcobalamin (4). For many years GIF was thought to be the only molecule that involves in gastro-intestinal VB12 absorption till 1998.

Studies reported from Finland, in 1998, indicated that a protein other than GIF, cubilin, is also involved in the internalization of VB12 (5). It was suggested that before internalization of VB12, GIF-cobalamin complex should be attached to cubilin. Recent molecular studies have shown that in the majority of Finnish patients with selective VB12 malabsorption, the cubilin gene (CUBN) is mutated. Contrary to this, molecular studies in Norway and other Scandinavian countries indicated that only a few of the patients with selective VB12 malabsorption had CUBN mutations. The study reported in 2003 by Tanner et al. showed that the majority of the Norwegian patients had mutations of a gene similar to the mouse amnionless (AMN) gene (6). Later studies revealed that cubilin and AMN are colocalizing epithelial proteins, which form a tight complex molecule (cubam) in the

gastrointestinal system, as well as in other organs such as the kidney. It was suggested that GIF cobalamin complex should be attached to cubam for internalization of cobalamin. Cubilin acts as a peripheral ligand-capturing membrane protein attached to the extracellular part of AMN, whereas AMN represents the trans-membrane unit important for membrane association and endocytic trafficking (4).

Although a relationship between congenital VB12 malabsorption and abnormalities in GIF have been reported in a few patients, many years ago (7, 8), molecular pathology in the GIF gene only recently, was reported to be associated with congenital selective VB12 malabsorption in 2004 (9).

Recent molecular studies in 10 of the Turkish patients among 35 unrelated families with selective VB12 malabsorption have shown that 6 of them have CUBN (2 novel mutations in 2 patients) or AMN mutations (2 novel and 1 common mutations in 4 patients) (10). Later studies have shown that in some of the patients, without cubilin and AMN mutations have GIF mutations (11). Examinations of the 4 of theTurkish patients without CUBN or AMN mutations have also revealed that they have several novel GIF mutations (12).

It was shown that in Turkish patients, AMN and GIF mutations were present with the same frequency, and that these 2 pathologies seem to be more common than cubilin gene mutations. Geographic distribution of the patients having mutations of these 3 genes showed no peculiar pattern. The majority of the mutations for each gene were novel; however, there was no common mutation for each gene to be identified as common for Turkish patients. Molecular study has indicated that among the 3 genes there is only one founder mutation that is in AMN (208 2A-G mutation). This mutation was reported in 2 unrelated Turkish families and in a patient of a Tunisian Jewish family living in Israel (10).

In a previous study conducted by A. Kara et al., it was shown that congenital or infantile VB12 deficiency, which resulted from maternal VB12 deficiency, was not rare in Turkish newborns and infants with several neurological abnormalities (13). Therefore, differentiation between selective VB12 malabsorption and VB12 deficiency due to poor diet in infants and young children is very important, since the first disorder requires lifelong treatment and medical attention, while the latter one is easily treated by adjusting the diet. The Schilling test was useful in the differentiation of malabsorption from adult pernicious anemia and dietary deficiency of VB12. During the last 5-10 years, the Schilling test has been abandoned for several reasons, making differential diagnosis even more difficult. It was fortunate that a laboratory diagnostic test for the differentiation of VB12 malabsorption and dietary deficiency of VB12, which depends upon measurements of serum holo-transcobalamin and transcobalamin saturations after giving small doses of oral VB12, was recently introduced (14). This test was used with our patients and VB12 malabsorption was detected in all of them. The second phase of the test using GIF manufactured by DNA technologies in order to differentiate adult pernicious anemia and some type of selective VB12 malabsorption are in preparation.

Although treatment and handling of the patients are the same, in patients with selective VB12 malabsorption due to these 3 different genetic pathologies, molecular studies are needed in order to understand the genetic make up of the underlying disorder. These types of studies would help in elucidating the mechanisms of the disorders and the functions of the products of related genes. There are still several patients who did not have any abnormalities in any of the 3 genes in question. This suggests that in the future there is a probability of the identification of new genes associated with VB12 absorption.

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Iron deficiency anemia: Why still an ongoing health problem?

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It is necessary to remind ourselves of the definition of health first: the widely accepted definition is that of the World Health Organization (WHO, 1946), stating that "health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity". In more recent years, this statement has been modified to include the ability to lead a "socially and economically productive life."

Following, let's have a look on the relatively recent data regarding iron deficiency and consecutive anemia.

According to the US CDC "... as many as 4–5 billion people, 66–80% of the world's population, may be iron deficient and approximately 2 billion people, more than 30% of the world's population, are anemic. It is estimated that more than half of the pregnant women in developing countries are anemic."

Moreover, it seems that the situation is even worsening with time since the 4th United Nations Report on The World Nutrition Situation, presented in January 2000, stated that "... iron deficiency and its anemia affect more than 3.5 billion people in the developing world, well over two persons out of every three."

Even last year, competent medical authorities are still issuing warnings: "... The statistics are staggering. More than two billion people worldwide, 30 percent of the earth's population, suffer from anemia, or severe iron deficiency."

Is this a problem only in underdeveloped and developing countries? Probably many think so, but the numbers do not correspond with this opinion. More close to the truth is the statement that it is "... a global public health problem that affects all parts of the world." Or, as many experts say: "... iron deficiency is the most common nutrition problem in the world today".

Attention of the public general is drawn by major media in the US in not so distant past, warning about the high incidence of iron deficiency among different gender subpopulations, different age subpopulations, as well as different ethnic groups.

In one European country iron deficiency is ranked among the 10 most frequent death risk factors. Mostly as the 10th ranking reason for impairments of national economies, iron deficiency is a problem in 16 well-developed European countries, the majority of them being members of the EU. The situation is more worrying in developing European economies. The European Health Report for 2005, issued by the WHO Regional Office for Europe, states that "... iron deficiency is responsible for 0.7% of DALYs (disability adjusted life years) in the European Region." And this does not refer only to countries outside of the EU! In the Eastern Mediterranean Region (EMR), for instance, measurements in several countries suggest that only 6%–10% of all anemia is due to causes other than iron deficiency.

Since iron deficiency and consecutive anemia are regarded as a deficiency state, attention to these conditions is drawn by the FAO Report for Europe in 2004, stating the statistical figures for undernourishment in the region. Not surprisingly, almost all of the countries represented in this

meeting are in the tables, with variable, but still not encouraging levels of undernourishment.

So, does iron deficiency really represent such a big problem? Medically speaking it is a condition quite successfully and easily managed, to the satisfaction of both the patient and the physician. But, we are only looking at medical issues and consequences. Apparently, some other experts have their views of the problem. Here are some of their quotes:

"... lowers the body's defenses to disease and diminishes body and brain functions."

"... has less obvious, developmental effects on children; they may be less intelligent, scoring up to 10 points lower on standard IQ tests, their school performance may be below normal and their future productivity may be diminished."

"... deaths of an estimated 50.000 women a year during childbirth."

"... impairs the cognitive development of children through to adolescence ... damages immune mechanisms, and is associated with increased morbidity rates ... impairs physical work capacity in men and women by up to 30% ... during pregnancy is associated with multiple adverse outcomes for both mother and infant, including increased risk of sepsis, maternal mortality, perinatal mortality, and low birth weight ... reduce learning ability and the work capacity of individuals and entire populations, bringing serious economic consequences and obstacles to national development."

"... damaging the health of one-third of the world's people and holding back the economic development of virtually every country in the southern hemisphere."

"... consigning some 2 billion people to lives below their physical and mental potential."

"... the wider impact of micronutrient deficiency on a nation's economy is unknown or unacknowledged among government leaders, politicians and economists, despite the fact that it traps not only individuals and communities but also entire countries in a cycle of poor health, poor educability, poor productivity and persistent poverty."

"... estimated losses of 2 per cent of GDP in the worst-affected countries."

Now, considering all of the above, it really does look pretty much big and significant!

So, what can we do about it?

Our first goal would basically be to recognize the problem and its existence, and make the public general and relevant authorities in every country aware of it. This should require a joint performance and effort from the health and pharmaceutical sector, economy and social government portfolios, food-processing industry, education sector, civil-society organizations and the mass media.

The WHO, UNICEF, FAO and other relevant organizations provide guidelines in dealing with the problem. These include food fortification, supplementation, education regarding food contents, dietary characteristics, etc., as well as medical measures. There are initiatives for periodical, regular screening of subpopulations at risk. The pharmaceutical industry provides a variety of prescriptions effective for treatment of existing anemic patients. Tight collaboration in this process is warranted between different medical specialty profiles, e.g. primary care, public health sector, family physicians, school physicians, pediatricians, gynecologists, gastroenterologists, of course hematologists, and other relevant specialties. With regard to expenses, country authorities could show truly very little concern and more tangible enthusiasm and support to such initiatives: balanced against the enormous damage this deficiency causes on the long run, one of the possible measures, food fortification, costs 2 USD per a person's lifetime, and supplementation costs only a few cents per year.

These measures and efforts would certainly contribute in a suitable extent to the achievement of 5 of the United Nations Millennium Development Goals:

Goal 1 – eradicate extreme poverty and hunger

Goal 2 – achieve universal primary education

Goal 3 – promote gender equality and empower women

Goal 4 – reduce child mortality

Goal 5 – improve maternal health

The problem exists, it causes considerable damage, but we have the prerequisites, knowledge, abilities, capacity and means to deal with it successfully.

Chronic autoimmune (idiopathic) thrombocytopenic purpura in adults. Where are we now?

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Immune (autoimmune, idiopathic) thrombocytopenic purpura (ITP) is defined by a low platelet count secondary to accelerated platelet destruction by anti-platelet antibodies. The diagnosis of ITP requires decreased platelets in the blood smear, normal or increased megakaryocytes in the marrow, and exclusion of other causes of thrombocytopenia (1). ITP can be classified on the basis of the absence or the presence of other diseases (primary or secondary), patient age (adult or childhood ITP), and duration of thrombocytopenia (acute or chronic). The presentation and management of ITP is different in adults and children. Childhood ITP is typically acute in onset, with boys and girls being equally affected, and often develops after a viral infection or vaccination. Although thrombocytopenia may be severe, it usually resolves spontaneously, from within a few weeks to up to 6 months (2). In contrast to the ITP of childhood, adult ITP is generally a chronic disease, of insidious onset, is predominant in women, and rarely resolves spontaneously. Annual incidence of ITP was reported as 5.5 per 100,000 persons when defined by a platelet count of ≤ 100,000/mm3, and 3.2 per 100,000 using a cut-off platelet count of 50,000/mm3 (3). The female-to-male ratio was estimated at 1.7. The incidence of ITP increases with age, being two-fold higher in populations over 60 years of age, as compared to individuals younger than 60 years of age (3,4).

Clinical features

Chronic ITP is traditionally defined as ITP with a platelet count of less than 150, 000/mm^3 for longer than 6 months (4). Bleeding symptoms are generally seen in patients who have platelet counts less than 30,000/mm^3. Purpura, epistaxis, menorrhagia, and gingival bleeding are common; hematuria, hemoptysis, and gastrointestinal bleeding are less common. Hemorrhagic bullae, which may develop in the buccal mucosa, reflect severe thrombocytopenia. Intracerebral hemorrhage is rare and generally occurs in patients with platelet counts below 10,000/mm^3. The incidence of life-threatening complications is highest in those older than 60 years (5–8). The history and clinical examination are usually normal. Family history is especially important to discriminate familial thrombocytopenic syndromes from ITP. The spleen is usually not enlarged (8,9). Constitutional symptoms such as fever and significant weight loss, marked splenomegaly, hepatomegaly, and lymphadenopathy strongly suggest an alternative diagnosis.

Laboratory features

Isolated thrombocytopenia is the major abnormality. Blood smear examination should exclude pseudo-thrombocytopenia. Platelets that are abnormally large or abnormally small may be seen; the former reflecting accelerated platelet production (10), the latter, with platelet microparticles, reflecting platelet destruction (11). The observation of giant platelets should trigger consideration of inherited platelet disorders. Bleeding time depends on the platelet count, and may be normal in patients with mild or moderate thrombocytopenia (12). The ultrastructure of ITP platelets viewed by electron microscopy is similar to that of normal platelets (13).

The hemoglobin concentration and hematocrit are generally normal. The presence of anemia that is not easily explained (for example, due to iron deficiency anemia in bleeding patients) requires further investigation. Autoimmune hemolytic anemia with a positive Coombs test and reticulocytosis (Evans syndrome) may accompany ITP. Leukocytosis and leukopenia with immature cells are not consistent with the diagnosis.

Marrow evaluation generally shows normal or increased megakaryocyte number, although a decreased number of megakaryocytes do not rule out ITP (14). It has been observed that many patients with ITP have less than maximal platelet production which may be related to the effect of anti-platelet autoantibodies (15). American Society of Hematology (ASH) ITP guidelines state that marrow aspiration is unnecessary in the initial evaluation of ITP if the patient is younger than 60 years of age, has a typical presentation, has a good response to first-line therapy, and if splenectomy is not being considered (1). Nevertheless, some hematologists recommend that the marrow be evaluated to rule out leukemia and myelodysplasia, especially in children and those over 40 years of age (16).

Antiphospholipid antibodies (lupus anticoagulant and anticardiolipin antibodies) are frequent in patients with ITP. Although some researchers have reported that these autoantibodies have no clinical significance, we have found an increased risk for thrombosis in ITP patients who had antiphospholipid antibodies (17).

Measuring platelet antibodies

Since ITP is caused by autoantibodies, specific measurement of such antibodies would be expected to provide useful diagnostic clues. Various anti-platelet antibody tests have been described, but none is sufficiently sensitive or specific to be of widespread clinical use. Three types of anti-platelet antibody tests have been developed:

In **phase 1 assays**, the patient's serum is incubated with control platelets, and platelet-dependent endpoints (platelet aggregation or agglutination, release of granular content, platelet lysis are measured, endpoints). Phase 1 tests are neither sensitive nor specific and are of no diagnostic value for ITP (17,18). In **phase 2 assays,** platelet associated IgG antibodies (PAIg) are measured. PAIgG is found to be increased in patients with ITP, but is also found in healthy subjects, and in patients with non-immune thrombocytopenia (19). Although sensitivity of phase 2 assays for ITP patients were reported as high as 91%, the specificity was very low (19-21). In **phase 3 assays** platelet glycoprotein–specific autoantibodies are measured. Three techniques are most widely employed: immunoblotting, immunoprecipitation, and glycoprotein immobilization assays (22,23). *Glycoprotein immobilization assays* include five different assays: the microtiter well assay, the immunobead assay, the modified antigen-capture enzyme-linked immunoadsorbent assay (MACA), the monoclonal antibody-specific immobilization of platelet antigens assay (MAIPA), and the platelet-associated IgG characterization assay (PAICA). These tests may be useful to discriminate immune from non-immune thrombocytopenia and for monitoring response to treatment. Although phase 3 tests are specific, they are still not sensitive for routine use for the screening of ITP.

Therapy

ITP may respond to various agents or manipulations, including glucocorticoids, splenectomy, intravenous immunoglobulin (IVIg), Anti-D, danazol, and antineoplastic drugs such as vincristine and azathioprine, the patient's symptoms and initial response to therapy should dictate the initial therapy.

Emergency treatment of acute bleeding due to severe thrombocytopenia:

Life-threatening bleeding may occur in patients with platelet counts below 10,000/mm^3. Emergency treatment should be instituted in patients with intracranial bleeding, gastrointestinal bleeding, massive hematuria, internal hematoma, or in patients who need emergent surgical intervention. These patients also require hospitalization and conventional critical care measures. High-dose parenteral glucocorticoid therapy (methyl prednisolone 1 g/day for 3 days), IVIg (1 g/kg/day for 2 days), or IVIg and parenteral glucocorticoid in combination are generally recommended for those patients (1, 5, 25). Platelet transfusions after infusion of IVIg may increase the platelet count, because the IVIg may improve platelet survival (26). Aminocaproic acid (initial dose 0.1 gram/kg over 30 minutes, then either continuous infusion at 0.5–1 g/hour or an equivalent intermittent dose every 2 to 4 hours), which inhibits fibrinolysis,

may also be used to reduce bleeding (27). Vincristine may also be used in combination with glucocorticoid and IVIg treatment (25).

Observation:

Patients with no bleeding and platelet counts over 50,000/mm^3 do not require treatment, and can be safely followed (5, 6, 24). Patients with platelet counts between 30,000 and 50,000/mm^3 generally do not experience clinically important bleeding, but may manifest easy bruising. Careful follow-up is necessary for those patients, because the clinical course is difficult to predict. Simple observation is not recommended in patients with platelet counts below 20,000/mm^3, or in those with platelet counts between 20,000 and 50,000/mm^3 and significant mucosal bleeding, or who have risk factors for bleeding, such as uncontrolled hypertension, peptic ulcer, or a vigorous lifestyle (1).

[1]Glucocroticoid therapy:

Oral prednisone at a dose of 1–2 mg/kg/daily is generally accepted for initial treatment in patients with ITP (28,29). In approximately two thirds of the patients, platelet counts increase to greater than 50,000/mm^3 within one week, but decrease again when the prednisone dose is tapered (1,5). Thus, in patients who respond it is recommended that glucocorticoid therapy be continued at a dose of 1 mg/kg/daily for a total of three weeks, before initiating the taper. Besides the standard 1–2 mg/kg/daily dose of prednisone, lower doses (30) and high doses (26,27) have been studied by several investigators. Common side effects of glucocorticoid therapy are facial swelling, weight gain, hyperglycemia, hypertension, cataracts, osteoporosis, opportunistic infections, and behavioral disturbances (1,31). [2]Sustained remission rates with glucocorticoids are infrequent, ranging from 5 to 30% in different series (1,18). If the patient does not respond to 3 weeks of prednisone therapy, other therapeutic options should be considered.

Splenectomy:

Splenectomy is indicated in adult ITP patients whose platelet counts remain below 10,000/mm^3, and in patients whose continue to experience excessive bleeding after 4–6 weeks of appropriate medical treatment. Splenectomy should also be considered in patients who have experienced a transient response to primary treatment and have platelet counts below 30,000/mm^3 after 3 months or who require continuous glucocorticoid therapy to maintain safe platelet counts (1,32). At least 2 weeks before splenectomy, patients should be immunized with polyvalent pneumococcal vaccine, hemophilus influenza b vaccine, and quadrivalent meningococcal polysaccharide vaccine (33). Two thirds of patients who undergo splenectomy achieve normal platelet counts (1,25,32). In the remaining one third, platelet counts recover only partially or transiently, and most of these patients relapse within 6 months of splenectomy. (34). The mortality rate associated with splenectomy is very low (less than 1%) even in patients with severe thrombocytopenia (1,33). Splenectomized patients should be informed to be alert for the symptoms and signs of such infections, and prepared for an emergency situation (1,33,34).

Laparoscopic splenectomy is an alternative to open splenectomy since the spleen is of normal size and vascularity in ITP patients. In experienced hands, laparoscopic splenectomy is cost-effective and safe; long-term and short-term benefits and complications are similar to those seen with open splenectomy (35,36). This procedure is limited by a high frequency of retained splenic tissue, especially in those with an accessory spleen, and increased risk of splenosis (25, 35,36).

Intravenous immunoglobulin (IVIg):

IVIg increases the platelet count in more than 75% of patients with chronic ITP, and normalizes the platelet count in approximately 50% of the patients. The effect of IVIg is transient, generally lasting only 3-4 weeks (1,31). The patient may become refractory with repeated infusions of IVIg (37). Postulated mechanisms for the action of IVIg include blockade of macrophage Fc receptors, which would slow clearance of antibody-coated platelets, and anti-idiotype neutralization of anti-platelet autoantibodies (31). The recommended total dose of IVIg is 2 g/kg administered as either 0.4 g/kg/day on 5 consecutive days or as 1 g/kg/day on 2 consecutive days. For maintenance therapy, 0.5–1 g/kg as a single dose may be used. Adverse effects of IVIg therapy include headache, backache, nausea, fever, aseptic meningitis, alloimmune hemolysis, hepatitis, renal failure, pulmonary insufficiency, and thrombosis. Anaphylactic reactions may occur in patients with congenital IgA deficiency; IgA levels should therefore be evaluated before IVIg infusions. The cost of

IVIg is considerable, and it is not recommended as initial therapy in adult patients with ITP, except in the setting of life-threatening bleeding (1).

Anti-(Rh) D:

Anti-(Rh) D binds Rh-positive erythrocytes and leads to their destruction in the spleen. Because splenic Fc receptors are blocked, more antibody-coated platelets survive in the circulation (38,39). A positive direct antiglobulin test and mild hemolysis occur in all Rh-positive patients after anti-(Rh) D infusion, generally without requiring a blood transfusion. Anti-(Rh) D therapy is not effective in Rh-negative patients, and response rates are very low in splenectomized patients. A single dose of 50–100 μg/kg is recommended, given by intravenous infusion over 3–5 minutes (38,40). Although it has been reported that anti-(Rh) D increases the platelet counts in over 70% of the patients who are Rh-positive and not splenectomized and may preclude the necessity of splenectomy, a recent randomized, controlled trial comparing anti-D with conventional therapy showed no differences in the rates of spontaneous remission or splenectomy (40).

Other Treatments:

Vinca Alkaloids. Both vincristine and vinblastine transiently increase the platelet count in approximately 70% of ITP patients within 5 to 21 days, but produce sustained remissions in only 10% of treated patients (1,6,25). The recommended dose of vincristine is 1–2 mg and of vinblastine is 0.1 mg/kg (maximum 10 mg), both given by bolus injection at one week intervals for a minimum of three courses. It has been proposed that vinca alkaloids bind to platelet microtubules, and are thereby transported to the spleen, where they subsequently inhibit the phagocytic functions of the macrophages. They may also stimulate megakaryocytopoiesis Peripheral neuropathy, neutropenia, jaw pain, alopecia, and constipation are complications of treatment with vinca alkaloids (41).

Cyclophosphamide. Cyclophosphamide can be used orally (50–200 mg/daily) or parenterally (1–1.5 gram/ every 4 weeks) in refractory ITP patients (42). It increases platelet counts in 60–80% of ITP cases, and 20–40% of those patients will stay in remission for 2–3 years (1). Its action in increasing the platelet count involves immunosuppression. The major complications of cyclophosphamide therapy are bone marrow suppression, hemorrhagic cystitis, infertility, alopecia, and secondary malignancy.

Azathioprine. It has been reported that azathioprine produces a sustained normalization of the platelet counts in up to 45% of refractory ITP patients (43). A dose ranging from 50 to 250 mg of azathioprine daily for at least four months seems to be necessary before its effectiveness can be evaluated. As with other immunosuppressive drugs, major adverse effects are marrow suppression, possible increased risk of secondary malignancy, and teratogenesis (1).

Danazol. It is postulated that danazol decreases Fc receptor numbers on phagocytic cells by antagonizing the effects of estrogens. Given at a dose of 800 mg/day for at least 6 months, reported response rates range from 10 to 80%. . Danazol should be avoided in pregnant women and in patients with liver disease. Because liver dysfunction is common with danazol therapy, liver function should be evaluated monthly (1, 6).

Many other therapies, including interferon-alpha (44), dapsone (45), immunoadsorbtion with staphylococcal protein A (46), cyclosporine (47), ascorbic acid (48), colchicine (49), and plasmapheresis (50) had been studied for refractory ITP cases, but none have been clearly demonstrated to be effective. Recent small case series suggest that rituximab, a monoclonal antibody against CD20, may be effective in the treatment of refractory ITP (51,52). Another approach, the stimulation of the platelet production by thrombopoietin mimetic agents (AMG 531 and elthrombopag) has been successful in preliminary studies, and may provide a new alternative therapy (53).

Where Are We Now in the Management of ITP?

Diagnosis of ITP still has some difficulties. Isolated severe thrombocytopenia and purpura in otherwise healthy individuals is typical presentation of ITP. A careful physical examination and peripheral smear examination may easily confirm the diagnosis of ITP in such patients. Major problem is to evaluate of otherwise healthy individuals with mild to moderate thrombocytopenia. The evaluation limits of such patients are still controversial. Pseudothrombocytopenia, familial thrombocytopenic disorders, infections (especially HIV, HCV and other viruses), drugs, autoimmune diseases such as antiphospholipid syndrome may

cause immune thrombocytopenia. A careful physical examination and peripheral smear examination are still very important in patients with mild to moderate thrombocytopenia, but usually not enough for exclusion of other thrombocytopenia causes. Measuring anti-platelet antibodies with current methods are insufficient, since these antibodies have also been detected in 10-20% of patients with non-immune thrombocytopenia. We need more sensitive and specific diagnostic tests for ITP patients.

Another important issue is the treatment of ITP. All current treatment modalities are designed to prevent platelet destruction, either by immunosuppression (mainly with glucocorticoid therapy) or by splenectomy. The side effects of glucocorticoid therapy and splenectomy are sometimes worse that disease itself. Since the therapeutic options for refractory ITP patients are many; therapy should be tailored to the individual patient. Bleeding symptoms rather than platelet count should determine if any treatment is required. Patients with platelet counts over 30.000/mm^3 may be followed, without therapy. The bleeding risk is very low in those patients. The treatment strategy is uncertain for patients with platelet counts below 30,000/mm^3. Observation without therapy may be appropriate if the patient has no signs of bleeding and has no risk factors for bleeding such as uncontrolled hypertension, peptic ulcer disease, etc. It is generally accepted that if splenectomy fails to produce a remission, other modalities are unlikely to produce a complete and long-lasting remission. The main goal of therapy in these patients is to prevent bleeding, not correcting the platelet counts in normal limits. New treatment modalities that stimulate the platelet production with thrombopoietic agents, may provide a new dimension in the treatment of refractory ITP patients.

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1ST BALKAN HEMATOLOGY DAYS

Poster Presentations

BSH001

INTRAVASCULAR LARGE B-CELL LYMPHOMA ASSOCIATED WITH HYPOPITUITARISM SUCCESSFULLY TREATED WITH IMMUNOCHEMOTHERAPY

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Introduction. Intravascular large B-cell lymphoma (ILBCL) is a rare generally fatal disease characterized by massive proliferation of lymphoid cells within the lumina of small and medium blood vessels. Circulating lymphomatous cells are rarely detected on blood smear analysis.

Case report. A 67-year-old woman presented with a fever, malaise and slight mental status change. There was splenomegaly of 150 mm in diameter and no lymphadenopathy. Laboratory results showed: Hb 79 g/l, WBC 4,3x109/l (bands 15%, segmented 60%, eosinophils 2%, lymphocytes 8%, monocytes 15%), platelets 64x109/l.SE 76 mm, fibrinogen 8,03 g/l, lactate dehydrogenase 1751 UI/l. Bone marrow histology revealed infiltration with lymphoid tumor cells confined within the lumina of sinuses. On immnohistochemistry neoplastic cells were CD20+++, CD79alfa+, CD3-, MPO-. Abdominal ultrasonography and CT scan were normal except slightly enlarged spleen which were of homogenous structure. On chest radiography right small pleural effusion was detected. Hyponatremia (128,0 mEq/L) was associated with normal kalemia and normal renal function tests. Hormonal levels revealed hypophyseal insufficiency with decreased serum levels of follicule stimulating hormone (FSH), luteinizing hormone (LH) and thyroid-stimulating hormone (TSH). Thyroxin (T4) was 7,10 (normal range 10,00-22,00 pmol/L). Prolactin level was 836 (normal 72,0-511,0 mlU/L). Cranial computed tomography and magnetic resonance imaging (MRI) of hypophyseos were normal. The diagnosis of ILBCL confined to bone marrow and pituitary gland was established.

The patient was treated with 6 cycles of R-CHOP protocol: rituximab 500 mg i.v., 1 day, adriblastin 50 mg i.v., day 2, oncovin 2 mg i.v., day 2, endoxan 1000 mg day 2, prednison 100 mg on days 1-5 and than continued with 20 mg. At the same time she got substitution for hypothyreosis. The patient entered complete remission but continued with therapy for hypothyresis.

Conclusion. This case present an unusual intravascular lymphoma associated with hypopituitarism and the efficacy of the current treatment of this group of patients with CHOP/rituximab. Endocrine gland dysfunction in ILBCL has been previously reported but hypopituitarism rarely.

BSH002

MUTATION OF THE P53 GENE IS ASSOCIATED WITH BLASTIC TRANSFORMATION IN CHRONIC MYELOID LEUKEMIA

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Chronic myelogenous leukemia (CML) is a clonal disorder of multipotenial hematopoietic stem cells characterized by excessive proliferation of immature and mature myeloid cells. Molecular mechanisms in blastic crisis remain largely unknown. However loss of functions of tumor suppressor genes such as P53 might be involved in disease progression. This work was planned to investigate P53 protein expression and gene mutation in chronic myeloid leukemia and to evaluate its role in blastic transformation. Mutant P53 protein expression by flow cytometry and P53 gene mutation by polymerase chain reaction – single strand conformational polymorphism (PCR-SCCP) and sequencing technique were assessed in 26 patients with newly diagnosed CML, 11patients at blastic crisis as well as 10 apparently healthy individuals selected to act as a control group. In this study mutant P53 protein expression-detected by flow cytometry – was found in 3.8% of patient at chronic phase and in 27.7% of patients at blastic crisis opposite to 0 %of control group. One patient had P53 mutant gene- as detected by PCR-SSCP% sequencing technique – in chronic phase in the form of tansition point mutation (thymine→cytosine) at codon 273 of exon 8. Three patients of blastic crisis had P53 mutant gene, one had similar pattern of sequencing as that in chronic phase while the other 2 patients had denovo P53 gene mutations. There is framshift mutation where there is insertion of cytosine at codon 250 of exon 7 in one patient and transversion point mutation (guanine→thymine) at codon 154 of exon 5 in the other patient. From this study we can conclude that expression of mutant P53 as a result of P53 gene mutation may be important in the genesis of blastic crisis of chronic myeloid leukemia.

BSH003

SERUM LEPTIN LEVEL IN ACUTE LEUKEMIA PATIENTS

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Leptin is a regulator of fat metabolism that is synthesized in adipocytes and released into circulation. Leptin is, in addition released locally in the bone marrow by adipocytes and stromal cells. We investigated systemic leptin levels before and after chemotherapy in acute leukemia patients which is related to clinical parameters (diagnosis, blast count and neutropenia) or not.

Serum samples were derived from 17 consecutive acute leukemia patients; 13 patients was acute myeloblastic leukemia (AML) and 3 patients with acute lymphoblastic leukemia (ALL). Median age was 40 years (range 16-65 years, 7 men, 10.women). The diagnosis of acute leukemia were based on clinical criteria as well as morphological, cytochemical, immunophenotypic, cytogenetic examination of peripheral blood and bone marrow specimens. Serum leptin level was studied in these patients and were compared with those in patients who with initial diagnosis, after chemotherapy with neutropenia and in 14 healty controls. Leptin levels in serum, enzyme-linked immunosorbent assays (ELISA) was

used (RayBio, USA) to determine leptin concentrations. There was no difference between age and sex in all patients and healty controls. Body mass index (BMI) did not differ between acute leukemia patients and control group. Serum leptin levels before and, after chemotherapy did not differ from those normal controls. Levels of leptin in acute leukemia patients did not correlated with BMI, CRP, blast count and febrile neutropenia.

Leptin levels are affected by many factors those are energy imbalance, fasting, acute infection, inflammation as well as hormones and many cytokines. We found no correlation between leptin levels and clinical parameters; diagnosis, after chemotherapy, blast count and neutopenia of acute leukemia patients. However, These data do not rule out the possibility of leptin production of leukemia blasts in bone marrow stroma which would create a high local concentration of leptin within bone marrow microenvironment. The biological and pathological roles of leptin in acute leukemia should be clarified by the further investigations.

BSH004

OUR INITIAL EXPERIENCE WITH BORTEZOMIB IN THE TREATMENT OF REFRACTORY MYELOMA

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Background: Proteosome inhibitors have been proven to be very effective for the treatment of refractory/relapsed multiple myeloma (MM). Bortezomib inhibits proliferation and angiogenesis and promotes apoptosis of myeloma cells; it is the first agent of this class to enter clinical trials. Aims: The aim of this study was to report our initial experience with Bortezomib in the treatment of patients with refractory myeloma. Methods: Four patients with MM (3 male and 1 female, median age 53.5 years, range 43-62 years, 2 with IgG type and 2 with BJP type) who had received 2 or 3 prior treatment protocols and were with refractory disease were treated with Bortezomib (Velcade) in the Department of Hematology, Clinical centre in Skopje. Bortezomib was administrated using the wildly published 3-weekly regimen (1.0 or 1.3mg/m (2) at days 1, 4, 8 and 11, followed by rest for 10 days). Results:Velcade was administrated as a monotherapy in three patients and in combination with Prednisolon in one patient. The patients were refractory to prior therapies including Thalidomide in three cases. The median time from diagnosis to initiating Velcade was 42.7 months, range 9-82 months. The average number of cycles administrated per patient was 3.8, range 1-6. After a median follow up of 5 months, two patients achieved CR (complete remission), one patient had PR (partial remission) and there was one early death, occurring within the first two months of treatment. One patient delayed therapy for infection (pneumonia); grade 3-4 thrombocytopenia occurred in one patient and one patient experienced constipation and fatigue. No patient developed peripheral neuropathy. The early death was detected in one patient who developed acute renal failure and required hemodyalisis. This patient had normal blood urea nitrogen and slightly increased value of serum creatinine at baseline (urea 7.8 mmol/l and creatinine165mmol/l) with a rapid increase of these values during the first four doses of Bortezomib (urea 12.9, 15.5 and 23.8mmol/l and creatinine 420, 588 and 980mmol/l). Intensive supportive treatment was conducted including hemodyalisis with a fatal outcome within 55 days from initiating the treatment with Bortezomib.Conclusions :Our preliminary data demonstrates that Bortezomib is extremely effective in patients with refractory MM with a RR (response rate)in 75%. The adverse events were temporary and well tolerated except the renal failure that was fatal for one patient. Renal adverse events with fatal outcome were not reported in up to now trials so, many questions remain doubtful: Is Velcade itself responsible for such an outcome in our patient? Had the patient already had disturbed renal function because of the disease with a rapid deterioration after Bortezomib? Futher investigations are necessary to answer these questions.

BSH005

BONDRONAT IS ESSENTIAL ACCESSESORY TRATMENT IN MULTIPLE MYELOMA WITH NEGLIGIBLE SIDE EFFECTS

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Background: Multiple myeloma (MM) is a malignant disease characterized by skeletal involvement. Osteolytic bone lesions from MM are associated with skeletal complications such as bone pain and pathologic fractures which have a negative impact on quality of life. Bisphosphonates have been shown to decrease the progression of osteolytic lesions, bone pain and fractures. Aims: Aim of this study has been to evaluate the efficiency of ibandronate (Bondronat, F.Hoffman-La Roche) on the course of bone lesions in myeloma patients and also its safety particularly concerning renal function. Methods: We analysed a group of 28 patients in clinical stage III-A or III-B (median age 59.9 years, range 42-77, male: female ratio 13:15) who were currently treated, independently from the adopted chemotherapy, with Bondronat given as an IV infusion over 1-2 hours every four weeks. Patients were clinically evaluated before, during and after Bondronat administration with the median time of observation 7.7 months (range 3-18). Evaluation of osteolytic lesions was performed during the study associated with skeletal complications such as bone pain and pathologic fractures. Routine serum chemical screening including creatinine, calcium, phosphorus and AP were performed. Markers of tubular damage (NAG, AAP, gamaGT and ß2M) were measured in urine before and after Bondronat administration. Results: Clinical improvement of skeletal pains was observed in 23 pts (82%): 12 pts (43%) had a complete pain relief with no more necessity for analgesic-drug use, and 11 pts (39%) had a minor effect, while 5 pts (18%) had no improvement. Nine patients had pathologic fractures at baseline (8 were on vertebral bodies and 1 on ribs) and all of them underwent radiotherapy. During the observed period we didn't find any new pathologic fracture.

There were no significant adverse effects associated with the administration of Bondronat. Twenty-one patient (75%) had normal renal function at baseline and the rest had varying degrees of renal insufficiency. No clinically relevant changes in serum creatinine occurred even in patients with existing renal impairment. Transient hypocalcaemia was detected in 3 pts (10%). The levels of NAG, AAP, gamaGT and ß2M were similar before and after administration of Bondronat. Conclusions: The treatment with Bondronat has reduced skeletal morbidity and was well tolerated in our group of myeloma patients; we didn't find any signs of acute renal toxicity in the observed period.

BSH006

A CASE REPORT OF A PATIENT WITH TTP IN COMA-IS PLASMA EXCHANGE ESSENTIAL FOR RESTORATION?

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Background:Thrombotyc thrombocytopenic purpura (TTP) was first described in 1924 by Moskowitz. It is a devastating, severe and potentially fatal disease with a broad spectrum of clinical manifestations. Later in 90, investigators identified an enzyme that is involved in the pathogenesis of TTP. This enzyme is a metalloprotease which is called vWF metalloproteinase and is involved in braking down the large multimers in bloodstream to smaller ones Aims: The aim of this report is to describe the evolution of the disease in a young person with recently diagnosed TTP and very serious clinical presentation. A 33-year-old man was admitted to the hospital in the Department of Gastroenterology because of haematemesis and melena. There was a prompt clinical deterioration with loss of conciseness and problems with respiration. The patient was transferred to the Intensive Care Unit for respiratory monitoring and reanimation. According to the clinical and laboratory data the diagnosis of TTP was established (anemia with Hb-42g/l, thrombocytopenia with platelets 41000/mikroL, reticulocytes 20%, total bilirubin 52-indirect 46, LDH 2549 IU/l, direct Cooms test normal and fragmented red cells in peripheral blood film present). Intensive plasmapheresis with plasma exchange was started. After three successive courses of plasma exchange there was a dramatic change in the disease evolution. The patient woke up but was still confused, disoriented and very aggressive. We continued the treatment with futher plasma exchange, Methylprednisolon, Dipyridamole, sedatives and symptomatic therapy until complete recovery of hematological values and clinical condition. Discussion: Plasma exchange is essential in the treatment of TTP with dramatic improvement in the clinical course particularly in extremely risky patients. As the disease course is sometimes fulminate and rapidly fatal, the initiation of therapy is urgent even in patients who do not fulfill all the criteria for TTP at presentation.

BSH007

RETINOIC ACID SYNDROME IN A PATIENT WITH PROMYELOCYTIC LEUKEMIA THAT MIMICS SEPTIC ARTHRITIS

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The most serious adverse effect of all-trans retinoic acid (ATRA) in patients with acute promyelocytic leukemia (APL) is retinoic acid (RA) syndrome that is characterized by fever, respiratory distress, weight gain, oedema, pleural and pericardial effusions, and unexplained hypotension.We present a case with RA syndrome that mimicking septic arthritis. A 72-year- old woman was diagnosed with APL based on bone marrow morphology, flow cytometry analysis, demonstration of t(15;17) on karyotypic analysis and the PML/RARα rearrangement by reverse transcriptase polymerase chain reaction (RT-PCR). Treatment with ATRA 45 mg/m² was initiated. On the 9 th day of ATRA, she developed fever, dyspnea and pain of right knee joint. On physical examination right knee joint was extremely painful,warm and swollen. Effusion was detected by performing ballottement on patella. Effusion was aspirated by arthrocentesis.Morphologic examination of snovial fluid showed infiltration of mature myeloid cells and occasional promyelocyte. Gram stains and cultures of material were negative. Diagnosis of RA syndrome was made and ATRA was stopped. Dexamethasone at a dose of 10 mg twice a day intavenously was initiated. On the 5 th. day of discontination of ATRA and initiation of dexamethasone all manifestations of joint improved.

BSH008

CASE OF PANCYTOPENIA ASSOCIATED WITH SHEEHAN'S SYNDROME

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Normochromic and normocytic anemia is a well-described hematologic manifestation of Sheehan's syndrome. However pancytopenia is rarely observed. A 57-year-old women was admitted to our hospital with complaints of generalised weakness,fatique and dyspne of effort.She had 9 children.At the end of the last delivery at age of 36,she had severe vaginal haemorrhage. Thereafter she had no normal breast activity and she had permanent amenorroea. Laboratory evaluation showed a leukocyte count of 3.3 x 109/l with a peripheral differential count of, %38 neutrophil, %6 monocyte and % 53 lymphocyte and %3 eosinophil. The hemoglobin level was 9.6 mg/dl and the platelet count was 100x

109/l. Plasma iron, ferritin and B12 levels was normal. Bone marrow examination revealed hypocellular marrow with decreased hematopoesis. Hormone tests showed decreased level of ACTH, cortisol,free T4, free T3, LH, FSH, GH. Pituitary gland MR images was consistent with empty sella. Diagnosis of Sheehan's syndrome was made. Hormonal therapy consisting of prednisolone and levothyroxine was initiated. After 2 months of therapy, clinical and hematological improvement was achieved.

BSH009

ISOLATED CENTRAL NERVOUS SYSTEM RELAPSE AFTER PERIPHERAL STEM CELL TRANSPLANTATION FOR ACUTE LEUKEMIA PATIENTS

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Isolated extramedullary relapsesof acute lymphoblastic and myeloid leukemia usually occure in testis, central nervous system (CNS) and skin. Little is known about the frequency, treatment and outcome of extramedullary relapse. For that reason there is not a standart treatment available for these patients.We investigated isolated central nervous system relapse in 2 patients with ALL and 1 patient with AML-M1 after HLA identical sibling donor allogeneic peripheral stem cell transplantation. (PSCT). 3 patients presented with isolated central nervous system relapse while their marrow showed signs of complete hematological remission (CR) with 100 % donor chimerism. (DC) The first patient with T-cell ALL, primarily succesfully treated with CALG-B chemotherapy regimen, developed leukemic cells within the central nervous system 3.5 months after PSCT. He treated with intrathecal ARA-C, methotrexate and dexamethasone. İn the other patient,diagnosed with ALL type T cell isolated CNS relapse occured 12 months after PSCT. He was treated with radiotherapy and the leukaemic cells disappeared after local radiotherapy. In the third patient (AML-M1) isolated CNS relapse were observed during 14 month period following PSCT. This patient was treated with local radiotherapy. All these patients are stil in CR with 100 % DC. Conclusions: Isolated CNS relapses following allo PSCT, although infrequent, continue tobe a therapeutic problem. Irradiation should be considered a first line therapy in selected cases, individualized treatment, such as intrathecal chemotherapy may be of value.

BSH0010

PITYRIASIS LICHENOIDES ET VARIOLIFORMIS ACUTA IN ASSOCIATION WITH ACUTE MYELOID LEUKEMIA

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Paraneoplasticdermatoses are non-neoplastic skin disorders which occur in the context of an underlying malignant neoplasm. The classic paraneoplastic dermatoses are mostly associated with solid neoplasms and rarely occur in the context of nodal or primary cutaneous lymphomas. Additionally; there are skin disorders reported to occur in close association with hematological disorders which can thus be regarded as paraneoplastic manifestations. We report a patient with pityriasis lichenoides et varioliformis acuta in association with acute myeloid leukemia.An 64 year old man presented to our clinic with complaints multiple skin lesions, fatique and fever.On physical examination he had hepatosplenomegaly and multiple erythematous and necrotic skin lesions. On labaratory examinations there were anemia, thrombocytopenia, leukocytosis and 60% blastic cells on the peripheral blood smears. Bone marrow smears contained 90 % blast cells. Morphological, cytochemical and immnophenotypic examination revealed AML M1. Skin biopsies and reviews of slides confirmed the diagnosis of pityriasis lichenoides et varioliformis acuta. He received induction chemotherapy with daunorubicine and ARA-C. After induction therapy complete remission achieved and all skin lesions disappered

BSH0011

SPONTANEOUS ACUTE TUMOR LYSIS SYNDROME IN A PATIENT WITH ACUTE T-CELL LYMPHOBLASTIC LEUKEMIA

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Acute tumor lysis syndrome (TLS) is a metabolic emergency that results from rapid destruction of tumor cells with release of cellular breakdown products into the systemic circulation. It usually occurs following therapy of malignancies. Spontaneous acute TLS before treatment without any precipitating factor is rarely observed. We present a patient with acute lymphoblastic leukemia (ALL) with pericardial effusion and a large mediastinal mass who developed acute TLS spontaneously before institution of chemotherapy. A 17-year-old woman was diagnosed with T cell ALL based on bone marrow morphology and flow cytometry analysis. She presented with non-oliguric ARF with extreme hyperuricemia, hyperphosphatemia and hypocalcemia on admission. Diagnosis of spontaneous acute TLS that results from ALL was made as there was no contributing factor for development of renal failure. She was successfully treated with aggresive intravenous hydration and allopurinol and then intensive chemoterapy (Hyper-CVAD program) was administered. She is still alive and in remission for 2 months.

BSH012
TRANSFUSION ASSOCIATED GRAFT-VERSUS-HOST DISEASE

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Transfusion associated graft-versus-host disease (TA-GvHD) is the most fatal complication of transfusion that results from the engraftment of viable T lymphocytes,usually from HLA homozygous donors,into immunocompromised recipients sharing one HLA haplotype with the donor. It is characterized by dysfunction of the skin, liver, gastrointestinal tract and bone marrow. A 44-year-old woman was admitted to our hospital with complaints of nausea,vomitting and fatique for 1 week. She underwent an operation for myoma uteri 2 weeks ago and one unit of blood was transfused from her brother before the operation. Physical examination was normal. On admission CBC was normal. AST,ALT,LDH,ALP and GGT was extremely increased. Disseminated erythematous maculopapular and bullous skin lesions, jaundice and diarrhea were developed an the 7 th day of admission. On labaratory examination pancytopenia occured.Skin lesions,,jaundice, diarrhea, pancytopenia and abnormal liver function tests suggested TA-GvHD. Skin and bone marrow biopsy was performed to establish the diagnosis. Bone marrow biopsy showed hypocellular bone marrow with decreased hematopoesis. Skin biopsy was consistent with TA-GvHD. Immunosuppressive treatment with prednisolone, anti-thymocyte globulin (ATG) and cyclosporine was initiated. Hypotension and respiratory failure developed and she died 12 th day of admission.

BSH013
RANKL AND RANKL/OPG RATIO ARE IMPORTANT MARKERS OF BONE METABOLISM IN PATIENTS WITH MULTIPLE MYELOMA

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Background: In the last years data accumulated which demonstrate that RANKL is a critical regulator of osteoclastogenesis and bone resorbtion in physiological and pathological conditions. Data in literature concerning RANKL levels and RANKL/OPG ratio in patients with multiple myeloma (MM) are a few.

Aim: To analyze the serum levels of RANKL and RANKL/OPG ratio in patients with MM, their clinical correlations with important parameters of the disease; their role as risk factors for development of myeloma bone disease (MBD); their prognostic significance for the duration of the remission.

Patients and Methods: We studied 66 newly diagnosed patients with MM, 29 male, 37 female, median age ± SD: 61,8 ± 8,6; range: 45-81 years. In I clinical stage (Durie et Salmon) were 13,7%, in II- 33,3%; in III- 53,0% patients. Renal failure (RF) was found in 40,9%, MBD in 84,8%, hypercalciemia in 31,8%. Serum levels of RANKL were analyzed by ELISA with kits of Biomedica, Vienna. A group of 32 healthy individuals was used for controls. Statistics were performed by variative, correlative, alternative analyses, non-parametric tests, Mann-Whitney-test, Kaplan-Meier with log rank test using SPSS v 11.0 for Windows.

Results: Levels of RANKL were significantly higher in MM compared with controls: 0,458 ± 0,046 pmol/l vs 0,203 ± 0,031 pmol/l (p<0,001). RANKL/OPG ratio was also significantly higher in MM compared with controls: 0,114 ± 0,013 vs 0,053 ± 0,003 (p<0,001). The levels of RANKL and RANKL/OPG ratio increase significantly and differentiate each clinical stage and each grade of MBD. The highest levels of RANKL and RANKL /OPG ratio were found in patients with MBD grade "2+3": 0,589 ± 0,076 and 0,162 ± 0,021 respectively (Tabl1).

The strongest correlations of RANKL and RANKL /OPG ratio were found with clinical stage (p<0.001 r=+ 0,524; p<0.001 r=+ 0,682 respectively) and MBD (p<0.001 r=+ 0,557; p<0.001 r=+ 0,651 respectively). RANKL and RANKL /OPG ratio also correlated significantly with bone marrow plasmocytosis, LDH and beta2-microglobulin in the group without RF. No correlation was found between RANKL, RANKL/OPG and the immune variant, serum levels of Ca, creatinin, hemoglobin and albumin. Using "odds ratio" method, included in the crosstabs test, we found that levels of RANKL>0,4 pmol/l increase 10 times the risk for development of MBD. When RANKL/OPG ratio is >0,11 this risk is 20-times higher. In patients with increased levels of RANKL>0,4 pmol/l we registered significantly shorter remission: 4 vs 7 months (p<0,001). In patients with RANKL/OPG ratio>0,11 the remission duration was only 2 months vs 7 months (p<0,001).

Conclusions: RANKL and RANKL/OPG ratio are significantly higher in MM patients, increase significantly with each clinical stage and grade MBD. The elevated serum levels of RANKL and RANKL /OPG ratio result in high risk of development of MBD and significantly shorter remission duration. They are important markers of bone metabolism in MM and can be used for selecting groups of patients who need intensive therapy and early administration of biphosphonates.

Table 1. RANKL and RANKL/OPG ratio in patients with MM according to clinical stage, MBD and compared with controls.

Group	*N*	*RANKLpmol/l Mean±SEM*	*p*	*RANKL/OPG mean±SEM*	*p*
Controls	32	0,203 ± 0,031	<0,001	0,053 ± 0,003	<0,001
MM patients	66	0,458 ± 0,046		0,114 ± 0,013	
I clinical stage	9	0,222 ± 0,022	I vs II: 0,012	0,038 ± 0,005	I vs II: 0,015
II clinical stage	22	0,346 ± 0,033	I vs III: 0,001	0,065 ± 0,007	I vs III: 0,001
III clinical stage	35	0,589 ± 0,078	II vs III:0,018	0,126 ± 0,021	II vs III 0,001
MBD gr 0	10	0,200 ± 0,030	0vs 1: 0,004	0,035 ± 0,006	0 vs 1: 0,009
MBD gr 1	20	0,350 ± 0,029	0vs 2+3:0,001	0,068 ± 0,008	0vs 2+3: 0,001
MBD gr 2+3	36	0,589 ± 0,076	1vs 2+3: 0,015	0,162 ± 0,021	1vs 2+3 :0,001

BSH014

CHRONIC MYELOLOID LEUKEMIA IN THE COURSE OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Case report: The coexistence of chronic myeloid leukemia (CML) and B-cell chronic lymhocytic leukemia (CLL) in the same patient is rare. In May 2001, a 56-year-old man was diagnosed as having CLL by the evaluation of blood smear, bone marrow biopsy and immunophenotyping. Blood examination showed white blood cell (WBC) count of 199x109/l with lymphocytes 90%.

The patient was treated with cyclophosphamide, vincristine, prednisolone(COP) regime. After 5 courses, we stopped drug treatment since indications of the patient was disappeared. In September 2003, WBC count of 65.1x109/l with lymphocytes 75%, low serum immunoglobulin levels, positive antiglobulin tests, progress of lymphadenopathy and B symptoms were observed. Then, we started therapy with cyclophosphamide, vincristine, doxorubucine, prednisolone(CHOP). After 6 courses, we stopped the treatment due to improvement in the clinical and laboratory findings. In September 2005, complete blood count revealed WBC 88.4x109/l, neutrophil 46x109/l, hemoglobin 13.7 gr/dl, platelets 230x109/l. The peripheral blood smear showed mature lymphocytes and granulocytosis with immature forms. Leukocyte alkaline phosphatase score was low. Then, CML was considered and cytogenetic test of fluorescence in situ hybridization (FISH) was performed. BCR/ABL and deletion of 13q14 were detected. After these results, hydroxyurea and then STI571 was commenced. Reduction of WBC level and splenomegaly were achieved.

Discussion: CLL and CML are two distinct heamatological neoplasms. CLL is associated with increased risk of second malignancy and this can occur in treated and untreated patients. Alkylating agents may contribute to the development of second malignancy. The impaired immune survaillance associated with CLL may also contribute to the increased risk of second neoplasms. Explanation for simultaneous occurance of both malignancies in the same patient could be that both diseases are biclonal origine and originate from a unique stem cell capable of differentiating into two different cell lines. In addition, a coincedental occurence of CML and CLL in the same patients can not be ruled out.

BSH015

THE MANAGEMENT OF SERIOUS UPPER GASTROINTESTINAL BLEEDING AND ANTIBODY DETECTION IN TWO PATIENTS WITH GLANZMANN THROMBASTENIA

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Glanzmann thrombastenia is a rare disorder characterized by a prolonged bleeding time, normal platelet count, and absent macroscopic platelet aggregation. The basis for their defective function is deficiency or dysfunction of the GPIIb-IIIa complex. Purpura, epistaxis, gingival hemorrhage and menorrhage are the most frequent bleeding manifestions in these cases., we aimed to look over the management of serious upper gastrointestinal bleeding and antibody detection in two patients with Glanzmann thrombasthenia

Case 1: 27-year-old men was admitted to the hospital with complaints of vertigo and melena for the last two days.. His parents were not relatives and has two brothers and two sisters. His brother was died at 12 years old the result of serious epistaxis The Ivy bleeding time was 19' 4" (Normal 1-9 minutes). PT, aPTT, INR and clotting time was normal. Case 2: A 25-year-old man was admitted to the hospital with complaints of epistaxis for ten days and melena for the last two days.. Template bleeding time was > 20' (Normal 1-9 minutes). PT, aPTT, INR,clotting time and VWF ag level was normal. Invitro bleeding time with PFA 100 kollagen/epi and Kollagen/ADP was > 300. Platelet aggregation were absent with ADP, collagen, epinephrine but it was normal with ristocetine both of patients. CD41 was detected as 1.47 % and CD61: 2.6% In flow cytometric analysisTransexamic acid 500 mg/d was initiated by infusion for 1 hour and desmopressine acetate (DDVAP) added in the 1nd day of the treatment. These two cases were examined for antibody against to thrombocyte. Sample of normal plasma which is thrombocyte agrogometry was normal and sample of patients plasma was mixed with 1:1 ratio. Then thrombocyte agregometry was performed and seen tthat agregation with kollagen was absent. It is thought that mixing test was positive due to iso-antibodies which are againts thrombocyte caused absence of agregation.

In conclusion, there is no exact treatment for Glanzmann thrombastenia. Platelet and packed-red-cells transfusion should be given. Because bleeding is a lifelong problem, the use of human leukocyte antigen-matched platelets should be considered to lessen the chance of refractoriness to transfusion due to platelet alloimmunization. In rare instances, thrombastenic patients have developed antibodies against normal GPIIb-GPIIIa following transfusion. We detected these antibodies by mixing test

BSH016

HAIRY CELL LEUKEMIA: A RETROSPECTIVE CLINICAL STUDY

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Hairy cell leukemia (HCL) is an uncommon chronic lymphoproliferative disease characterized by splenomegaly, pancytopenia and infiltration of bone marrow with lymphocytes and good therapeutical response using purine analogues. We have revised clinical evaluation and therapeutic response of this disease. We reviewed four HCL cases diagnosed by histopathologically and immunohistochemically between 2003 to 2006 years in our center. Mean age was 45 years. The most frequent cause of diagnosis in HCL patients were anemia, neutropenia and splenomegaly. Cinical and laboratory findings are shown in table 1.

All cases treated with cladribine. The median response time was 6 months. None of those patients were relapsed. As reported here, all patients with HCL responded to initial treatment with cladribine. Long-term follow-up of patients with HCL treated with cladribine shows that this agent is a safe and highly effective in the treatment of HCL. HCL has a long survival though the age of presentation is advanced. Patients who relapse have several options available, including another cycle of cladribine, treatment with alternative purine analogs, or treatment with novel agents such as rituximab and BL22. Based on long-term experience with cladribine in HCL should be agents of choice in the initial treatment.

BSH017

MODIFIED IDARAM CHEMOTHERAPY REGIMEN TO R-IDARAM FOR PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA:

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Primary central nervous system lymphoma (PCNSL) is an agressive non-Hodgkin's lymphoma that arises within brain, eyes, leptomeninges, or spinal cord in the absence of extracerebral tumor manifestation and metastases. Currently PCNSL; accounts for 3.3 % of all brain tumors. Herein we report three PCNSL patients presenting with mass lesions of the central nervous system (CNS). We carried out a specifically CNS-targeted chemotherapy combination.

All three patients were male and the mean age was 30 (range, 17-48). In all the patients tumor was diagnosed as B-cell lymphoma according to the revised European-American classification of lymphoid neoplasms (REAL)/World Health Organization (WHO) classification of neoplastic diseases of the hematopoietic lymphoid tissues. The IDARAM regimen was developed as CNS targeted chemotherapy. It was found to be effective in PCNSL. It includes idarubicin 10 mg/m2 IV days 1 and 2; dexamethasone 100mg/m2 12 h. infusion days 1,2,3; cytosine arabinoside 1 gr/m2 1 h. infusion days 1 and 2; methotrexate 2 gr/m2 6 h. ınfusion, days 3. (with folinic acid rescue); and cytosine arabinoside 70 mgr plus 12 mgr intratechally days 1 and 8 and colony-stimulating factor. Chemotherapy cycles were repeated at 3 weeks intervals. We modified IDARAM regimens to R-IDARAM by adding following three changes: 1) Rituximab 375 mgr/m2 day 1, intravenously 2) dosage of MTX increased from 2 gr/m2 to 3 gr/m2, 3) two more courses of R-IDARAM after cranial RT. Following complete staging after course 2, radiotherapy was applied at a dosage of 3600-4140 cGy in conventional schedule (180 cGy or 200 cGy per day) to whole brain (with 3600 cGy to eyes in one case whose eyes were involved), and then 2 more courses of R-IDARAM (totally four courses) regimen were applied. Response to R-IDARAM regimen was evaluated with cranial MRI after completion of pre-RT chemotherapy, after RT and after the fourth course of R-IDARAM regimens. In 1st and 3th patients CR were achieved after first two cycle of R-IDARAM. We achieved CR after the fourth courses of R-IDARAM chemotherapy regimen in the 2nd patient.

Currently, there is no standart treatment modality for PCNSL. But it seems that HDMTX and cytosine arabinoside play a major role. In conclusion, we believe that increased dose of MTX, adding rituximab and consolidation of the IDARAM regimen to R-IDARAM may improve disease control and outcome of patients. Large multicenter clinical trails are necessary to investigate these issues in the future

BSH018

A RARE CAUSE OF PROXIMAL JEJUNUM OBSTRUCTION: PRIMARY JEJUNAL EXTRAMEDULLARY PLASMOCYTOMA

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A case of 35 years old woman with solitary extramedullary plasmocytoma of jejunum is presented. She had history of weight loss and intermittant upper abdominal pain. Abdominal and pelvic tomography revealed diffuse lobulated wall thickening of the proximal jejunum. The jejunal mass was well marginated, heterogenous (max diameter 4 cm) arising from the wall of the small intestine on the left upper abdominal quadrant. Histopathologic examination of surgical specimen showed diffuse infiltration of atypical plasma cells. Immunohistochemical examination revealed positive reaction with CD38 and monoclonality of plasma cell population with kappa light chain. A subsequent work up for multiple myeloma was performed. Bone marrow aspiration and biopsy showed 3 % of plasma cells. Immunoelectrophoresis and immunofixation did not show M protein in serum and urine.

The presenting clinical features of Jejunal EMP are similar to those of carcinoma, lymphoma, other solitary or multiple polipoid lesions and inflammatory stricture of the intestine. Diagnosis on clinical level alone is impossible and radiologic findings are not distinctive. Histopathologic examinatoins of biopsy specimen or surgical material are the earliest oppurtinity for diagnosis.

In conclusion Jejunal EMP is a rare differential diagnosis of an intraabdominal tumor. The need to consider unusual potentially treatable tumors that cause jejunal obstruction should be kept in mind. These patients should be evaluated further and followed-up for life with the repeated bone marrow aspiration and protein studies to detect the development of multiple myeloma.

BSH019

THE PROGNOSTIC SIGNIFICANCE OF MYELOID-ASSOCIATED ANTIGENS EXPRESSION ON BLAST CELLS IN CHILDHOOD ALL

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Assessing the prognostic significance of expression of myeloid-associated antigens on blast cells and their association with other classical prognostic factors of childhood acute lymphoblastic leukemia are aimed.

We retrospectively studied 96 cases newly diagnosed and treated with modified Berlin-Frankfurt-Muenster (BFM) 2000-based protocol between August 2000 and September 2005 in Bakirköy Maternity and Childhood Hospital-Hemathology Department. Complete immunophenotyping was performed in all cases with flow cytometer. Three myeloid cluster groups (CD13, CD14 and CD33) were analyzed. Criteria for MyAg expression (MyAg+ALL) included positivity of one or more MyAg on at least 20% of blasts. All children with ALL were classified to standard, intermediate and high risk groups according to Trall-BFM 2000 protocol. Presenting features (age, sex, white blood cell count, hemoglobin level, presence of hepatomegaly-splenomegaly and lymphadenomegaly, central nervous system involvement, mediastinal mass involvement, extramedullary involvement, FAB morphology, immunophenotype, translocation, risk groups, peripheral blast count at day 8) and treatment outcome (bone marrow blast rate at day 14 and 33, relapse and exitus status) of MyAg+ALL patients were compared with MyAg-ALL patients.

Eightysix patients were classified as B-cell precursor ALL, 1 patient as biphenotypic ALL and 9 patients as T-ALL. Expression of myeloid-associated antigens were demonstrated in 49 cases (51.1%) of childhood ALL. Patients without myeloid antigen expression were classified as group I. Fourtytwo of cases expressed only one MyAg (group II), while 7 cases expressed two or more (GroupIII). The 5-year event-free survival (EFS) for all patients was 79.9% with median follow-up of 45.58 months. EFS of group I, II and III was as follows: 81%, 77% and 68%. This was not statically significant between the three groups (p= 0.871). Our analyses showed that the difference between the groups with regard to age, sex, white blood cell count, hemoglobin level, presence of hepatomegaly- splenomegaly and lymphadenomegaly, central nervous system involvement, mediastinal mass involvement, extramedullary involvement, immunophenotype, translocation, risk groups, peripheral blast count at day 8, bone marrow blast rate at day 14 and 33, relapse and exitus status was not significant, except FAB morphology.

Myeloid antigen expression is fairly common, but it lacks prognostic value in childhood ALL.

BSH020

RADIOSYNOVECTOMY WITH YTRIUM-90 AND RHENIUM-186 IN HEMOPHILIC ARTHROPATHY

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Repeated intraarticular bleeding episodes in hemophilic patients result in chronic arthropathy that causes permanent disabling in joints. Our aim was to assess the efficacy of radiosynovectomy in hemophilic arthropathy.

Between January 2004 and May 2006 we performed radionuclide synovectomies on 14 knee joints (with Yttrium-90 silicate) and 2 ankle joints (with rhenium-186) of 16 patients with hemophilic arthropathy. All of the patients were male, with a mean age of 13,23,2 (range 9-21). Patients who fulfilled the following prerequisites were included for Y-90 synovectomy application: (1) more than four hemorrhagic episodes in six months (2) at least a Stage II hemophilic arthropathy according to the classification of Arnold and Hilgartner (3) persistent synovitis. Our goal was to prevent joint destruction by eliminating frequent joint bleeding before the onset of arthropathy. All patients were admitted to the hospital and treated with factor replacement so as to raise the factor level of the patient to 80% the following morning and 50% for three days thereafter. The effusion in the joint was evacuated before the injection of the radiocolloid. Radiographs, both pre- and post-synovectomy were reviewed in each patient. Each joint was coded according to the Petterson scale with a possible scale of 0-13 points.

The frequency of hemorrhage just before the injection ranged from two episode per month to six episodes per month. The mean number of hemorrhages was three per month. The mean number of hemorrhages became one episode per month in six months after treatment. There was no improvement or little change roentgenologically.

The most striking and significant difference was the marked decrease in hemorrhagic frequency in patients. Radiocolloids seem to be an effective method in the management of the hemophilic arthropathy.

BSH021

FACTOR X DEFICIENCY PRESENTING WITH RECURRENT INTRACRANIAL BLEEDING AND APCC PROPHYLAXIS

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Deficiency of Factor X is a rare bleeding disorder inherited autosomal recessively. Intracranial bleedings during the newborn and infancy periods were reported. Our patient had also recurrent intracarnial bleedings and he was diagnosed as Factor X deficiency. In this presentation we evaluated aPCC usage in our patient during the attacks and prophylaxis of the bleedings.

The patient is now 14 months of age. He was admitted to the neonatology unit at the first day of his life due to the bleeding disorder of the newborn.He was treated with vitamin K and fresh frozen plasma (FFP) and was discharged from the hospital.

At the 42th day he was once more admitted with severe anemia, convulsions and left hemiparesis. A large hematoma of the left frontal lobe and area of infarction were present at this cranial CT. The activity of Factor X was 1.75%. He was internailzed and followed first at the intensive care unit for 10 days. He was treated with FFP for 16 days. No regression was seen in the control CT. He was given aPCC for 7 days. After this regimen regression was seen.

At the 4 ½ month of age, another episode of intracranial bleeding occured. He was given FFP for 5 days and aPCC for 9 days. Prophylaxis with aPCC as twice a week dose was started at 6 months of age.

He was being followed up since 05/12/2005 and no new bleeding or thrombosis were documented.

BSH022

EVALUATION OF OUR CASES WITH GLANZMANN THROMBASTHENIA

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Glanzmann Thrombasthenia is a rare bleeding diathesis occuring due to the dysfunction or absence of glycoprotein IIb-IIIa present on the thrombocyte membrane. Severity and frequency of the bleeding episodes may change from patient to patient. In this report, clinical and laboratory features of 26 patients that were followed between 1990-2000 were evaluated.

They were 13 males (50%) and 13 females (50%). The age of onset was between 1 month and 7 years (mean 2.8 years). Familial consaguity was present in 14 (53.8%) of the patients. Bleeding time was prolonged and thrombocyte function tests were correlated with Glanzmann disease. Flow cytometric tests were done in 22 patients. Type 1 disease was present in 3 (13.6%), type 2 in 7 (31.8%), type 3 in 12 (54.5%) of the patients. The bleeding types seen in our patients were as follows; superficial skin bleedings (84.6%), epistaxis (69.2%), oral mucosal bleedings (76.9%), menorrhagia (23%), bleeding due to trauma (7.6%) and intracranial bleeding (3.8%). The mean annuel bleeding number was 48. The mean bleeding episode per year and per patient was 1.84/year.

Local therapies, antifibrinolytic agents, oral contraceptives and if severe bleeding is present thrombocyte suspensions were given. For three of our patients with 4 bleeding attacks rFVIIa was used. One of our patients died due to tuberculous pericarditis and dilated cardiomyopathy.

Follow up duration was between 1 and 16 years (mean 9.2 years). There was not any documanted alloimmunization. Twenty five of our patients are still alive and being followed up.

BSH023

FX DEFICIENCY: CHARACTERISTICS AND TREATMENT OUTCOME OF SEVEN CASES

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Congenital factor X deficiency is an autosomal recessively inherited rare disorder that is diagnosed 1 in 1000000. During the newborn period, bleeding episodes might occur in moderately or severely affected patients. Umblical cord bleedings, epistaxis, mucosal bleedings, menorrhagia, hemarthrosis and central nervous system bleedings were reported.

In this study, we are presenting the retrospective analysis of the 7 patients that were diagnosed as congenital factor X deficiency between 1990-2005. Five of the patients were males and 2 were females. The ages of the patients were changing from 2 months to 19 years. The activity of factor X was under 1% in 3 patients, between 1-5% in 2 patients and above 5% in 2 patients. When the bleeding episodes were evaluated; soft tissue bleeding was seen in 71,4%, epistaxis in 42,8%, intracranial bleeding in 42,8%, gingival and mucosal bleedings in 28,5%, intratricular bleeding in 28,5%, menorrhagia in 14,2%. In 2003, all the bleeding episodes were treated by tranexamic acid and fresh frozen plasma. After 2003, during the first bleeding episode rFVIIa and aPCC were used together and during the second bleeding episode aPCC were used alone.

In this presentation we are discussing the need for prophylaxis in patients having recurrent intracranial bleedings.

BSH024

KIMURA DISEASE

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Case Report. Swelling in the neck is present in the 68 year-old male patient. In his medical history, noninvasive in situ bladder carcinoma and local chemotherapy 10 years ago is present. The case who is in remission period fort the bladder Ca, has no additional diseases. 1 cm axillary and inguinal mobile lymphoid tissue is detected in the physical examination. 60% eosinophils were found in the peripheric blood smear. Serum IgE level was increased (1.82 IU/L). Other blood parameters were normal. In the neck, thorax and whole abdominal CT scan, there were 2-2.5 cm bilateral jugular and submandibular lymph nodes in the cervical area, 1.5-2 cm lymph nodes in both axillary areas in thorax and 2 right inguinal lymph nodes having 2 cm diameter in the abdomen. Excisional biopsy was performed from the lymph tissue in the cervical area.

A structure rich of histiocytes was determined in the areas of visible lymphoid tissue in which the lymphatic nodular structure was typically impaired. Extensive eosinophil infiltration was seen in all the areas and eosinophilic necrosis was detected in the infiltrated areas.

Discussion. Though Kimura disease has been known for 60 years, its etiology remains unclear. The disease is seen frequently in the far east countries in particular. Its prevalance increases in the second and third decades, and males are generally effected. Lymph node biopsy is required fort he diagnosis of the disease. Kimura disease can be defined as a granulomatous, autoimmune and eosinophilic disease which is accompanied by lymphadenopathy. Eosinophilia and increased serum IgE levels are among the most characteristic features of this disease.

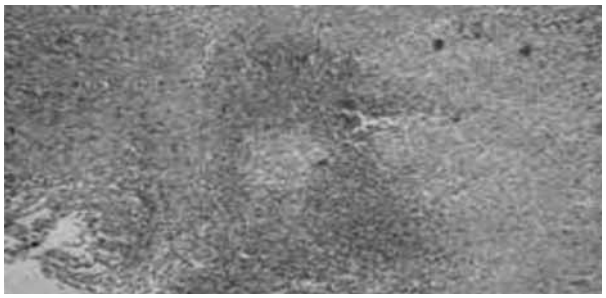

Figure 1. Eosinophilic abscess is seen in the middle. Histiocyte group is present in the middle of the abscess (HEx40).

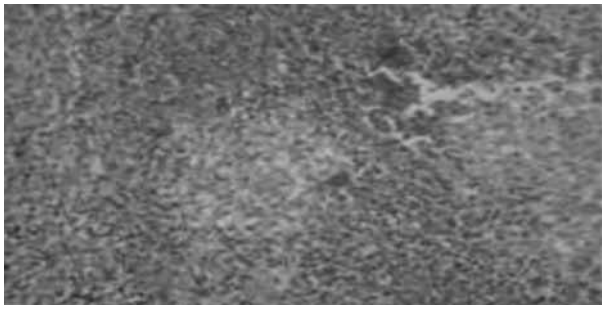

Figure 2. Close-up view of the eosinophilic abscess (HEx100).

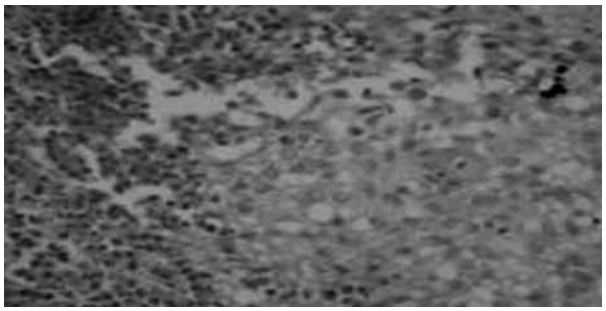

Figure 3. Histiocyte groups is seen on the right, and eosinophils on the left (HEx200).

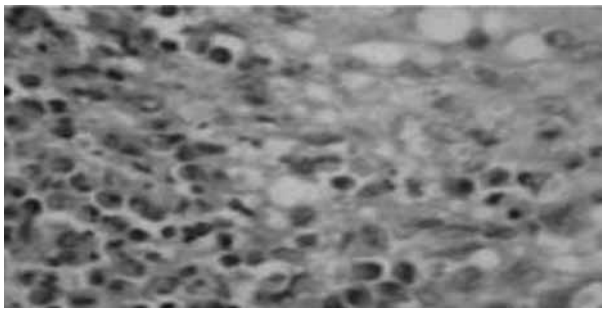

Figure 4. Close-up view of the eosinophilic infiltration (HEx400).

The germinal cores of the lymph follicles are pathologically infiltrated by the eosinophils. Fibrosis and eosinophilic microabscesses can sometimes be detected. Post-capillary venous proliferation is noted. It may be confused with eosinophilic angiolymphoid hyperplasia (ALHE) because of these pathologic features. Kimura disease and ALHE used to be defined as the same single disease in the past. However, Kimura disease was considered as a separate disease following the better understanding of its clinical and pathological characteristics. Unlike Kimula disease, ALHE appears commonly in females. Cutaneous rushes are more frequently observed in the course of ALHE. In ALHE, serum eosinophil and IgE levels are not elevated as much and frequently in Kimura disease, moreover, lymphadenopathy is observed more commonly in Kimura disease, compared to ALHE. In the pathologic distinguishing of these two diseases, vascular proliferation, atypia in the endothelium cells and vascularization are more significant in ALHE, compared to Kimura disease. Kimura disease can be confused with drug or hypersensitivity reactions because of its pathologic characteristics. Medical history of the patient, laboratory tests and radiologic measures can be helpful in distinguishing Kimura disease from drug or hypersensitivity reactions.

In the beginning of the disease, only lymphadenopathy is seen as well as the various clinical features of the disease, other pathological findings appear thereafter in time. Various treatment options are employed for the disease, while there is no definite treatment yet. These are corticosteroids, cyclosporine A, vincristine, radiotherapy, cryotherapy, surgical excision and laser. If steroids are considered for the treatment, the initial dose should be 60 mg/m2. If the patient is considered to be refractory to steroids, other treatment options should be selected. The patients are generally refractory to steroid therapy in Kimura disease when nephropathy overweighs. Vincristine therapy (1 mg/m^2) may be considered in these patients. In our case, apart from the classical neck and head lymphadenopathies in Kimura disease, we detected lymphadenopathies in the axillary and inguinal areas. Skin findings were lacking in our case, unlike the classical appearances. Steroid treatment was initiated (60 mg/m2). Regression was observed in the dimensions of lymph nodes in the follow-up of the patient. No relapse was seen in the one-year follow-up of the patient. Blood eosinophils of the patient decreased to 1-3% and decreases in the serum IgE levels were observed (1.0 IU/L).

BSH025

HYPOLIPEMIANT BESIDES ANTILEUKEMIC EFFECT OF IMATINIB MESYLATE (CASE REPORT)

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Introduction. The tyrosine kinase inhibitor imatinib mesylate (IM)(formerly STI571; now Gleevec in the United States and Glivec in Europe [Novartis]) specifically inhibits the ki-

nase activity of ABL, platelet-derived growth factor receptor beta (PDGFRβ), and c-KIT tyrosine kinases and has impressive clinical efficacy in chronic myeloid leukemia (CML) [1]. Besides anti-leukemic and anti-tumoral effects, IM prevented also the development of atherosclerotic lesions and diabetes-induced inflammatory cytokine overexpression. [2,3].

Case report. A 36 years old male patient with a history of acute myocardial infarction and double coronarian by-pass eight years ago was admitted for the first time on December 2005 complaining of wheigt loss, intermittent discomfort in upper left abdomenum, polydipsia.and polyuria. By the examination of the peripheral blood and of the bone marrow the diagnosis of chronic phase CML-Ph(+) has been established. Simultaneously, type II diabetes mellitus (serum glucose level 290mg/dL, urine glucose 250 mg/dL) and dyslipidemia (Table) were discovered. After a short course of Hydrea, associated with diet and oral antidiabetic therapy (Glicazid 60 mg/d), Glivec 400 mg/d has been started on April 2006. The hematologic remission and a concomitant reduction of the serum levels of cholesterol, and of low-density lipoproteins has been obtained (Table).

The dynamics of the peripheral blood counts and lipidic profile during Glivec therapy.

Comments and conclusions. The effects observed in this reported case and in other similar patients [4] indicates that the treatment with IM induces a favourable control not only on cell growth and replication but also on the homeostasis of lipoproteins and glucose. A possible mechanism of action could be the inhibition by IM of PDGFR-dependent phosphorylation the low-density lipoprotein (LDL)-receptor–related protein (LRP)[2]. IM appears to be a novel therapeutic option to retard the development of atherosclerosis, specifically in the context of diabetes. The drug appears superior to other hypolipemiant treatments and could be the treatment of choice for patients suffering of CML and dyslipidemia.

Parameters	*Date of control (month)*			
	April	*May*	*June*	*Jully*
Hemoglobin (g/dl)	9,6	11,1	12	12
Leukocyte count (x10^9/l)	5.2	4.6	7.5	7.8
Platelet count (x10^9/l)	168	287	210	215
Serum total cholesterol (mg/dl)	310	189	157	152
Serum LD lipoproteins (mg/dl)	219	128	85	87
Serum HD lipoproteins(mg/dL)	49	53	52	50

BSH026

GORHAM-STOUT SYNDROME; A RARE CASE

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A 7 year old girl was referred to our pediatric oncology unit from another hospital with the probable diagnosis of primary bone tumor or bone metastasis of lung tumor.

From her history and reports it was learned that she had complaints of cough and back pain. She had been coughing for a month. On her chest X- ray, pneumonia was detected and she had been treated with ceftriaxone therapy. Her radiographic findings had not regressed so meropenem had been added. But, as the same findings were preasent, an ultrasound had been performed and a chest tube had been placed. A hemochylous exudate had been obtained and incidentally a bony destruction had been seen over her left humerus. Her bone scans had shown increased activity of both humerus and thoracal vertebral bodies. She had been evaluated for tuberculosis but no correlation had been found so was discharged and sent to our hospital. Her C-reactive protein was mildly positive and erythrocyte sedimentation rate was 22 mm/hr. She did not have leukocytosis. She was afebrile. Bone marrow biopsy was performed and it was normal. A malignancy was not a very possible diagnosis. She was thought to have lymphangiomatosis, hemangiomatosis or Gorham-Stout syndrome with the present findings of chylous exudate, bony destruction, near normal acute phase response. She was unresponsive to antibiotic therapy. A chest tube was again placed but the chylous material still was coming, she was consultated with thoracic surgeons, and they decided to make thoracotomy and perform open biopsy from lung, enlarged thymus, pleura and costa. The biopsy was examined by the pathologists and the diagnosis was Gorham-Stout syndrome with capillary and lymphatic proliferation and their bony infiltrations to the surrounding bones. We reviewed all the literatures for the therapy but we had seen that there was only very few cases with few treatment modalities, like radiotherapy, steroid therapy, interferon therapy and watchfull follow-up. As the age of the patient is very young we did not think of radiotherapy. We started steroid therapy, contineud for 2.5 months but she did not respond. So interferon treatment was discussed but due to some insurance problems and the possible complications the therapy could not be started. So now she is being followed up with middle chain fatty acid diet, without any therapy. Her effusion increased but the bony lessions began to regress. She feels well and does not have any respiratory problems.

This case is presented due to the rarity of the disease and also for pointing out the disease in the differential diagnosis of bone infiltrating tumors and primary bone tumors.

BSH027

GROWTH, DEVELOPMENT AND PUBERTAL STATUS OF THE PATIENTS WITH THALASSEMIA MAJOR

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Evaluation of patients diagnosed as thalassemia major for their growth and development, puberty status and growth hormone deficiency is aimed.

The reports of 53 patients (27 female, 26 male) were analysed retrospectively. Their ages ranged between 25 and 226 months (mean 129.4 month). Blood transfusions were done with 3-4 week intervals. Ferritin levels of the last 6 month were obtained. Anthropometric measurements of the patients were taken and the height of the parents were measured. Standard deviations were calculated. Standard deviations of the parents were accepted as the target height standard deviations of the patients.

Their puberty status were also evaluated according to Tanner-Whitehouse system. The bone ages of all the patients were also analysed. Blood chemistry and hormone levels were studied 10 days after blood transfusions. Standard deviations of all the parameters were calculated. In 35% of the patients, heights were below – 2SD. As the ages increase, this percentage also increases. There was no correlation between ages,heights, SDs, ferritin levels, bone ages and the frequency of the transfusions. In 5.2% of the patients growth hormone levels were under normal levels after growth hormone stimulation test. This points out the limited role of growth hormone deficiency in the development of growth retardation. Pubertal delay was found in 9.4% of the patients. It was assumed to be due to hypophyseal deficiency.

Cranial hemosiderosis was detected in the cranial MRI of 3 patients with growth hormone deficiency, 3 patients with pubertal delay and 1 with primary amenorrhea. This implicates the importance of imaging techniques in patients with endocrinological abnormalities.

With a better follow-up and early optimum therapy, it is probable to prevent the possible endocrinological complications.

BSH028

A DRAMATIC RESPONSE TO RITUXIMAB IN A PATIENT WITH RESISTANT THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP) WHO DEVELOPED ACUTE STROKE

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Refractory condition can occur in 10%-30% of all cases of thrombotic thrombocytopenic purpura despite increased frequency of total plasma exchange. The usefulness of immunomodulatory agents is unreliable in refractory disease. We report the case of a woman with refractory thrombotic thrombocytopenic purpura who developed a near-comatose state and right hemiplegia while under plasma exchange, corticosteroid, and vincristine therapy. After initiation of rituximab (375 mg/m2 weekly for 4 weeks), a dramatic response occurred and the patient's neurologic function recovered fully within days. Sustained remission was achieved, and the patient was well 1 year after her admission, while she was on azathioprine treatment.

BSH029

A SINGLE INSTITUTION (NCHT SOFIA, BULGARIA) EXPERIENCE WITH AUTOLOGOUS STEM CELL TRANSPLANTATION FOR THE TREATMENT OF HODGKIN'S AND NON HODGKIN'S LYMPHOMA.

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Background: Conventional chemotherapy can cure patients with Hodgkin's (HD) and non-Hodgkin's lymphoma (NHL), but those who relapse or are refractory have a poor prognosis. High-dose chemotherapy (HDCT) with autologous stem cell transplantation (ASCT) can improve the outcome of these patients. Here we evaluate and present our first results of high-dose chemotherapy and autologous stem-cell transplantation (HDC/ASCT) in patients with malignant lymphomas.

Purpose: To analyze clinical outcome and some prognostic factors for overall survival (OS) and disease free survival (DFS) in a group of 31 patients with Hodgkin's and non-Hodgkin's lymphoma treated between June 2004 and September 2006..

Patients and methods: Thirty-one patients with Hodgkin's (n = 22) and non-Hodgkin's (n= 9); male to female ratio 14/17; median age 33yrs (22-55) patients were enrolled. Patients with histologically proven with primary progressive or relapsed aggressive NHL or relapsed or refractory HD,age 18-65 years,were eligibility for the study. All patients received salvage therapy prior to transplant (DHAP or ICE) and mobilization with chemotherapy + GSCF. Conditioning regimen consisted of BEAM (carmustine, etoposide, cytarabine and melphalan) in 18 patients, and LACE in (carmustine, etoposide, cytarabine and cyclophosphamide) in 13. Toxicity was tolerable.

Results: After HDC/ASCT, 30/31 patients were evaluable for response. Only 1 patient was not assessed for response because of early death due to infection (day + 25). The 100 day mortality rate was 3.2%.Thirteen patients achieved a complete remission (CR), 7 were in stable disease (SD) and 10 had progression of the disease (PD), 7 from them died.

Conclusions: Our results show that HDC/ASCT is acceptable to treat patients with HD or NHL who do not obtain CR to front-line chemotherapy or with relapse. Disease status before ASCT is the most important prognostic factor for final outcome; thus, transplantation should be considered in early stages of the diseases. However, there are many unanswered questions as to the role of high-dose therapy in certain subtypes of lymphoma, the timing of transplant, and even the type of transplant to perform. An attempt will be made to clarify many of these unanswered questions. The utilization of high-dose therapy for non-Hodgkin's lymphoma is recommended for most patients who have relapsed after initial therapy. Resistant patients are not good candidates for HDT and they should be offered newer strategies.

BSH030

LEMIERRE SYNDROME VARIANT CAUSED BY SOIL-BASED STAPHYLOCOCCUS AUREUS INFECTION

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Lemierre syndrome is a rare and potentially fatal entity characterized by septic emboli from thrombosis of the internal jugular vein after oropharyngeal infection. The etiologic agent is not always an anaerobic bacterium. We report a patient with a Lemierre syndrome variant who presented with thrombosis of both the right internal jugular vein and the splenic vein as well as septic pulmonary emboli caused by Staphylococcus aureus, which proved resistant to methicillin, amoxicillin, and ciprofloxacin. The patient was thought to have acquired the infection during the exploration of a river cave in Turkey 10 days before his admission to the hospital. Such caves are natural reservoirs of infectious microorganisms. After having undergone a 6-week course of antimicrobial treatment and anticoagulant therapy, the patient recovered from the infection with no residual complications, and the signs and symptoms of Lemierre syndrome resolved.

BSH031

PLASMA EXCHANGE IN CRITICALLY ILL PATIENTS WITH SICKLE CELL DISEASE

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The therapeutic value of plasma exchange in treating potentially life-threatening complications in patients with sickle cell disease who do not respond to automatic red cell exchange is not well known. Plasma exchange with fresh-frozen plasma as replacement fluid was performed in seven selected critically ill patients with sickle cell disease unresponsive to red blood cell exchange.The aim of this retrospective study was to identify the clinical course of sickle cell disease in patients who underwent plasma exchange. During10 hospitalizations from 2004 to 2006, seven patients with sickle cell disease were eligible for plasma exchange. The median age of those patients was 20 years (range, 9-50 years). The presenting symptoms included dyspnea in 70% of the patients, progressive jaundice (100%), pleuritic chest pain (10%), painful crisis (40%), palpitations (100%), emotional instability and/or somnolence (60%), and fever (40%). The median duration of symptoms before plasma exchange procedures was 4 days (range, 1-9 days). The median time interval between automatic red cell exchange and plasma exchange was 3 days (range, 1-9 days). Infections, incompatibility with blood transfusions, pregnancy, hepatic necrosis, and hip surgery were the predisposing factors for poor clinical condition. During each hospitalization, multiple organ dysfunction occurred. Only one plasma exchange procedure was performed during each hospitalizatiton, and one plasma volume per procedure was exchanged. All procedures except for 1 resulted in the stabilization of the patient's clinical condition, and all stabilized patient were discharged from the hospital after 1 to 12 days.

Plasma exchange may help in the treatment of severe illness in patients with sickle cell disease in whom automatic red cell exchange therapy has failed.

BSH032

MISDIAGNOSIS OF BONE METASTASIS IN A SICKLE CELL PATIENT WITH BREAST CANCER

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Cancer antigen (CA) 15-3, which is associated with breast carcinoma, is an epithelial mucin and a product of the MUC1 gene. Recent evidences show that MUC1 can also be expressed in the haematopoietic lineages. Sickle cell disease is a particular inflammatory disorder characterized by chronic hemolysis, endothelial activation and vaso-occlusion. It was suggested that the serum concentration of CA 15-3 should thus be considered when malignant disease is suspected in patients with sickle cell disease.

In this report, we describe a sickle cell patient with breast cancer whose severe bone pain was mimicking bone metastasis.

The patient was a 45 year-old female who was diagnosed with infiltrative ductal carcinoma of the breast four years before admission. She received combination chemotherapy of CMF. She went into complet remission. Then she began to use tamoxifen regularly. One year before admission, the patient complained of severe hip and back pain that referred to the rib regions. The pain was difficulty managed with simple analgesic. This required narcotic drugs. Elevated CA 15-3 levels above 1000 IU/L was found in the patient's serum. An MRI and CT scan were performed in the region of interest. This showed evidence of metastatic foci of breast cancer. Based on that diagnosis, treatment with local radiation therapy was commenced ten months before admission. However, the severity of the pain was unchanged. Aspirated bone marrow smears and biopsy material revealed erythroid hyperplasia. There were no metastatic cells. For this reason an automatic erythrocyte exchange was applied, and the pain resolved rapidly with automatic red cell exchange. Serum CA 15-3 level decreased from 1199 IU/L to 824 IU/L.

Increased serum CA 15-3 has been reported among patients with homozygous β-thalassaemia, sickle cell-thalassemia and homozygous sickle cell anemia. We would like to call attention to an uncommon abnormalities related with bone and tumor antigen mimicking cancer metastasis that seems to be present in sickle cell anemia with cancer.

BSH033

SOCIOCULTURAL FACTORS LINKED WITH VOLCANIC SOIL PICA IN CHILDREN AND ADOLESCENTS FROM CAPPADOCIA, TURKEY

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The primary objective of the present cross-sectional study was to broaden the scope relate to the disordered eating habit in a group of children and adolescents living in Cappadocia. A 50-item parental bonding instrument, supplemented with a structured interview, was conducted with 171 participants with pica and 145 control individuals, from September 1992 to April 2005. In addition, the rates of anemia, iron and zinc deficient participants that may be associated with volcanic soil pica are investigated. The response rate was 100%. Volcanic soil was the most common non-nutritive material consumed in the pica group. A higher proportion of the pica group was observed to have aggressive fathers, uncaring mothers, and child abuse. The rate of zinc deficient participants in the pica group was significantly lower than that in the control group. This Cappadocia study concludes that the pica group was subjected to an obvious increase in the number of individuals consuming volcanic soil. It seems poor family relationships have a negative impact upon the soil pica. The frequencies of anemia, iron and zinc deficient participants in the pica group were below expected, indicating that this factor may need to pay more attention in future investigations.

BSH034

COCAINE INDUCED PLATELET FUNCTION DEFECTS: CASE REPORT

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Background: Cocaine use has increased in recent years. Nasal insufflation of cocaine injures the nasal mucosa, causes nasal bleeding and perforation of the septum. The underlying mechanisms leading to these complications have not beeen well defined. Cocaine induced vasoconstriction resulting in ischemia and certain coagulation disorders, especially related to platelet dysfunction may be responsible for these complications.

Case report: A 22 year-old man was admitted to hospital because of recurrent epistaxis. No family or previous medical history of bleeding diathesis was evident. He did not have hypertension and liver functional tests were normal. The patient declared that he was on cocaine during the last 2 years. During physical examination, no nasal mucosal injury and perforation of the septum were observed. The laboratory evaluation revealed the following results; Hb:14.2 g/dL (13.5-17.5 g/dL), Platelet count 154x103/mm3 (130-400x103/mm3), PT 13.7 s. (11-15 s.), aPTT 32 s. (25-40 s.), Bleeding time 4 min. (7 min.), Coagulation time 9 min. (5-15 min.), Fibrinogen 2.89 g/L (2-4.5 g/L), Ristosetin cofactor (VWF activation) 61.3 (50-150%), VWF antigen 110 (60-150%), FVIII 133 (50-150%) and FXIII normal. The platelet function tests evaluated the following abnormalities; ADP:32.3 (83-100%), Collagen: 7.4 (80-100%), Ristosetin: 85.7 (70-100%), Epinephrine: 23(26-59%).

These results support the hypothesis that cocaine inhibits human platelet aggregation, induced by the platelet agonists ADP and collagen. It was also revealed that a transfusion of one bag plasmaphresied platelet suspansion could lead to positive clinical response regarding the underlying platelet function defects.

Discussion:Although cocaine induced platelet functional defects has beeen documented in vitro; platelet aggregation abnormalities in cocaine abusers have not been reported in the literature yet.

BSH035

DEVELOPMENT OF MULTIPLE MYELOMA IN THE COURSE OF CHRONIC LYMPHOCYTIC LEUKEMIA

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B-cell chronic lymphocytic leukemia (CLL) and multiple myeloma (MM) are chronic B cell malignancies that origin from different phases of B cell development. These two diseases have many parallel and different characters. They may be rarely seen together. Clonal relation between MM and CLL have not been clarified yet. We would like to share our experience on a case with CD5 (+) B-cell CLL who developed MM after three years.

Case report: A 64 year-old male diagnosed with phase 0 CLL was being followed up without any chemotherapeutic agent and the complete blood counts were stabilized. Bone marrow aspiration and biopsy were performed after the development of hyperglobulinemia, increased BUN and sedimentation rate values. Bone marrow aspiration revealed an increase of 30-40% atypical plasma cell count and 30-35% lymphocyte count. Bone marrow biopsy was consistent with CLL/SLL interstitial small lymphocyte infiltration and lambda coloured immunohistochemical monoclonal plasma cell infiltration. Del 13q was determined in 34% of cells with FISH (Flourescence in situ hybridisation) analysis. In serum immunoelectrophoresis IgG lambda monoclonal gammopathy was observed. The excess of BUN, lytic lesions on head radiographies and osteoporosis of thoracic 9-10 vertebra were accepted as treatment indications. Treatment agents were methyl prednysolon and melphalan that effect both of the two diseases. Although no alkylating agent was used, additional devolopment of MM clone to CLL is remarkable. Scan of literature showed that this situation was seen rarely. We aimed to present this case to understand the molecular basis of B cell oncogenesis.

BSH036

TREATMENT OF SINUSOIDAL OBSTRUCTION SYNDROME WITH DEFIBROTIDE: A SINGLE CENTER EXPERIENCE

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Sinusoidal obstruction syndrome (SOS) formerly known as venoocclusive disease of the liver (VOD) is a frequent, troubling and a potentially fatal complication of hematopoietic stem cell transplantation. Defibrotide (DF) is a single stranded polydeoxyribonucleotide, obtained from controled depolymerisation of porcine intestinal mucosal cells with an anti-thrombotic, anti-ischemic, anti-inflammatory and thrombolytic properties without significant side effects. Despite promising results with defibrotide (DF), a standart accepted treatment of SOS has not been established yet. We retrospectively evaluated the charts of 94 consequtive patients, with 101 hematopoietic stem cell transplants for hematologic malignancies. The incidence of SOS was 14,7 % (15 of 102 transplants); 10 patients (one with haploidentical transplant) with myeloablative allogeneic SCT, 4 patients with reduced intensity allogeneic SCT and one patient with autologous SCT. The results of DF treatment in 15 patients with SOS are presented in this study.

Fifteen patients, 9 male and 6 female (median 41 years, range 16-46 years) were diagnosed to have SOS. Clinical diagnosis of SOS was based on Seattle criteria which is; jaundice (bilirubin ≥ 2 mg/dl) with two or more of the following: hepatomegaly, right upper quadrant pain, ≥ 5% weight gain from admission, and ascites. Diagnosis of severe SOS was based on the presence of multiorgan failure in addition to SOS, which was defined as either an oxygen requirement with an oxygen saturation of < 90% on room air and/or ventilator dependence, and/or renal dysfunction (doubling of baseline creatinine and/or dialysis dependence) and/or encephalopathy. Disease severity was classified as severe in 7 (46,6 %), moderate in 4 (26,6 %) and mild in 4 (26,6 %) patients. We treated 15 patients with DF for SOS for a median of 8 days (range 4 – 39 days). DF was administered intavenously in normal saline in 4 divided doses over 1 hour with a total dose of 10 mg/kg/per day. For non-responding patients daily dose was increased to 20 mg/kg per day. All 15 patients received DF as soon as possible after the diagnosis of SOS mostly on the day of diagnosis. Among 15 patients treated with DF, 4/7 patients (57,14 %) with severe SOS and all of the patients with mild to moderate SOS responded to treatment with complete resolution of SOS related signs and symptoms. Ultrafiltration was performed in 7 patients for volume overload and 3 patients required mechanic ventilation. All patients responding to DF were alive on day +100 post transplantation. There was no significant drug related side effects in SOS patients treated with DF.

SOS is one of the major regimen-related toxicities in HSCT patients. Disease risk is high in patients with hepatitis C, non-alcoholic steatohepatitis, systemic bacterial or viral infection before the start of cytoreductive therapy, previous radiation therapy that involved the liver, chemotherapeutic agents such as cyclophosphamide and TBI. While mild and moderate cases have a self limited course even without treatment; severe SOS cases are associated with very high mortality rates inspite of aggressive therapeutic approaches. Identifying high risk patients for the development of severe SOS is very critical for the early initiation of treatment. Heparin plus tissue plasminogen activator (rh-tPA) have been used in the treatment od SOS patients with a response rate of 23 – 29 %. However with rh-tPA clinically significant bleeding has been reported in 35 % of cases with a fatality rate of 3 %. Our promising result with 57.14 % complete response rate in severe SOS cases suggests that DF is the best available agent with minimal side effects in treating SOS. Patients with high risk of developing SOS prior to transplantation should be identified and considered for prophylactic treatment.

BSH037

A SINGLE CENTER EXPERIENCE OF THE FIRST 100 HSCT :GAZI UNIVERSITY FACULTY OF MEDICINE STEM CELL TRANSPLANTATION UNIT

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Hematopoietic stem cell transplantation (HSCT) is a curative treatment approache in hematologic malignacies. Here we report the results of our first 100 transplants between September 2003 and July 2006, in a cohort of 93 patients with hematologic malignancies.

Fourty-five autologous HSCT was performed for 44 patients (24 male, 20 female) with a median age of 45 (range 16-71 years). The diagnosis of patients were multiple myeloma (MM) in 30 patients (68,18 %), Hodgkin's disease in 7 patients (15,9 %), Non-Hodgkin lymphoma (NHL) in 4 patients (9,09 %), ALL in 1 patient (2,27 %), POEMS syndrome in 1 patient (2,27 %) and primitive neuroendocrine tumour (PNET) in 1 patient (2,27 %). One patient with MM was retransplanted for relapse 1 year after the first transplant. Conditioning regimen was melphalan for patients with MM and BEAM for lymphoma. Main causes of death were relapse in 4 patients (8,8 %), febrile neutropenia in 2 patients (4.4 %) and secondary malignancy in 1 patient (2,2 %). Treatment related mortality within 100 days posttransplantation was observed in 2 patients (4,4 %). Median number of CD34+ cells infused were 7.86 x 106/kg (range 2.1-17,04). Stem cell source was peripheral blood in all patients. Median days to neutrophil and platelet engrafment were 14 days (range 8-21 days) and 11,5 days (range 9-30 days) respectively. With a median follow-up of 511.5 days (range 9-1014) 84,4 % of patients are still alive.

Additional non-myeloablative HSCT from HLA identical sibling donor was performed in 5 patients [2 male and 3 female with a median age of 28.5 years (range 15-42)] after autologous SCT; 2 MM (40 %), 2 NHL (40 %) and 1 HD (20 %). Conditioning regimen for allogeneic HSCT was fludarabine/TBI in 4 patients (2 MM and 2 lymphoma) patients and busulfan/fludarabine/ Cy in 1 lymphoma patient. Graft versus host disease (GVHD) prophlaxis was cyclosporine (CsA) and methoteraxate on day 1, 3, 6 and 11. The causes of first 100 day mortality were disease progression in 1 patient with NHL and pulmonary

toxicity in addition to infection in a heavily pretreated patient with NHL. Median number of CD34+ cells infused were 3,84 x 106/kg (range 2.2-6,17). Stem cell source was peripheral blood in all patients. With a median follow-up of 272.5 days (range 31-514) 60 % of patients are still alive. One patient with MM relapsed 1 year after the allogeneic SCT and partially responded to Thal/Dex treatment. Other patient with MM developed acute hepatic GVHD and during the 3rd month CNS involvement with N. Hypoglossus paralysis which partially responded to bortezomib/Cy/Dex and radiationtherapy. One patient with HD is still alive with partial response and receiving involved field radiationtherapy. Acute GVHD developed in 2 patients; one grade II GIS GVHD and the other with grade 1 hepatic GVHD. Both patients responded to prednisolone.

Fifty allogeneic HSCT was performed in 44 patients with a median age of 32,5 years (range 16-53 years) (29 male, 15 female). The diagnosis of patients were AML in 18 patients (40,90 %), ALL in 10 patients (22.72 %), severe aplastic anemia (SAA) in 8 patients (18,18 %), CML in 4 patients (9,09 %), and HD in 4 patients (9,09 %). Six retransplants were performed; for graft rejection in 2 patients with SAA and for engraftment failure in a patient with AML and finally for relapsed disase in 2 patients with acute leukemia. Two patients with relapsed/refractory acute leukemia receieved haploidentical transplants and both died of transplant related toxicities. Stem cell source was peripheral blood in all patients except 3 patients with bone marrow harvest. GVHD prophlaxis was CsA and methoteraxate on day 1, 3, 6 and 11. Conditioning regimens for leukemia patients were Bu/Cy in 15 patients (30 %), intravenous Bu/Cy in 10 patient (20 %) and TBI/Cy in 6 patients (12 %). Cy and/or ATG was the conditioning regimen for patients with SAA. Bu/Cy/Fludarabine was used as conditioning regimen for 1 patient with AML with cardiac dysfuction and 4 patients with lymphoma. Median number of CD34+ cells infused were 4,63 x 106/kg (range 1.07-7.7). Median days to neutrophil and platelet engrafment were 17 days (range 13-30 days) and 17 days (range 8-54 days) respectively. Main causes of death were infection in 5 patients (10 %), sinusoidal obstruction syndrome in 3 patients (6 %), acute GVHD in 2 patients (4%) and serum sickness associated with ATG in 1 patient (2 %). The total incidence of transplant related mortality (TRM) on day 100 was 20 %. Mortality due to relapse was detected in 13 patients (26 %). At the time of transplantation 6 heavily pretreated patients with leukemia were not in remission and 5 of these patients died within 100 day posttransplant. Acute GVHD was detected in a total of 7 patients (15,9 %) and chronic GVHD in 9 patients (20,45 %). We achieved acceptable TRM in a high risk group of patients.

BSH038

GEMCITABINE AND VINORELBINE AS A SALVAGE REGIMEN PRIOR TO HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN RELAPSE/ REFRACTORY HODGKIN'S DISEASE: PRELIMINARY REPORT

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Although the majority of patients with Hodgkin's disease (HD) are cured by first line treatment 20-30 % of patients require salvage treatments because of refractory or relaps disease. Salvage chemotherapy followed by autologous stem cell transplantation is the treatment of choice for refractory or relaps patients. In this study we evaluated the role of gemcitabine and vinorelbine as a salvage regimen in the treatment of refractory or relaps HD patients prior to hematopoietic stem cell transplantation.

Between February 2005-August 2006 10 previously treated refractory or relaps HD patients (3 female, 7 male with a median age of 39.5, range 18-56 years) were enrolled. Confirmed histologic diagnosis was nodular sclerosis in 7 patients, mixed cellularity in 2 and lymphocyte depleted in 1 patient. The clinical features of the group were generally unfavorable: 8 patients were heavily pretreated and in 2 of these patients relapse occured after autologous hematopoietic stem cell transplantation (AHSCT). Three of these patients were primary refractory. Patients received Gemcitabine 1000 mg/m2/day and Vinorelbin 30 mg/m2/day on days 1.,8., and 15. of a 28-day schedule for a total of 6 cycles. All cycles were delivered in an outpatient setting. In 3 patients this chemotherapy regimen was also used for peripheral blood stem cell mobilization at the end of cycle 6 and a median CD34+ cell count of 7.03x106 (range 4.4-11.6) was achieved. At the end of 6 cycles of chemotherapy 3 patients were refractory (% 30), 3 patients achieved complete response (%30) and 4 achieved partial response (% 40). Three patients (30 %) required hospitalization for neutropenic fever. Treatment related toxicity was NCI grade III-IV neutropenia in 5 patients (50 %), grade II febril neutropenia in 4 patients (% 40) and grade III hepatotoxicity in 2 patients (% 20). No treatment related death was reported. Supportive therapy with G-CSF was used for patients with grade III-IV neutropenia. Two patients with relapse after AHSCT and 2 patients with poor mobilization received non-myeloablative allogeneic HSCT from HLA matched sibling donore. One patient with poor prognostic criteria received additional allogeneic HSCT after AHSCT from a HLA matched sibling donore. Other 5 patients received AHSCT after conditioning treatment with BEAM. Neutrophil and platelet engrafment was reported on median day 12,5 (range 9-26) and 15.5 (range 11-54) respectively. Transplantation related mortality was reported in 1 patient (10%) with refractory disease after gemcitabine/vinorelbine because of hepatic sinusoidal obstruction syndrome. Disease status was assessed in 9 patients on day 30 after transplantation; 5 patients with complete response (% 55,5), 3 patients with partial response (% 33,3), and 1 patient with refractory disease (% 11.1). One patient with refractory disease and 1 patient with partial response after gemcitabine/vinorelbine obtained complete response after AHSCT. Patients with rezidual disease after transplantation achieved involved field radiationtherapy.

In this study with heavily pretreated HD patients we obtained complete response in 3 patients(30%) with an overall response rate of 70 % after gemcitabine/vinorelbine salvage treatment. Even with the limitation of the small size of the patient cohort and short follow-up our results in heavily pretreated HD patients with gemcitabine/vinorelbine seems as an effective alternative salvage regimen.

BSH039

MAINTENANCE TREATMENT WITH THALIDOMIDE AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA: PRELIMINARY REPORT

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Despite therapeutic advances, multiple myeloma (MM) remains an incurable disease. Although early administration of high dose chemotherapy with autologous hematopoietic stem cell transplantation (AHSCT) improves survival most patients develop recurrent disease after undergoing transplantation. The aim of this retrospective analysis is to evaluate the effect of posttransplantation low dose thalidomide maintenance.

Between September 2003 and June 2006, 30 patients with MM (17 female, 13 male; median age 50 range 33-66) received AHSCT after conditioning with melphalan 200 mg/m2/day except one with chronic renal failure who received melphalan 140 mg/m2/day. After transplantation 16 patients received thalidomide 100 mg per day for maintenance. Thalidomide was started at day 100 posttransplantation. Disease status prior to transplantation was complete response in 8 (26.6 %) patients, very good partial response in 1 (3.3 %), partial response in 16 patients (53.3 %), minimal response in 1 patients (3.3 %) and nonresponders in 4 (13.3 %) patients. During transplantation 1 patient died because of fungal infection. In 9 patients disease status improved after transplantation; 8 patients achieved complete response (26.6 %) and 1 patient achieved partial response (3.3 %). After a median of 19 months follow up (range 2-36 months) 3 patients (10 %) died; 1 patient with secondary gastrointestinal system malignancy, 2 patients with progressive primary disease in which one with secondary malignancy and one with progressive disease were on thalidomide maintenance. Median follow-up of thalidomide maintenance was 11.5 months (range 1-20 months). Under thalidomide maintenance 6 patients (37.5 %) preserved their best disease status achieved after transplantation. However 9 patients (56.25 %) progressed. Median progress free survival under thalidomide maintenance was 23.5 months (range 3-29 months). Only one patient with minimal response improved his disease status to partial response under thalidomide maintenance.

Although the results of AHSCT are superior to standart dose chemotherapy, transplantation is not a curative strategy in patients with MM. Effective maintenance treatments are required to prolong the duration of response. In recent reports posttransplantation thalidomide seemed to improve the survival of patients. In our small number of patients we could not achieve an additional benefit of low dose thalidomide in improving best disease status after transplantation. Low dose thalidomide maintenance does not seem to further improve the results of autologous HSCT. Larger patient cohorts are required to conclude on the benefit of low dose thalidomide in the posttransplantation maintenance.

BSH040

77 YEARS OLD PATIENT WITH MYELODYSPLASTIC SYNDROME / MYELOPROLIFERATIVE DISEASE UNCLASSIFIABLE AND TRISOMY 8

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Background : The myelodysplastic/ myeloproliferative diseases (MDS/MPD) are clonal myeloid disorders that possess both dysplastic and proliferative features but are not properly classified as either myelodysplastic syndromes or chronic myeloproliferative disorders. (CMPD). Myeloid disease that shows features of both MDS and CMPD but does not meet the criteria for chronic myelomonocytic leukemia, juvenile myelomonocytic leukemia and atypical chronic myeloid leukemia is designated as myelodysplastic/ myeloproliferative disease unclassifiable (MDS/ MPD-U).Diagnostic criteria and clinical characteristics of MDS/MPD-U are known. But no cytogenetic or molecular findings are available that are spesific for MDS/MPD-U. Case Report:

A 77 years old woman was admitted to our hospital with fatique, and abdominal distension. Her physical examination revealed tacipnea and hepatosplenomegaly. Laboratory features typically included anemia and dimorphic erythrocytes on the peripheral blood smear.Thrombocytopenia (Platelet count <50x109 /l) and leukocytosis (White blood cell count > 30.000 x 109) were present. The bone marrow smears were hypercelluler and exhibited proliferation all of myeloid lineages. Dysplastic features were present in all cell line. Cytogenetic and FİSH studies 12/36 metaphases showed a trisomy of Cr.8. No Philadelphia chromosome or BCR/ABL fusion gene, del 5 q, t (3.3) or inv (3).She received red cell packed transfusions for associated anemia, supportive measures and chemotherapy with hydroxyürea. Now the patient under observation. Discussion: The karyotype found (trisomy 8) is frequent in acute myeloid leukemia patients. But this cytogenetic finding is unusual for MDS/CMPD-U cases

BSH041

EFFECTIVENESS OF BORTEZOMIB IN PERICARDIAL INVOLVEMENT DUE TO MYELOMATOUS INFILTRATION

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Multiple myeloma is a malignant disorder clinically characterized by bone pain with lytic bone lesions, anemia, hypercalcemia, renal function impairment and the presence of extramedullary involvement.The soft tissue plasmacytomas have been reported in 15-20% at the time of diagnosis and in an additional 15 % during the course of disease.

Pericardial involvement, a rare complication of multiple myeloma, is caused by amyloidosis, bleeding abnormalities, infections and plasma cell infiltration. Optimal treatment for malignant involvement of pericardium in multiple myeloma has not been established.

In the recent years, several novel agents particularly thalidomide, bortezomib and lenalidomide, have been introduced in the treatment of multiple myeloma. There is a little

information on the effect on bortezomib on extramedullary myeloma. Here, we report a patient with multiple myeloma treated with bortezomib who had pericardial involvement under the treatment of thalidomide and dexamethasone (Thal/Dex).

A 66 year-old woman was diagnosed with Ig A-kappa myeloma stage IIIA. She was given 3 cycles of melphalan and prednisone chemotherapy. Because she did not respond to melphalan and prednisone treatment, combination chemotherapy with vincristine, doxorubicine and dexamethasone was initiated. She was also refractory to 3 cycles of this regimen and she did not accept high dose chemotherapy with autologous peripheral blood stem cell support. Because of this Thal/Dex regiment was started. On the fifth month of treatment, progressive dyspnea, orthopnea developed. There was no evidence of pulmonary embolism in the CT scan of the thorax. Echocardiogram showed a massive pericardial effusion and mild diastolic dysfunction. Pericardiocentesis with echocardiographic guidance was performed and 600 mL of hemorrhagic fluid was removed. The cytological and flow cytometric analysis of the pericardial fluid showed plasma cell infiltration. Pericardial fluid cultures were negative. Complete blood cell counts and serum immunoglobulin levels were normal. Serum immune electrophoresis revealed an Ig A kappa monoclonal gammopathy. Bone marrow examination showed infiltration of plasma cells less than 10%. At this time bortezomib was added to Thal/Dex therapy. Bortezomib was administered intravenously at an initial dose of 1.3 mg/m2 on days 1, 4, 8, and 11 every 3 week. After the 3rd cycle of bortezomib therapy, echocardiography was repeated. There was no evidence for progression. Nowadays, at the end of 6th cycle, the patient survives with mild pericardial effusion, and has no progression in multiple myeloma.

Bortezomib is an active antimyeloma agent which produces responses in about one-third of patients with resistant disease. We reported this patient because during the serologic response to thalidomide and dexamethasone therapy, pericardial involvement developed, and controlled by the addition of bortezomib.

BSH042

RISK FACTORS FOR CARDIAC TOXICITY IN STEM CELL TRANSPLANTATION

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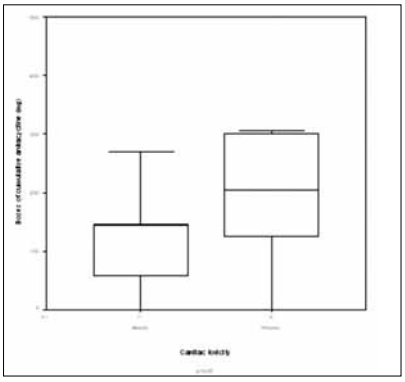
Background: Serious cardiac complications such as arrhythmia, cardiac failure and tamponade due to stem cell transplantation (SCT) conditioning regimen were reported with ratios of 1 to 26% in different series. Conditioning regimens including high-dose cyclophosphamide and/or total body irradiation (TBI) are the main reasons of cardiac toxicity (CT) in SCT. There are reports showing that, low ejection fraction before SCT and cumulative dose of anthracyclines are also effective. Aims: To determine the frequency of CT among the SCT patients in our department and the factors effective on CT retrospectively. Methods: Between September-2003 and May-2006, 83 SCT patients [38 females and 45 males, 16-71 (mean 36,8±14,2) years]were included the study respectively. There were 15 AML, 11 ALL, 27 MM, 10 SAA, 6 NHL, 10 HD, 4 CML and 37 autologous SCT, 27 allogeneic SCT were done to these patients. Age, cumulative dose of anthracyclines before SCT, mediastinal radiotherapy, transplantation type, conditioning regimen, EF values evaluated with transthorasic echocardiography and radionuclide ventriculography (MUGA) before and 1, 3, 6 and 12 months after SCT and Bearman CT evaluation were recorded. Results: EF evaluated with echocardiography was ≥ 50% for all patients (50-77, median %67), where as EF evaluated with MUGA was median 55 (36-74). CT was determined in 12 (14,4%) patients. There were grade I CT in 8 patients (%9,6), and grade II CT 4 patients (%4,8). Cumulative dose of anthracyclines before SCT were higher in the patients in whom CT occurred compared to the patient in whom CT was not occurred (196,2 and 129,9 mg) ($p<0,05$). CT was determined more frequent in the patients were applied 200mg and/or higher doses anthracyclines (17/6, %9,1 and 66/6, %35,3) ($p<0,01$) and mediastinal radiotherapy (9/4, %44,4 and 74/8, %10,8) ($p<0,01$). There was no difference between CT frequency according to conditioning regimen and transplantation type ($p>0,05$). EF values evaluated with echocardiography and MUGA before SCT were not different ($p>0.05$) between the CT and the other group. But 1, 3 and 6 months after transplantation, the % decreases of EF values with echocardiography of the patients in whom CT was determined, compared to the values before the transplantation were significantly lower ($p<0,01$, $p=0,01$ and $p<0,05$), but EF values evaluated with MUGA were not different ($p<0,05$). EF values evaluated with both techniques at 12 months after SCT were not different($p>0,05$). Conclusions: Since the EF values were ≥ 50% for all patients, there was no serious CT in our series. Cumulative dose of anthracyclines higher than 200mg and mediastinal radiotherapy may be risk factor for CT due to SCT also in the patients without cardiac comorbidity. Acceptable CT occurs with myeloablative conditioning regimens in the patients with EF≥50 before SCT. CT is reversible; MUGA has no additional benefits on estimating and follow-up of CT pre and posttransplantation period for the patient in whom serious EF decreases were not detected with echocardiography.



Cardiac toxicity and doses of cumulative anthracycline

BSH043

RISK FACTORS OF ORAL MUCOSITIS IN PATIENTS RECEIVING ALLOGENEIC STEM CELL TRANSPLANTATION

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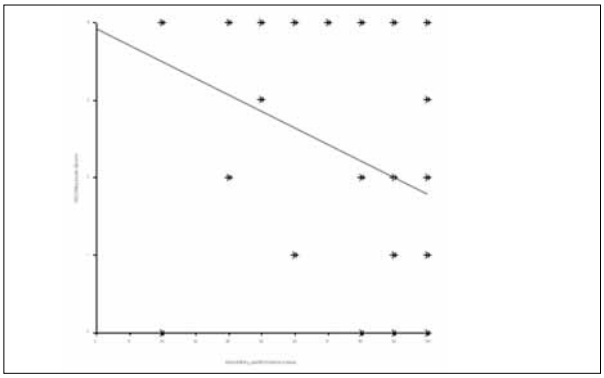
Background: Mucositis is one of the early complication increasing risk of infection and haemorrhage. Oral mucositis determined after allogeneic stem cell transplantation (ASCT) is associated with conditioning regimen and methotrexate. It is believed that, lower folate status due to MTHFR C677T polymorphism aggravated with MTX worsens mucositis. Aims: The aim of the present study is to determinate the oral mucositis frequency in the patients with ASCT, and to investigate the difference of MTHFR mutation frequency and the other probable factors according to serious mucositis existence or not. Methods: Fifty-nine patients with ASCT [21 females and 38 males with a mean age of 30,1±10,7 (16-63 years)]were included the study respectively and retrospectively between November 2003 and August 2006. There were 22 AML, 12 SAA, 11 ALL, 5 HD, 4 CML, 2 NHL, 2 MM, 1 MDS patients. Myeloablative ASCT was done to 53 patients and reduced intensity conditionig regimen was done for 6 patients. MTX (15mg/m2 1st day, 10mg/m2 3rd, 6th and 11th day) was used for GVHD prophylaxis in all patients. Chemotherapy protocols, Karnofsky performance status, MTHFR C677T mutation, ABO compatibility, sex match with donor and conditioning regimen was recorded. NCI toxicity scoring was used for determining the presence and severity of the mucositis. Results: There were Grade I or II mucositis in 18 cases and Grade III or IV mucositis (severe) in 26. MTHFR C677T polymorphism was evaluated for 53 patients and in 25 cases (42,4%) homozygote normal, in 24 (40,7%) heterozygote and in four (6,8%) homozygote mutation were detected. No significant difference was found about the mucositis frequency and severity between the patients with or without MTHFR C677T mutation, ABO compatibility and sex match (p>0,05). Severe mucositis was detected in males, in the patients who had two or more chemotherapy regimens before SCT and in the patients in whom busulfa n+cyclophosphamide or total body irradition was used for conditioning regimen more frequently(p<0,05). More slight mucositis was observed in the cases who had ASCT during first complete remission (p<0,05). Significant correlation in the correlation analysis was observed between Karnofsky performance status at the beginning of transplantation and NCI mucositis scores in the patients with severe mucositis (p<0,05, r=-0,320). Logistics regression analysis showed that sex (p<0,05), performance status at the beginning of transplantation (p<0,05) and two or more chemotherapy regimen application (p<0,01) are independent risk factors for severe mucositis. Conclusions: Risk factors for severe mucositis may be effective on the choice of conditioning regimen. Complications associated with mucositis may be prevented by early transplantation for the patients in whom ASCT indication is present. Performance status, sex, two or more chemotherapy regimen before transplantation and conditioning regimens including busulfan+cyclophosphamide or TBI were found as risk factors for oral mucositis. No correlation between MTHFR C677T mutation and mucositis was found.



The relationship between Karnofsky performance status before SCT and oral mucositis scores

BSH044

IMPLEMETATION OF THE AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION AS STANDARD THERAPY FOR MULTIPLE MYELOMA IN FUNDENI CLINICAL INSTITUTE

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Fundeni Clinical Institute

Background: First autologous stem cell transplant (ASCT) for Multiple Myeloma was performed in Fundeni Clinical Institute in Nov 2003. We increased our activity year by year, during 2006 we performed 8 autografts for Multiple Myeloma (MM) patients.

Aims: to introduce the autologous pheripheral stem cell transplantation as standard therapy for eligible Myeloma patients

Methods: We established the protocols for eligibility of Multiple Myeloma patients for peripheral stem cell transplant; for harverst and criopreservation of stem cells, for conditioning and for monitoring after autotransplant.

Results: Between Nov.2003 and Aug. 2006, 16 patients with multiple myeloma (MM), 1 stage I, 5 stage II and 10 stage III at diagnosis, were treated with myeloablative high-dose therapy followed by autologous peripheral stem cell transplantation (ASCT).

The median time from the diagnosis to transplantation was 36,9 months (3-300). The ASCT was performed below 1 year after initial Myeloma diagnostic at 12 cases and as a late treatment (4 patients). At the first response evaluation after autograft (day +100) we obtained: 6 patients in complete remission (CR), 5 partial remission (PR) and 1 progressive disease. Mobilization of stem cells was performed with Ciclophosphamide + G-CSF in 14 cases, G-CSF alone in 1 case (second mobilization) and other regimens in 2 cases. The conditioning regimen consisted in high-dose Melphalan in 15 cases and high-dose BCNU in 1 case. The median time for engrafment was 11 days (9-13) for leucytes and 14 days (12-17) for platelets. The main post transplant complication was febril syndrome (12 cases). Transplant related mortality (first 100 days after ASCT) was 0. Post transplant 9 patients receive Interferon Alfa as maintenance therapy. First

6 months evaluation post ASCT (10 patients) shows a 90% stable response (CR and PR) and 1 patient with relapse.

Summary: The ASCT represents a safety procedure as first line therapy for eligible patients with Multiple Myeloma, with a minor/medium toxicity and a very good response.

BSH045

NEPHROTIC SYNDROME AND ACUTE RENAL FAILURE IN NON-HODGKIN LYMPHOPLASMOCYTIC LYMPHOMA WITH MONOCLONAL GAMMOPATHY

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Introduction. Lymphoplasmacytic lymphoma is a rare disease that occurs in older adults, characterized by proliferation of small B lymphocytes, plasmacytoid lymphocytes or plasma cells with or without production of serum monoclonal protein. Renal lesions may occur quite rarely and may be the result of amyloidosis or depositing of monoclonal immunoglobulins or their subunits in one or more renal compartments.We report two patients with lymphoplasmacytic lymphoma and monoclonal gammopathy of IgM and IgG type, nephrotic syndrome and acute renal failure.

Case report.A 58-year-old man previously treated for pre-B acute lymphoblastic leukemia, developed 3 years later nephrotic syndrome as a complication of lymphoplasmacytic lymphoma and high paraprotein IgM kappa type. Immunofluorescent analysis of kidney biopsy showed extensive IgM and light kappa chain deposits which caused membranoproliferative glomerulonephitis. Treatment with cyclophosphamide was ineffective and patient died 2 months later. The second patient is 42-year-old female diagnosed with lymphoplasmacytic lymphoma and paraprotein IgG lambda type. The course of the disease was fulminant with developing nephrotic syndrome and fatal acute renal failure. Immunofluorescent and light microscopic studies of kidney biopsy showed signs of immunotactoid glomerulonephritis with deposits of IgG and C3. Haemodyalises and cytostatic therapy were without response and she died after 45 days.

Conclusion. In both cases cytorediction therapies were inefficient so the production of light chains remained after initiation of therapy indicating an aggressive course of disease and finally both patient died a short time after diagnosis.

BSH046

A STUDY OF IgG, IgM AND IgA IMMUNOGLOBULIN IN IRON DEFICIENCY ANEMIA

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Background: Immunoglobulins or antibodies are a group of glycoproteins found in the serum and lymphatic fluids of all mammals. They are produced in large amounts by plasmocytes which originate from B lymphocytes. Of the five class, of human immunoglobulins, three classes in cluding IgG, IgA and IgM are of greater importance against foreign bodies. They play an important role in controlling infections factors. Among the various nutritional problems anemia relating to iron deficiency has been known as an important general health problem due to its high prevalence and adverse effects. 'Aim: We wnted to see if the level of the blood immunoglobulins chenge in iron deficiency anemia.

Method: 5cc of venous blood were obtained and poured into the single test tubes from both go subjects showing the clinical signs of anemia without any other disease and go healthy subjects of the health screening project at Yazd central laboratory. The subjects were bone genious in both control and experimental groups.Four hours after obtaining the blood samples. The serum was separated and kept in freezer at 800c the immunoglobulins IgG, IgM and IgA were measured using the SIRD method. The amount of ferettin was measured using ELIZA of the go subjects showing ferretin deficiency, 17% (18,22) were in the age group 1-19 year 2-29 year old including 5 men (males) and 38 women (females). 30 subjects (% 33.33) belonged to the age group 30-59 including 6 men (males) and 38 women (female).

Results: The average of IgG in the subjects having feretin deficiency was 877.33 mg/dl, while for the subjects with normal feretin level. It was 1048.7 mg.dl showing a statistically significant difference (P=0.001). The IgM average in subjects having feritin deficiency was 134.2mg/dl. While in the subjects with normal ferretin level it was 138.92 showing no statistically significant difference (P= 0.487). The IgA average in the subjects with ferretin deficiency was 149. 82 mg/dl, while it was 179.33 for the subjects with normal ferritin level showing a statistically significant difference (P= 0.002).

Summary: the average of IgA and IgG in the subjects with ferritin deficiency was significantly lower than that of the subjects with normal ferritin, but there was no significant difference in the average IgM level in both groups.

BSH047

THE PREVENTION OF THROMBOEMBOLISM IN PATIENTS WITH MULTIPLE MYELOMA

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Introduction: There is an increased risk of thrombotic complications in patients with multiple myeloma. Following risk factors play role in aetiopathogenesis: a) the presence of monoclonal immunoglobulin and related hyperviscosity syndrome with fibrine structures, b) production of pro-coagulation acting antibodies, c) effect of inflammatory cytokines to endothelium, d) frequent incidence of aquired activated protein-C resistance /Zangari 2002/, high level of both vWF:Ag /Minnema,2003// and FVIII /Zangari.2002/.

The treatment with thalidomide especially in combination with dexamethasone and doxorubicine as well as therapeutic regimens containing high dose dexamethazone significantly increase the risk of thromboembolism.

Prophylactic administration of LMWH in the risk groups

of patients with multiple myeloma decreases the risk of thromboembolism by 50%.

Objective: Evaluation of selected haemocoagulation parametres (vWF:Ag, F VIII:c) in patients in various stage of multiple myeloma and their possible relation with the prediction of thromboembolic risk.

Methods: We examined 18 patients with multiple myeloma in clinical stage I.-III.(Durie, Salmon) for both vWF:Ag and F VIII and compared with group of 25 healthy controls.

Results: The level of vWF:Ag was significantly increased in patients with multiple myeloma compared to control healthy group (p=0.00086). The level of F VIII:c was significantly higher (p=0.00020) in patients as well.

Conclusion: Our findings confirmed the significant increase of vWF:Ag and F VIII in patients with multiple myeloma, which contribute to the thromboembolic risk. The analysis of these results, taking also the other risk thromboembolic factors into consideration, may be helpful to decide the correct prophylaxis of thrombembolism. To confirm these results the larger randomized patient groups and following-up the changes of selected haemocoagulation parameters depending on activity of the disease are required.

BSH048

PULMONARY FUNCTION TESTS IN CHILDREN WITH SICKLE CELL ANEMIA

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Pulmonary involvement accounts for acute and chronic morbidity and mortality in patients with sickle cell anemia (SCA). There is conflicting data concerning pulmonary function test (PFT) results of SCA patients in different populations. In this study, we aimed to investigate PFTs in a group of children with SCA (aged 5-16 years, 31 patients) and compare these results with those in age-matched controls (20 healthy children). We also investigated the impact of acute chest syndrome (ACS) attacks on PFTs in patients with SCA. We found that mean results of forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) were significantly lower in children with SCA ($p<0.05$ and $p<0.01$, respectively). There was a negative correlation between the number of ACS attacks and FEV1 ($r=-0.36$, $p<0.05$). Our results imply that PFTs are adversely affected in children with SCA. We suggest PFT of SCA patients, especially those who had frequent ACS attacks, should be followed closely.

BSH049

SUCCESSFUL TREATMENT WITH PERCUTANEOUS VERTEBROPLASTY FOR OSTEOPOROTIC FRACTURE IN A PATIENT WITH SICKLE CELL ANEMIA

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Vertebroplasty involves the injection of a bone cement to strengthen a vertebra. This minimally invasive method is reported to provide quick pain relief in 90% of patients, with mostly minor complications. This technique has been used for the relief of pain in the spine as a result of tumor involvement, vertebral hemangioma, focal Paget's disease, and osteoporotic vertebral fracture. The bone is also commonly affected, with infarcts, and osteoporosis in patients with sickle cell anemia.

Herein, we report for the first time a patient with sickle cell anemia who underwent percutaneous vertebroplasty. An 22-year-old female patient with sickle cell anemia presented with acute back pain that manifested after minor activities. Thoracolumbar MRI at the time of presentation with back pain revealed compression fractures at T-11 and L-4. She had a history of asthma bronchiale and she was under treatment of corticosteroid (methyl prednisolone) for two years. Percutaneous vertebroplasty was performed by injecting 1 to 2 mL PMMA into the anterior third of the compressed vertebral body. The patient experienced a 80% reduction in pain immediately after treatment; 3 months later she were walking and reported minimal back pain while undergoing treatment for osteoporosis. One year after procedure the patient remains pain free, and ambulatory.

The patients with sickle cell anemia have the wide variation in the shape and size of the vertebral structure means that these patients are susceptible to cement leakage-related complications. Percutaneous vertebroplasty relatively with small volume injection resulted in prompt pain relief and rapid rehabilitation in the described patient with sickle cell anemia.

BSH050

CD38 EKSPRESSION AS PROGNOSTIC FACTOR ACCORDING TO RAI STAGING IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS

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In the recent years, new prognostic factors in CLL except RAI staging (Ig VH Mutation, B2 microglobuline, cytogenetical analysis, timidincynase activity, ZAP 70, CD38) were determined and the treatment was oriented according to these factors.

Between 2004 and 2005, 95 cases with CLL were investigated immunophenotypically in flow cytometry and CD38 positiveness according to RAI staging was examined, in Ankara Numune Training and Research Hospital, Heamatology Section.

Clonal lymphocyte presence more than 5000/mm3 in peripheral blood and 30% in bone marrow was accepted as CLL prognosis criteria. CD5+CD19+CD23+, Anti-Kappa or Anti-Lambda +, CD22-, FMC7-, CD79b-, and CD38 positiveness in cells (for CD38 cut off value was accepted as 30%) were investigated from the peripheral blood samples of these patients with three color flow cytometry (with a panel consisted of CD5, CD11c, CD19, CD20, CD22, CD23,CD25, CD38, CD79b, FMC7, Anti-Kappa, Anti-Lambda and Anti-HLADR). Clinical staging of the patients were done according to RAI Staging System.

There were 25 cases (26%) Stage 0, 35 cases (37%) Stage I, 17 cases (18%) Stage II, 11 cases (11,5%) Stage III and 7 cases (7,3%) Stage IV. CD38 positiveness according to RAI Staging; CD38 was positive for 4% in Stage 0, for 22,8% in Stage I, for 29,4 % in Stage II, for 72% in Stage III and for 100% in Stage IV.

There is an evident correlation between RAI staging and CD38 positiveness but, we believe that evaluation of CD38 with the other prognostic factors is more suitable than alone.

BSH051

PROTEIN A IMMUNOADSORPTION THERAPY FOR CHRONIC REFRACTORY ITP

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Extracorporeal plasma immunoadsorption for the removal of immune complexes and IgG in patients with chronic refractory ITP is a life saving method in some cases. This method was employed in 4 patients who could not be adequately treated previously with established treatments. The mean baseline platelet count of the patients was 8000/μL. Immunoadsorption was performed 6 times in 3 patients and 4 times in one patient. The platelet count reached to 50.000/μL with the method administered for a mean duration of 10 to 14 days. No method-related side effect was observed during treatment. These patients still maintain normal platelet values without receiving any treatment. The mean follow-up of the treatment is 3 years and continues at present.

Table 1-Patients and the previous treatments administered

Table 2-The baseline platelet counts and the platelet counts during treatment

All patients had widespread petechia and ecchymosis with 2 patients additionally experiencing menometrorrhagia. The mean time elapsed between the first diagnosis and the present time was calculated as 40 months for these patients. The patients had previously received standard/high dose steroid, IV immunoglobulin, immunosuppressive therapy and except one patient, splenectomy; these patients had also underwent postoperative investigation for accessory spleen but given negative results. During this procedure, the patients were administered 32 mg methylprednisolone daily. The procedure was performed for 6 cycles in the first 3 patients and for 4 cycles in the last patient. The patients were administered 2-4 U/day fresh frozen plasma, and platelet infusion for the first 3 days (2 U/day). No complications developed during this procedure. Apheresis was performed via immunoadsorbent method, Fresenius Home Care PROSORBA Column Therapy.

Berchtold et al. stated that immunoadsorbent method was better than the other alternative therapies with respect to long-term response in patients with chronic refractory ITP (1). Nevertheless controlled studies of this treatment method by Snyder et al. demonstrated a long-term response in 46% of the patients (2). However splenectomy was performed in 49 of the 72 patients in this study while this method was administered after performing splenectomy in 3 of the 4 patients in our study; the high response rate obtained suggested that administration of the method after splenectomy could be effective as well as the small number of patients included.

It should be noted that this treatment can yield good outcomes in cases of chronic refractory ITP where no response could be achieved with other therapeutical methods and that it should be considered as an alternative treatment method.

References

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Patients	*Gender*	*Age*	*Steroid*	*IVIG*	*Splenectomy*	*Other treatment*
Patient 1	Female	40	+	+	+	Azathopurine
Patient 2	Female	28	+	+	-	Azathiopurine
Patient 3	Female	66	+	+	+	Cyclophosphamide
Patient 4	Female	23	+	+	+	Cyclophosphamide

Days	*Patient 1(/μL)*	*Patient 2(/μL)*	*Patient 3(/μL)*	*Patient 4(/μL)*
Baseline	8.000	10.000	10.000	4.000
Day 1	13.000	13.000	16.000	8.000
DaY 3	17.000	20.000	20.000	23.000
Day 5	18.000	20.000	25.000	43.000
Day 7	28.000	27.000	28.000	53.000
Day 9	37.000	35.000	36.000	65.000
Day 11	50.000	50.000	44.000	80.000
Day 13	60.000	55.000	55.000	100.000
Day 15	65.000	70.000	60.000	120.000
Day 17	70.000	90.000	75.000	127.000

BSH052

BORTEZOMIB REFRACTORY PLASMA CELL LEUKEMIA

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Plasma cell leukemia (PCL) is a rare and aggressive form of monoclonal gammopathy.

PCL characterized by the circulating plasma cells (PCs >2.000/mm3 and more than %20 PCs in the peripheral blood differential count). Two types of PCL have been described, primary PCL and secondary PCL.The prognosis for primary

and secondary PCL is reported as being poor with median survival of about 6-8 months.Currently, no standard therapy is available, but intensive polychemoterapy aspecially autologous and allogeneic stem-cell transplantation appears to more effective than the conventional therapy. Bortezomib, a proteasome inhibitor is shown as a effective agent in relapsed or refractory myeloma. Some case reports describing the efficacy of bortezomib in the treatment of PCL. We report the case of patient in which circulating plasma cell persisted during thalidomide plus bortezomid treatment.

A 52 year -old male presented in May 2005 with abdominal pain and fatigue. His laboratory studies mild anemia and thrombocytopenia. He had a white blood cell count of 7000/mm3 (%56 atypical lymphoplasmacytic cell on peripheral blood smear). Serum protein electrophoresis and immunofixation study revealed monoclonal lambda light chain. Bone marrow examination revealed % 90 cellularity with replacement of normal elements by sheets of plasma cells. Flow-cytometric studies performed on the peripheral blood confirmed CD38, CD138 expression by PC. Three cycles of VAD (vincristine 0.4 mg days, 1-4 day, adriamycin 9 mg/m2 days, 1-4 day and dexamethasone 40 mg days 1-4,9-12, 17-20day) chemotherapy were given to patient, which yielded partial remission. The patient did not accept high dose chemotherapy with autologous peripheral blood stem cell support. Thalidomide and dexamethosone therapy was started. At this time bortezomib was added to thalidomide and dexamethosone therapy. Bortezomib was administered intravenously at an initial dose of 1.3 mg/m2 on days 1,4,8, and 11 every 3 week. After the 3rd cycle of therapy new multiple soft tissue mass was developed. Unfortunately, there was no effective reduction of peripheral plasma cell count or bone marrow infiltration.

Primary PCL is a rare haematologic malignancy characterized by the presence of high number of circulating plasma cells. PCL represents the most aggressive form of monoclonal gammopathy for which new treatment approaches are needed.

BSH053

RECURRENT ABORTION RELATED TO FACTOR XI DEFICIENCY

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Introduction: Pregnancy can be depend on undesirable results for the women with coagulation factor deficiency. Among these results the most common is the bleeding during labor. However, some literatures report spontaneous abortion, abruption placenta and premature birth related to especially factor X, von Willebrand factor(vWF) and factor XI deficiency

Case: A thirty years old patient with recurrent spontaneous abortions applied us for thrombophilia screening. The patient had 6 spontaneous abortions. These abortions occurred during the initial 8 weeks. Her parents had no thrombosis history. Protein C, S, antithrombin III, lupus anticoagulant, homosistein level, active protein C resistance, factor V Leiden, methylenetetrahydrofolate reductase, and prothrombin 20210A gene mutation was normal. Active partial thromboplastin time(APTT) was prolonged. Although factor VIII, IX, XII levels were normal, factor XI was 1.1%. The patient had no bleeding history.

Discussion: Factor deficiencies are rare cases among recurrent abortion reasons. Recurrent abortion was reported commonly for the patients with vWF deficiency. It is rare for the patients with factor XI deficiency. A retrospective study showed that only one of twenty-eight pregnancies with factor XI deficiency had spontaneous abortion. The reasons of abortion due to factor XI deficiency and its treatment are not certainly clarified yet. Several studies for other deficiencies suggest replacement therapy during pregnancy. But, risks of fresh frozen plasma usage should be considered. Since the patients with factor XI deficiency may not have bleeding problem even sometimes lifetime, these patient may live long period without diagnosis but only APTT is prolonged. So, it is important to evaluation APTT assay when reasons of recurrent abortion are searched.

BSH054

SECONDARY LIPOSOMAL AMPHOTERICIN B PROPHYLAXIS DURING STEM CELL TRANSPLANTATION IN PATIENTS WITH A HISTORY OF FUNGAL INFECTION

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Background:Fungal infections have become one of the major causes of mortality and morbidity in patients with hematological malignancies. In stem cell transplant recipients, relapse of previous fungal infection remains an important clinical problem, despite the advances in antifungal treatment schedules.

Aims:The secondary amphotericin B prophylaxis in patients with a history of fungal infection may reduce not only the risk of relapse of the prior fungal infection, but also transplant related morbidity and mortality as well.

Methods:Ten patients (five acute myeloblastic leukemia, three acute lymphoblastic leukemia, one hodgkin's disease, one non hodgkin's lymphoma) who underwent allogeneic stem cell transplantation were included the study. The median age of the patients was 28 years (range 18-53). The conditioning regimen was busulfan – cyclophosphamide in six patients, total body irradiation – cyclophosphamide in two patients and busulfan – cyclophosphamide – fludarabine in two patients. Five patients (50%) had probable fungal infection with radiological and microbiological diagnosis, while the others (50%) had possible fungal infection with radiological diagnosis. Aspergillus was isolated in 20% of the specimens.

Results:Low dose liposomal amphotericin B prophylaxis was administered at a dose of 1 mg/kg/day in nine (90 %)

patients. Since there was an active probable fungal infection in one patient, the dosage of amphotericin B was increased to 5 mg/kg/day. In seven patients (70%), receiving low dose amphotericin B prophylaxis, the dose had to be increased to 5 mg/kg/day because of the clinical and radiological progression of the current infection. The median administration period of liposomal amphotericin B was 34 days (range 11-63). Combination of more antifungal agents was necessary in six patients (60%), since an objective response could not be obtained with liposomal amphotericin B alone. The median neutropenic period of patients was 18,1 days (range 12-27). There was no infection related mortality.

The side effect profile of liposomal amphotericin B was tolerable. The concomittant use of other nephrotoxic agents, such as cyclosporine A, did not influence the renal functions.

Conclusions:Prior documented fungal infection is not considered to be an absolute contraindication for stem cell transplantation. However, transplant related morbidity and mortality remain a life threatening problem in this subgroup of patients. Liposomal amphotericin B prophylaxis, with a favorable toxicity profile, can certainly play an important role to prevent the recurrence of the previous fungal infection during stem cell transplantation.

BSH055

A LOW INCIDENCE OF NONTUBERCULOUS MYCOBACTERIAL INFECTIONS IN PEDIATRIC HEMATOPOIETIC STEM CELL TRANSPLANTATION RECIPIENTS

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Hematopoietic stem cell transplantation (HPSCT) is being used to treat a wide spectrum of clinical disorders but opportunistic infection remains an important factor determining outcomes for these patients. Nontuberculous mycobacterial (NTM) infections are being reported more frequently in HPSCT recipients and the incidence of NTM infections in adult recipients is reported to be 0.4-4.9%. However, the incidence and severity of NTM infections are less well described in pediatric HPSCT recipients. Centers for Disease Control guidelines were used to define definite and probable NTM infection among 132 children undergoing 169 HPSCT between January 2000 and December 2004 at our institution. NTM infection was diagnosed in 5 of 132 pediatric recipients (3.8%). The actuarial incidence of NTM in all HPSCT recipients and in allogeneic HPSCT recipients was 3.6% (CI95:0.5-6.8) and 6.4% (CI95:0.8-11.9), respectively. The mean age of the HPSCT recipients who developed NTM infections was 8 years (range: 2-19 years); 3 were male and 2 were female. Four conditioning regimens included alemtuzumab and 3 had ATG. Of the 5 patients with NTM infections, 2 met the criteria for definite infection and 3 for probable infection. Of the 2 patients with definite NTM infection, 1 had disseminated disease with M. avium complex (MAC) and the other had M. chelonae catheter-related bloodstream infection. The probable NTM infections were 1 skin infection with M. kansasii and 2 lower respiratory tract infections with MAC. Median time to NTM infection was 115 days (range: 14-269 days) following HPSCT. Two patients had GVHD at the time of NTM infection. All 5 patients received 3-4 anti-mycobacterial drugs and all NTM infections resolved. In summary, the incidence of NTM infection in pediatric HPSCT recipients appears similar to that described in adult HPSCT recipients and the outcome appears to be excellent with the proper antibiotic therapy. The increased use of anti-T-cell antibodies appear to be associated with an increased risk of NTM infections in pediatric HPSCT recipients. Multi-centered studies are needed to identify the risk factors, early diagnostic criteria and optimal therapy.

BSH056

THE IMPORTANCE OF THE THROMBOPHILIA MARKERS FOR THE VENOUS THROMBOEMBOLIC COMPLICATIONS AFTER TOTAL KNEE ARTHROPLASTY

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Background: Venous thromboembolic complications are the most frequent preventable death causes for orthopaedic surgery. There are several hereditary (ATIII, PC and PS deficiency, FV Leiden mutation, protrombin P20210A mutation, MTHFR mutation, hyperhomocysteinemia) and acquired (old age, immobilisation, obesity, surgery, trauma, tourniquet) factors increasing the risk of thromboembolic diseases.

Coagulation and fibrinolysis are in equilibrium. Thrombin activatable fibrinolysis activator (TAFI) is a procarboxipeptidase downregulating plasmin formation, thereby causing a tendency for thrombosis development. It was reported that, high TAFI levels elevate the thrombosis risk in the patients with venous thrombosis. Aims: In the present study we aimed to investigate the hereditary and acquired risk factors for deep vein thrombosis and thromboembolic complications in the patients with total knee arthroplasty and to determine the correlation between the risk factors and the TAFI levels.

Methods: Between January 2005 and October 2005 at Ankara Numune Training and Research Hospital 5th Orthopaedic Clinic Sixty patients with a mean age of 64.2 years (45-82), treated with total knee arthroplasty, were included the study. There were 11 males and 49 females. In our study group, TAFI, ATIII, PC, PS, APCR, FV Leiden mutation, protrombin P20210A mutation, MTHFR mutation, FVIII, PLT and fibrinojen levels were studied. Deltaparin was started 24 hours before the surgery. Lower extremities of the patients were investigated with Doppler USG preoperative, postoperative 8th to 14thday and 45th day for the deep vein thrombosis. Results: There was deep vein thrombosis in eight patients. In our study group, there were 11 heterozygote mutation and

49 patients with normal FV Leiden gene, 13 heterozygote, nine homozygote mutations and 38 patients with normal MTHFR, four heterozygote mutation and 56 patients with normal P20210A gene. APCR was positive in nine patients. PC, PS, ATIII levels were normal in all patients. There were significant correlation between FVL mutation, APCR and higher TAFI levels in seven of eight patients with deep vein thrombosis (P<0.001). P20210A mutation was heterozygote and TAFI level was normal in the other patient with deep vein thrombosis.

Conclusions: According to these findings, we believe that, investigating FV Leiden mutation, APCR and TAFI levels preoperatively may be beneficial on preventing the deep vein thrombosis and venous thromboembolic complications after orthopaedic surgery.

BSH057

STEM CELL TRANSPLANTATION FOR FANCONI ANEMIA WITHOUT RADIATION: A SINGLE CENTER EXPERIENCE IN TURKEY

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Hematopoietic stem cell transplantation offers the only curative potential for the marrow complications of Fanconi anemia (FA). FA patients are most susceptible to alkylating agents and radiation at dose ranges commonly used for allogeneic stem cell transplantation (SCT). Successful transplantation has been achieved in some patients using radiation and cyclophosphamide but studies demonstrated that regimen-related toxicity was significant barrier to success in these patients. In order to reduce toxicity, recently fludarabine based protocol has been used as a part of conditioning regimen. Eight patients were transplanted at our center between 1998 and 2006. Median age of the patients was 12 years (6.5-14 y). All patients had aplastic anemia and 7 of them received androgen and steroid therapy prior to SCT. All patients received 2 different preparative regimens without radiation. First 2 patients were transplanted with low dose busulfan (6.0 mg/kg), cyclophosphamide (40 mg/kg). After 2004, conditioning regimen consisted of fludarabine 150 mg/m2, cyclophosphamide 20 mg/kg, ATF-Frasenius 35-40 mg/kg. First 2 patients received cyclosporine A (CsA) plus methotrexate, other 6 patients who transplanted with fludarabine based regimen received only CsA for GVHD prophylaxis. All donors were HLA identical, 7 were siblings and one was father. Stem cell sources were bone marrow in 3, peripheral blood in 5 patients. All the patients were engrafted with a median neutrophile and platelet engraftment time of 10 days (9-15) and 13 days (11-35) respectively. One patient whose donor was father and received conditioning regimen which include busulfan died because of hepatic VOD. Fludarabine based regimen was well tolerated with minimal transplant related toxicity. Only one patient had chronic GVHD involving oral mucosa. Overall and event free survival were found as 87.5 % and mortality rate was 12.5%. Median follow-up period is 19 months (range: 7-71 months) and during this time no patient developed secondary malignancy. Based on our data, fludarabine based non TBI conditioning redimen is safe and associated with low organ toxicity and effective immunosupression for the stable engraftment in FA patients undergoing SCT with matched related donors.

BSH058

BORTEZOMIB: AN EFFECTIVE AGENT WHICH CAN BE USED SAFELY IN OSTEONECROSIS

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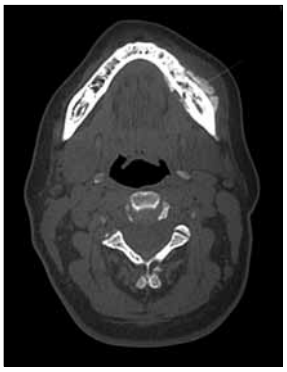
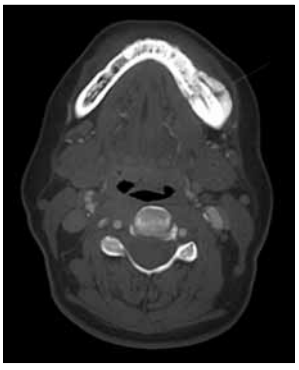
Proteasome is an enzyme complex that degrades many targeted intracellular proteins. Some of the target proteins of proteasome, such as NF-kB, tumour suppressor protein p53, Bax are also involved in malignancies. Since it was shown that the proteasome inhibitors potently inhibit both intracellular proteolysis and cell proliferation, only one of them which is called bortezomib is being started to use in cancer therapy especially in multiple myeloma (MM). Herein we reported the different effect of bortezomib in a patient with relapsed MM. Fifty-eight year-old female patient was diagnosed as Ig G kappa MM 4 years ago. After 3 cycles of VAD chemotherapy, she received high dose melphalan therapy with autologous PBSC support and then she was controlled with monthly zolendronic acid. One year later, she had a tooth extraction and then she was admitted with gum hyperplasia. Since no clinically associated disease was found to explain gum hyperplasia, it was surgically resected and histopathological evaluation revealed normal histological findings. A year after she was admitted with the complaint of a mass and pain on same side angulus mandibulea with extracted tooth and a very few days later an inflammatory material was began to leak. CT scan revealed osteitis and widespread periost reaction in her mandibulea, biopsy of involved bone was revealed osteonecrosis and mixed inflammatory reaction. Because of osteomyelitis, daily 4X2 g Ampicillin/sulbactam therapy was started. Although fistula was improved, the mass and pain on her mandibulea was not regressed. According to her clinical course, CT scan and histopathologic examination, the patient was diagnosed as the ostenonecrosis of mandibulea due to zolendronic acid. During this period MM was also progressed. Bortezomib as single agent was started on days 1, 4, 8 and 11 at the dose of 1,3 mg/m2. After the first cycle of bortezomib (Velcade) the swallowed mass was regressed and the pain was improved. A CT scan was performed after the second cycle of bortezomib and it was displayed, the resolved soft tissue mass, moderate callus formation and as a result moderately improved osteonecrosis. There are three possibilities about this patient. The mass was a plasmositoma which was respond to bortezomib. There was an inflammatory reaction due to osteonecrosis and/or osteomyelitis. The exact mechanism of bisphosphonate induced jaw osteonecrosis has not been elucidated yet, but it was suggested that cumulative ischemic

effect may be the causative event and it was reported that osteonecrosis induced by bisphosphonate does not improve with surgical rejection or antibiotics. Bortezomib is the first proteasome inhibitor which is used in the relapsed multiple myeloma. The main effect of this drug is NF-kB inhibition leading to decrease transcription of genes important in tumour survival, proliferation, invasion and metastasis, and angiogenesis. It has also an anti-inflammatory effect via the NF-kB pathway. It was also shown in preclinical studies that bortezomib decreases osteoclast function due to inhibition of NF-kB pathway. Proteasome inhibition not only decreases osteoclast function but also enhances osteoblastic bone formation but neither osteoclasts nor osteoblasts have an effective role in osteonecrosis pathology if the fracture of involved bone does not associate. We can suggest that probably the anti-inflammatory effect or the other effects of bortezomib which we don't know exactly yet, may cause the relief of our case and the micro fractures in involved bone may have been started the callus formation which bortezomib enhanced. This is the first report about the effect of bortezomib in zolendronic acid induced osteonecrosis. As conclusion, bortezomib can not only be safely used in patients with jaw osteonecrosis but also contributes the improvement of both osteonecrosis. Probably it can be used in osteonecrosis and other inflammatory bone disease which arise due to other factors. This should be proven with cumulative studies.



CT scan before bortezomib



CT scan after bortezomib

BSH059

THE EFFECT OF DIFFERENT BCR-ABL TRANSCRIPTS ON IMATINIB MESYLATE THERAPY

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Chronic myeloid leukaemia (CML) is a myeloproliferative disorder characterized with BCR-ABL fusion gene. According to breakpoint regions in BCR gene, p190, p210 and p230 proteins are encoded. The importance of these different BCR-ABL fusion genes and proteins are not known exactly yet, but it was shown that patients who express p190 have prominent monocytosis and their clinical presentations are between CML and chronic myelo-monocytic leukaemia. Although the different genes encode different proteins, all these proteins lead to activation of tyrosine kinase. After the introduction of selective tyrosine kinase inhibitor imatinib mesylate (IM) the approach to CML therapy was changed. But it was shown that some patients disclose complete haematological and molecular response, some patients do not and some of them relapse after molecular response. Recently, the resistance mechanisms of IM have been reported. In this research, For the reason that we couldn't find any report about the effect of different BCR-ABL genes on IM therapy, we tried to search this issue. Fourty-nine early chronic phase (<12 months) CML patients from two different centres were included in the study (median age 51, range 17-79). The patients did not receive any medication before IM other than hyroxyurea. The RNAs were extracted from peripheral blood samples before and during the IM therapy. The BCR-ABL levels were quantified in all these samples with LightCycler and qualitative nested- reverse transcriptase polymerase chain reaction (RT-PCR) was performed solely on the samples which were driven before IM therapy. All of the patients' p190 and p210 transcripts were checked according to Biomed I protocol. IM was started 400mg/day and the patients were followed at least 12 months. The dose of IM was increased to 600 mg/day in the patients who did not respond and/or who progressed. Molecular response was determined if the decrease in BCR-ABL levels was more than %0,1 together with haematological response. The results were given in table 1. The 64% of patients with p210 transcript (25/39) and 71% of patients with p190 transcript (5/7) responded. We couldn't find significant difference between these transcripts (p=1). The molecular response was observed 64,5 % of the patients who express b3-a2 and 54,5% of the patients with b2-a2 (p=0,65). The 60% of patients who express only P210 transcript and all the patients who express both p210 transcripts responded (4 patients). All the patients with P190 e1-a2 transcript also responded (4 patients). The disease in 3 responder patients with p210 transcript were progressed (12%, 3/25), but it was progressed in 20% of the responder patients with p190 transcript (1/5) (secondary resistance). Within the patients who express single p210 and p190 transcript progression rate was found 12% but it was 28% (p=0,296) in the patients who express more than one transcript. The progression rate in the patients with only b2-a2 and b3-a2 transcript was 9,5%, it was 25% in the patients who express both of them (p=0,422). IM dose was increased in 17 patients who did not respond and 6 of these patients did not respond to dose increment also (primary resistance). Within these 17 patients, 9 of 14 who expressed p210 did not progress. Clinical and haematological response was observed in these patients but BCR-ABL levels decreased less than 0,1% (partial response). There were two patients in this group who expressed p190 and one of them did not progress. As a result, especially the different p210 transcripts do not have an effect on IM therapy, if there are association of different transcripts, response rate is slightly higher but progression rate is also higher. There may be

significant results if the patients with p190 transcript can be increased.

		P210				P190	P210 +P190	total
		b2a2n (%)	b3a2n (%)	b2a2+b3a2n (%)	e1a2n (%)	e1a3+e1a2n (%)	n (%)	n (%)
Molecular response (+)	Progression (-)	6 (55)	13 (54)	3 (75)	3 (75)	1 (33,3)	1 (33,3)	27 (55)
	Progression (+) (secondary resistance)	0	2 (8)	1 (25)	1 (25)	0	1 (33,3)	5 (10)
Molecular response (-)	Primary resistance	2 (18)	3 (13)	0	0	1 (33,3)	0	6 (12)
	Partial response	3 (27)	6 (25)	0		1 (33,3)	1 (33,3)	11 (23)
		11	24	4	4	3	3	49

BSH060

REDUCTION OF HEPATITIS B SEROPREVALENCE IN BLOOD BANKING UNITS BY COMBINED UTILISATION OF SELF EXCLUSION FORMS AND CLINICAL EVALUATION OF BLOOD DONATION CANDIDATES IN A DEVELOPING COUNTRY; TURKEY EXPERIENCE

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The primary goal of blood banking facilities is to provide safe blood products to health care units. To achieve this objective various precautions should be taken including the evaluation of blood donation candidates and serologic screening of donated blood units for hepatitis B virus (HBV), hepatitis C virus (HCV), human immune defiency virus (HCV) and syphilis. 170 million individuals are infected with HCV worldwide and this represents a five fold prevalence than HIV infections. When HBV infection are taken into account it reveals an enormous problem throughout the world in means of international public health since it affects over 350 million persons worldwide. Hepatitis B (HBV) and hepatitis C (HCV) infections deserve a more pivotal concern than human immune deficiency virus (HIV) infections in countries like Turkey. But its prevalence varies considerably between different geographic areas. In Turkey for instance HBV carrier rates vary between 2, 1 and 4 % of the whole population.

Blood transfusions are a major risk factor in spreading these viral infections. All types of blood products undergo a series of serologic tests including enzyme linked immunosorbent assay (ELISA) for the detection of HbsAg, Anti-HCV, Anti-HIV 1-2 and rapid plasma reagin (RPR) test performed to exclude syphilis. Additionally, screening tests of higher technology are utilized in certain developed countries, like nucleic acid testing (NAT), but due to its high economic burden these expensive methods can not be used in developing countries. An additional precaution is the combined utilization of self deferral forms and clinical evaluation by questioning of the medical history and brief medical examination. These methods have been proved to be effective in reducing infections spread via blood transfusions. Health care professional should spend as long time as possible in evaluating the forms and current literature supports its advantage in reducing transfusion related infections. But there is no performed study investigating the relation of qualifications of the health care professionals who are in charge of evaluating these forms and the improvement of blood transfusion safety.

In the Blood Banking Center of Gazi University School of Medicine, self exclusion forms were evaluated by staff nurses until March 2004. In the following period this task has been performed by a family physician. Our objective in this study is to investigate the relation between the permanence and specifications of the health care professionals and decrease of hepatitis seropositivity This retrospectively designed study was aimed to evaluate the influence of the permanence and qualifications of health care professionals on blood disposal rates due to hepatitis seropositivity. A decrease of 44, 2% of disposed blood units due to hepatitis B seropositivity was observed in the second period where self exclusion forms and blood donation candidates were evaluated by a family physician. But a similar decrease of disposal rate due to hepatitis C seropositivity was not observed. As a result, health care professionals working in blood banks should be qualified for this task. In this study we aimed to stress the importance of this issue and wanted to emphasize that at least hepatitis B seroprevalence can be reduced and transfusion safety increased by application of these precautions. This is especially important in countries where laboratory tests of higher technology can not be utilized due to their high economical burden. A comprehensive evaluation of self exclusion forms and a brief examination prior to donation will remarkably increase transfusion safety. Unfortunately there are blood banks in our country where no physicians are employed at all. Immediate precautions should be taken to solve this problem.

BSH061

TRANSIENT SEVERE CYTOPENIA FOLLOWING DOUBLE INTRATHECAL CHEMOTHERAPY AFTER COMPLET REMISSION IN AN ELDERELY AML PATIENT

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The effect of intrathecal (IT) chemotherapy on the haematological profile of patients with leukemia during remission is unclear. Herein, a case of a female patient with complicated cranial involvement secondary to acute myeloblastic leukemia is reported.

A 62-year-old woman, hospitalized with a chief complaint of headache was diagnosed as having primary acute myeloblastic leukemia (AML) M2 with clonal inv (16) chromosomal anomaly. Repeated neurological examination, cerebrospinal fluid examination, and cranial CT scans showed evidence of subdural haemorrhagia, and central nervous system involvement. On the third day of her admission, the patient suddenly lost her consciousness due to acute increased intracranial pressure, and emergency ventricular drainage was performed for therapeutic and diagnostic purposes. Malig-

nant cells were found in cerebrospinal fluid obtained from a ventricular drainage and dura material. After improvement of her clinical condition, she was given a standart combination therapy with idarubicin and Ara-C for remission induction. She went into complet hematologic and molecular remission. Five weekly doses of double intrathecal (IT) chemotherapy (methotrexate, and Ara-C) was administered intrathecally starting after first consolidation treatment with high-dose ARA-C. On the subsequent weeks after initiating IT therapy, severe persistant leukopenia and thrombocytopenia were confirmed during her rutin control visits, but no evidence of leukemia relapse was found by morphologic, immunophenotypic and cytogenetic examination. Cessation of IT chemotherapy was followed by a hematological improvement after 6 days.

Double IT chemotherapy after remission of acute myeloblastic leukemia as used in described patient appears to be associated with increased susceptibility to cytopenia.

BSH062

PANCYTOPENIA DUE TO VITAMIN B-12 DEFICIENCY IN A BREAST-FED INFANT

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Megaloblastic anemia due to vitamin B12 deficiency is rare in childhood. It can be caused by decreased ingestion or impaired absorption or utilization of B12. In non-vegetarian populations, one of the most common causes of B12 deficiency is a specific malabsorption of B12, or Imerslund-Grasbeck syndrome. Nutritional B12 deficiency is a rather uncommon disorder resulting from nutritional inadequacy. Most cases are due to maternal insufficiency, resulting in deficient stores and intake in what is generally an exclusively breast-fed infant. We report the case of a 9 month old breast fed infant who presented with a history of pallor, and failure to thrive. Investigations showed severe nutritional vitamin B12 deficiency with pancytopenia. Treatment of the infant with vitamin B12 resulted in a rapid clinical and haematological improvement. The cause of the vitamin deficiency was a maternal dietary deficiency and prolonged breast-feeding. The importance of early recognition of significant maternal vitamin B12 deficiency during pregnancy and lactation is emphasized so that appropriate supplementation can be given and irreversible damage in the infant prevented.

BSH063

COMPARISON OF THE FENWAL AMICUS AND FRESENIUS COM. TEC CELL SEPARATORS FOR AUTOLOGOUS PERIPHERAL BLOOD PROGENITOR CELL COLLECTION

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Peripheral blood progenitor cells (PBPC) are commonly used as a stem cell source for autologous transplantation. This study was undertaken to evaluate blood cell separators in terms of separation results and content of the harvest. Forty autologous PBPC collections were performed with either Amicus or COM.TEC cell separators in patients with hematological malignancies. The median product volume was lower with Amicus compared to COM.TEC (125 ml vs. 300 ml; $p<0.001$). There was no statistically significant difference in terms of the median number of CD34+ cell/kg in product between Amicus and COM.TEC (3.0x10e6 vs. 4.1x10e6; $p=0.129$). There was a statistically higher mean volume of ACD used in collections on Amicus compared to COM.TEC (1040±241 ml vs. 868±176 ml; $p=0.019$). There was a statistical difference in terms of platelet (PLT) contamination of the products between Amicus and COM.TEC (0.3 x10e11 vs. 1.1x10e11; $p<0.001$). The median % decrease in PB PLT count was statistically higher in COM.TEC compared to Amicus (18.5% vs. 9.5%; $p=0.028$). In conclusion, PBPCs could be efficiently collected with both instruments. However, Amicus has the advantage of lower PLT contamination in the product, and less decrease in PB platelet count with larger product volume and more ACD consumption in autologous setting.

BSH064

THE EFFECT OF THEREUPEUTIC PLASMA EXCHANGE ON HEMODYNAMIC PARAMETERS IN SEPSIS: A PILOT STUDY

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Sepsis and septic shock are an extremely serious condition with a high mortality rate. The term "therapeutic plasma exchange" (TPE) is used when patient's plasma is removed and replaced with allogeneic plasma. It is known that bacterial toxins and inflammatory cytokines cause multiple organ failure in patients with sepsis and septic shock. TPE may be an alternative approach to eliminate these toxic mediators from the systemic circulation. Our primary aim is to determine the effect of TPE on hemodyanamic parameters in the treatment of patients with sepsis and septic shock. Twelve patients (seven men, five women, and 55±18 years old) who were diagnosed sepsis and septic shock were treated with conventional sepsis treatment and TPE in the medical intensive care unit (MICU), Medical Faculty of Erciyes University, from March 2003 to June 2004. We recorded patients' heart rate, systolic blood pressure, diastolic blood pressure, central venous pressure, pulmonary artery pressure, pulmonary artery wedge pressure, pulmonary vascular resistance, cardiac output, cardiac index, systemic vascular resistance, mix venous O2 saturation, arterial blood O2 saturation, blood pH, partial O2 pressure, partial CO2 pressure, HCO3 levels, hemoglobin, hematocrit levels, white blood cell count, platelet levels, coagulation parameters (PT, aPTT, fibrinogen), renal function tests (BUN, creatinine), liver function tests (AST, ALT and bilirubin), dopamine and dobutamin doses, and Acute Physiology and Chronic Health Evaluation (APACHE) Score II two times, baseline and after third session of TPE procedure. TPE was performed employing a Fresenius AS-

204 continuous flow aphaeresis machines using veno-venous access. During each exchange session a volume of 40 ml/kg bodyweight of patient's plasma was exchanged with an equal volume of fresh-frozen plasma from healthy donors. The duration of TPE session was 118±6 min. The mean exchange plasma volume was 2172±254 ml. Mean systolic blood pressure and diastolic blood pressure were increased after third session of TPE compare to baseline from 123.6±14.3 mmHg to 134.3±19.6 mmHg (p=0.05) and from 64.5±13.5 mmHg to 72.5±14.5 mmHg (p=0.007), respectively. There were statistically decreases in patients' mean APACHE score II after third session of TPD compare to baseline (22.7±8.3 vs. 21.0±9.1, p= 0.022). Mean dopamine dose was decreased from 25.7±11.8 μg/kg/min to 13.2±6.0 μg/kg/min after treatment with TPE (p=0.0001). However, there were no changes in other parameters and variables except for these parameters. Four of 12 patients (33%) died within 14 days after the last TPE. This study showed that TPE has a positive effect on hemodynamic parameters, APACHE Score II and use of vazopressör/inotrope therapy in patients with sepsis and septic shock. It appears a safe procedure in the treatment of septic patients. Therefore, TPE may be an important adjuvant to conventional treatment to improve morbidity and mortality rates. However, prospective randomized multicentre trials are warranted to confirm these results.

BSH065

THE INFLUENCE OF IPI AND KI67, BCL-2, BCL-6 ON SURVIVAL IN PATIENTS WITH DLBCL TREATED WITH R-CHOP REGIMEN- SERBIAN LYMPHOMA STUDY GROUP (SLG) EXPERIENCE

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Several clinical parameters including International Prognostic Index (IPI) as well as biological parameters (Ki67, Bcl-2, Bcl-6), are considered to have prognostic relevance in diffuse large B-cell lymphomas (DLBCL).

Retrospective analysis was performed on 30 patients (19 male/ 11 female) randomly selected from a large group of patients (pts) diagnosed and treated with R-CHOP regimen in three years' (yrs) period. In all pts, initial IPI was determined as well as biological markers by immunohistochemical staining on formalin fixed, paraffin embedded lymph node biopsy specimens.

Forty three percent of pts were in clinical stage I and II, while 56.6% had advanced stage of disease, clinical stages III and IV. The average age of the whole group was 53 yrs (63% pts were under age of 60). During the period of three yrs the survival was achieved in 67%. The group of pts with CS I and II had better survival- 84% comparing to those with advanced stage of disease- 53%. Seven patients had low IPI, 13 pts were with intermediate IPI and 10 pts had high IPI risk. The survival in the high risk group was 30% (7 pts died), intermediate risk group 75% and low risk group 86%.

The Ki67+ >60% was detected in 19/30 pts (63%). In the group of pts with high Ki67, Bcl-2 was positive in 11/19 pts and Bcl-6 was positive in 15/19 pts; in this group of pts, 9 of them died, and 7 of had high IPI risk.

Eleven of 30 pts (37%) had lower Ki67+; Bcl-2 was positive in 9/11 and Bcl-6 was positive in 7/11 pts; in this group only 1 patient died.

According to our findings, advanced stage, high IPI risk, Ki67+>60%, Bcl-2+ could be considered as bad initial prognostic profile.

BSH066

NON-HODGKIN LYMPHOMA - EXTRADURAL LOCATION-CASE REPORT

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Extradural location at onset is rare.

We describe a man with thoracic and lumbar vertebral pain, without sensitivity and motility troubles at the legs.

MRI described a spinal extradural tumor T11-L1 with paravertebral extension to the right side, 72mm diameter, irregular border, with foraminal invasion L1-L2 to the right side.

Emergency surgical debulking was carried out through T12- L1 laminectomy and tumor reducing mass.

The histopathological exam and IHC (IHC- CD56+, CD79a+, Cla+,L26/CD20+) - large cell nonHodgkin lymphoma.

The patient refused the chemotherapy and after six months the tumor recidivated to the same place and a hypodense tumor to the VIII segment of the right lobe of the liver was identified to abdominal CT.

We started the chemotherapy R- CHOP and a T12 vertebral reconstruction was done; the evolution appears favorable.

BSH067

AGRESSIVE INITIAL TREATMENT OF HODGKIN LYMPHOMA- SERBIAN LYMPHOMA STUDY GROUP (SLG) EXPERIENCE

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Clinical staging is very important parameter that helps clinicians to define the most appropriate treatment strategies. Patients with stage III/IV, bulky disease or the presence of B symptoms require combination chemotherapy with or without additional radiotherapy. But, 20-25% of Hodgkin patients fail primary standard therapy. One possible way of improving treatment results is by dose escalation of effective cytotoxic drugs, and BEACOPP is one of such regimens.

During the 18 months period, 47 patients were treated with BEACOPP regimen: 41 de novo and 6 patients with relapsed disease. The inclusion criteria was CS II/IIIB (bulky with high International prognostic score- IPS) and CS IV. Twenty one patients were treated with baseline, 19 with escalated doses, and 7 with IV escalated/IV baseline schedule. Characteris-

tics of patients were: male 32 (68%) and 15 female (32%), median age 31 (range 18-60). Nine patients died during the treatment and 3 pts reveled relapses and 1 patient had progression of disease. In one patient we changed regimen due to adverse effects. Thirty-three patients finished VIII cycles and 7 are still receiving therapy. 25/32 had CR (97%) and 1 patient had PR. The main reason of death was aplasia, but in 3/9 pts who died, BEACOPP was given as secondary therapy. G-CSF was administrated in all pats that were treated with escalated doses. The majority of pts had moderate anemia (mean value of: Hb 90g/L), and the highest frequency was noticed in later cycles. Almost half of them were treated with erythrocyte transfusions, but platelet transfusions were not reported.

We concluded that in our series of patients, BEACOPP is effective therapy, but we need a comparison with the group of patients that were treated with ABVD.

BSH068

RESULTS OF GLEEVEC TREATMENT IN ARGES DISTRICT BETWEEN 2002 -2006

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Imatinib mesylate is a selective tyrosine kinase inhibitor who revolutionized the management of patients with chronic myeloid leukemia; it has become the current standard therapy for newly diagnosed patients with CML in chronic phase.

We treated 18 patients with Glivec in Arges district between 2002- 2006, 11 female patients, 7 male; at 16 patients we started the treatment in chronic phase and at 2 patients in accelerated phase; the age at diagnosis was between 18 and 76 years; all patients had leucocytosis (maximum 411.000/ mmc) and thrombocytosis

(maximum 1.026.000 /mmc); Ph crz. was identified at 17 patients in 100% of metaphases and at 1 patient in 65% of metaphases.

The treatment with Glivec was started between 0-40 months after the diagnosis with 400mg/ day.

We evaluated the response to treatment after the most recent criterias of Baccarani:

4 patients showed no complete haematological response after 6 months treatement; 4 patients showed complete citogenetic response (Ph crz <1%) at 12 months ; 18 months evaluation could not evaluate the response for the whole group due to the fact that 6 patients initiated the treatement less than 12 months, and 4 patients did not show major molecular response.

For any patient we could not arise the dose to 600 mg/day to evaluate the response to the optimal dose.

BSH069

NONHODGKIN LYMPHOMA WITH EXTRANODAL SITUATION IN ARGEŞ DISTRICT

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The nonHodgkin's lymphomas are a heterogeneous biological and clinical group of malignancies with an increasing incidence of extranodal disease.

We studied a group of 186 patients with nonHodgkin lymphoma between 2002 – 2006 and we evaluated the incidence of extranodal disease.

In our group, extranodal presentation of NHL occurred in 26% of patients, the incidence of extranodal NHL increase year after year, the most common site was gastrointestinal tract (37%), but any site is possible (stomach - 9, small intestine - 3, large intestine - 6, rectum - 1, spleen - 5, mediastinum - 3, pulmon - 4, bone - 4, epidural - 1, extradural - 1, skin - 7, brain - 2, tonsils - 2, thyroid -1, thymus - 1, pericardium - 1). The median age was 52 years, 22 patients were female and 29 male, sex ratio M/F = 1,32/1.

The most frequent histopathological form was large cells diffuse lymphoma – 12, small cells diffuse – 6, anaplastic NHL – 3, follicular – 3, MALT - 5, mantle – 3, marginal of spleen – 1, T peripheral – 1, a part remained uncharacterized histopathological.

We concluded the increasing incidence of extranodal diseases and a large variety of presentation site in NHL.

BSH070

ACUTE DISSEMINATED ENCEPHALOMYELITIS IN A CHILD AND PARTIALLY RESPONSE TO PLASMAPHERESIS

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Acute disseminated encephalomyelitis (ADEM) is usually a monophasic inflammatory demyelinating disease process that affects the brain and spinal cord and typically occurs after a febrile (often presumed to be viral) prodrome or vaccination. The typical presentation is that of multifocal neurologic disturbance accompanied by change in mental status.Treatment with corticosteroids is considered to hasten recovery and is accepted as the mainstay of therapy. Intravenous immunoglobulin (IVIG) has been reserved for patients who do not respond to corticosteroids. Use of plasmapheresis to treat this condition is limited to case reports.Our patient who received plasmapheresis after failure to respond to corticosteroids had partial recovery with a residual spastic paraparesis. Plasmapheresis has a definite role in the treatment of neurologic conditions that are presumed to be immunologically mediated, such as Guillain-Barré syndrome, chronic inflammatory demyelinating polyradiculoneuropathy, polyneuropathies associated with monoclonal gammopathies, and myasthenia gravis. It has been suggested that plasmapheresis also may have a role in treating patients with acute demyelination, including MS and ADEM. However, there have been few reports of its use in ADEM.To our knowledge, only 3 children who underwent plasmapheresis to treat ADEM after failure to respond

to corticosteroids and/or IVIG were reported previously. All 3 reports documented improvement after plasmapheresis. Plasmapheresis should be considered as a treatment option for patients with ADEM, especially when the course is aggressive or severe disease has not responded to corticosteroids and IVIG. It is unclear whether the use of plasmapheresis early in the course of the disease would alter the prognosis. A multicenter prospective study to address the outcome of the use of different therapeutic modalities in the treatment of ADEM is warranted.

BSH071

THE EFFECT OF NEEDLE DIAMETER ON HEMATOPOIETIC STEM CELL VIABILITY IN HEMATOPOIETIC STEM CELL APPLICATIONS (EX-VIVO EXPERIMENTAL STUDY)

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Introduction: Bone marrow derived stem cells (BMDSCs) have been under extensive evaluation by stem cell researchers. Currently, different cellular implantation techniques have been performed including transepicardial, intracoronary, percutaneous transfemoral endoventricular, and coronary venous or systemic intravenous routes in clinical trials. This study evaluated if there is any difference between different diameters of catheters for implantation and the flow rates through the needle with regards to BMDSC viability. Method: Eighteen patients (1 Female/17 Male) in whom bone marrow was harvested were enrolled in our study. After the bone marrow harvest and process using Cobe Spectra cell separator to erythrocyte and volume depletion, we used three different sizes of needles with 16g, 18g and 22g and two different infusion rates (1cc/sec. 2cc/sec.) for the evaluation of the viability invitro. The infusion rates were measured by using an angio pump device (Angiomat LF 6000). Every infusion rate was evaluated for each different type of implantation catheter, respectively. Cytometric evaluation of cell viability was measured by 7-AAD solution (Coulter, France). The data were analyzed using SPSS 10.0 package program. Results: When the viability was compared as to three different needles, no differences were found (Table 1). Besides, the infusion rate did not show any effect on the viability in each type needles. In conclusion, different diameters of implantation catheters for BMDSCs and the infusion rates do not affect their viability.

Years	*Viability /*	*Flow Rate*	*2002*
Diameter (needle types)	*1cc/sec*	*2cc/sec*	*p*
16 g	87.7% (75.1%-96.4%)	91.3% (70.9%-96.0%)	0.77
18 g	87.9% (69.5%-97.2%)	87.9% (68.2%-96.6%)	0.99
22 g	87.1% (71.1%-96.8%)	88.6% (70.6%-97.4)	0.86
p	0.98	0.96	527

BSH072

TWO INDEPENDENT EFFECTS OF IMMUNOGLOBULIN-LIKE RECEPTOR(KIR) ALLELE MATCHING BETWEEN SIBLINGS: INHIBITORY KIR(IKIR) MISMATCHES ARE ASSOCIATED WITH GRAFT VERSUS HOST DISEASE(GVHD) WHILE ACTIVATORY KIR MATCHES(AKIR) AND CGVHD ARE ASSOCIATED WITH GRAFT VERSUS LEUKEMIA (GVL)

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Background: Activatory KIR receptors are observed less frequently than inhibitory KIRs (iKIR). Although the role of donor iKIRs and recipient ligands have been analyzed extensively, the effect of mismatches(mm) at aKIR or iKIR between donors(D) and recipients (R) have been addressed in only two studies (Gagne, Hum Immunol.2002 and Verheyden, Leukemia 2005): an aGVHD inducing effect of aKIR mm between unrelated donors and a protective effect of aKIRs: 2DS1 and 2DS2 against relapse were reported. The evaluation of other factors ie GVHD on GVL (related transplants) or GVL effects(MUD study) were lacking in these studies. Aim: In this prospective study we aimed to analyze the role of both D and R iKIR, aKIR and KIR-ligand match/mismatches on OS and DFS and made a multivariate comparison of all factors effecting outcome.

Methods: A total of 79 patients with a median age of 34 (M/F: 42/37, AML/CML: 37/33, PBSCT/BMT: 59/20, sex mm: 49 %, ablative/nonablative conditioning: 63/16, BuCy: 72 %) transplanted from their HLA matched siblings. All D and R were typed for KIR genes (2DL1, 2DL2, 2DL3, 2DL4, 2DL5a, 2DL5b, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL2, 3DS1) using the KIR Genotyping SSP Kit (Pel-Freeze, Dynal Biotech, USA). The frequency of GVHD was acute:44/76, chronic: 54/74. Statistical analysis were done using the SPSS 13.0 for Windows. The frequency of relapse in relation to m/mm at iKIR or aKIR alleles are summarized in table 1. Results: Overall KIR mm was observed in 75% of R-D pairs. 37 pairs had mm at the six iKIR loci (33% 2DL5a), 57 pairs had mm at the seven aKIR loci. The analysis on D iKIR / aKIR and the relevant R-ligand m/mm didnot reveal any effect on the frequency of GVHD or DFS. However when we compared the R and D KIR genotypes we were able to show a correlation between cGVHD and m vs mm at iKIR (62.5% vs 85%, p=0.041) and aGVHD (50% vs 67%) but not aKIR.The effect of aKIR mm was not influenced by stem cell source or diagnosis. Among the aKIR only 2DS5 and 3DS1, alone (20/30, 17/25) or together(16/21), resulted with more frequent aGVHD than pairs with other aKIRs (23/45). GVHD was associated with a decrease (aGVHD: p=0.032) or increase (cGVHD: p=0.076) in survival. cGVHD resulted with a decrease in relapse rate(-): 11/20 vs (+): 9/54: p=0.001). aKIRm was associated with an increase on DFS (p:0,031). Factors, other than KIR, known to influence outcome ie stem cell source, sex mismatch, conditioning regimen, disease type were analyzed in the multiple logistic regression and did not reveal any significant results. Conclusion: This study material enabled us to minimize the effect of HLA but

PBSCT being the major source of stem cells potentiated the role of alloreactive T cells and cGVHD. Although only two of the donor aKIRs, 3DS1 and/or 2DS5 were associated with aGVHD, D-R match between all aKIR and cGVHD exerted a protective effect against relapse(0/15). Mismatching for iKIR was also associated with GVHD but GvL wasnot independent of GVHD. Thus, we may conclude that D-R aKIR, iKIR genotyping may help to predict GvL in related transplants.

Frequency of relapse

	Stem Cell source		*aGVHD*		*cGVHD*	
	PB n=59	*BM n=20*	*(+) n=44*	*(-) n=32*	*(+) n=54*	*(-) n=20*
aKIR m n=25	2/17	1/8	2/12	1/11	0/15	3/7
aKIR mm n=54	13/42	6/12	12/32	7/21	9/39	8/13
iKIR m n=44	10/31	4/13	8/21	6/21	4/25	9/15
iKIR mm n=35	5/28	3/7	6/23	2/11	5/29	2/5

BSH073
MULTIPLE MYELOMA AND ITS OCULAR MANIFESTATIONS

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Multiple myeloma is a plasma cell malignancy charaterized by monoclonal protein production and either diffuse osteoprosis or lytic bone lesions. Pathological fractures may be seen on skeletal survey. This disease mainly contained within the bone marrow. Bone marrow biopsy shows uncontrolled proliferation of plasma cells. Ophtalmic manifestations of multiple myeloma tend be rare and variable. Most of these can be classified as ocular, orbital or neuro-ophtalmic. We present ophtalmic manifestations of multiple myeloma patients who were evaluated in our department,

This study was carried out prospectively between 2004 and 2006 at the Ophthalmology, and Hematology department of Gaziantep University Medical School. Sixteen patients were included in this study. 10 were males and remaining 6 were females with a mean age of 54 years (range 40-74). Diagnosis of multiple myeloma was established according to increasing of plasma cells in bone marrow examinations. The monoclonal immunglobulin (M protein) in serum and urine. All patients underwent a complete ophtalmological evaluation for the ophthalmic manifestations during the study. One patient (6.25%) showed corneal involvement. Three patients (18.75%) presented retinal microaneurysm. One patient (6.25%) revealed unilateral retinal ven occlusion in the admission into the hospital. Ocular manifestations of the multipl myeloma tend to be rare. They may appear at the initial presentation of the myeloma or occur late in the disease process. The knowledge of these manifestations can be important for diagnosis, to differentiate earlier this disease and have positive influence on the disease course.

BSH074
A COMPARISON OF MYELOABLATIVE VERSUS REDUCED-INTENSITY CONDITIONING (RIC) ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN ACUTE LEUKEMIAS

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Allogeneic hematopoietic stem cell transplantation (ALLOSCT) can cure some patients with a suitable HLA-matched allogeneic donor. Reduced-Intensity Conditioning (RIC) allogeneic hematopoietic stem cell transplantation is increasingly been used in patients with hematologic malignancies in recent years. In this retrospective study, we have compared the disease-free (DFS) and overall survivals (OS) of the patients with acute leukemias who underwent myeloablative (MA) (Cohort 1 = The patients who underwent myeloablative allogeneic transplantation at the Gazi University Hematology-BMT Unit) versus RIC ALLOSCT(Cohort 2 = The patients who underwent RIC allogeneic transplantation at the Hacettepe University Hematology-BMT Unit) at the two separate referral transplant centers in Ankara-Turkey.

The standard myeloablative conditioning for allogeneic transplantation used in cohort 1 was oral busulfan for 4 days (1 mg/kg q6hours x 4d)for a total dose of 16 mg/kg followed by cyclophosphamide 60 mg/kg/day for 2 days (BUCY) or Cyclophosphamide (60 mg/kg/day for 2 days) plus TBI 1200 cGy (CYTBI). In cohort 1, most of the patients received oral BUx4 days plus CY (Standard BUCY regimen), but only 6 patients received CYTBI conditioning regimen. The RIC conditioning used in cohort 2 was IV fludarabine 30 mg/m2 on days -10 thru -5 for 6 days and IV busulfan (Busulfex®) 0,8 mg/kg every 6 hours on days -6,-5 for 2 days followed by Anti-Thymocyte Globulin (ATG) for 2-4 days (IV Fludara/BU/ATG) as previously described by Slavin et al.1. The GvHD prophylaxis used was cyclosporine (3mg/kg IV inf.) then administered based on blood levels plus short-course methotrexate (CsA plus MTX).

The median follow-up time of all the patients was 11 months. The median follow-up time for cohort 1 (myeloablative ALLOSCT) was 6 months and for cohort 2 was 17 months, respectively. The total number of patients who underwent MA and RIC allogeneic transplantation was fourty-seven (n=47). There were 32 male and 15 female patients, M/F ≈ 2/1 (Table 1). The two cohorts involved the patients with acute leukemias (acute myeloblastic and lymphoblastic) in first or second remission (most of the patients were in first remisson).

The Kaplan-Meier estimate of the DFS for all the 47 patients who underwent allogeneic transplantation for acute leukemias was % 44,5 and OS was % 44,5 at 60-month. The Kaplan-Meier estimate of the DFS and OS for cohort 1 (24 patients who underwent myeloablative conditioning) was % 31,2 and % 26,7 at 34-month, respectively. Kaplan-Meier estimate of the DFS for cohort 2 (23 patients who underwent RIC con-

ditioning) was % 55,5 and OS was % 60,4 at 60-month. RIC ALLOSCT was superior in comparison to the myeloablative conditioning for acute leukemias which was statistical significant when compared with logrank both for DFS and OS (p = 0,023 and p = 0.001, respectively) (Figures 1, 2).

In conclusion, in this retrospective study, reduced-intensity conditioning (RIC) allogeneic hematopoietic cell transplantation seems promising and may be a viable transplant option in patients with acute leukemias who are in remission. Prospective controlled randomized clinial trials should be performed in order to better define the role of RIC allogeneic transplantation in this clinical setting.

Reference

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BSH075

APLASTIC ANEMIA ASSOCIATED WITH NON-A, NON-B, NON-C HEPATITIS

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Aplastic anemia (AA) is an acquired rare disease characterized by the replacement of hemopoietic tissue with fatty tissue. While in Europe, incidence of AA is 2 new cases per 1 million population per year, in Far East it occurs much more common. Causes such as drugs, chemical substances, and radiation and collagen tissue diseases were determined in one-third of cases, while two-third is idiopathic. The viruses related are mainly hepatitis viruses; Epstein-Barr virus (EBV), parvovirus 19, human immune deficiency virus (HIV) and cytomegalovirus (CMV). A rare syndrome of AA was defined after jaundice and high levels of transaminases related with non-A, non-B, non-C hepatitis. Here, a case hospitalized for increased bilirubin and hepatic function tests and investigated because of pancytopenia is presented in whom AA was diagnosed.

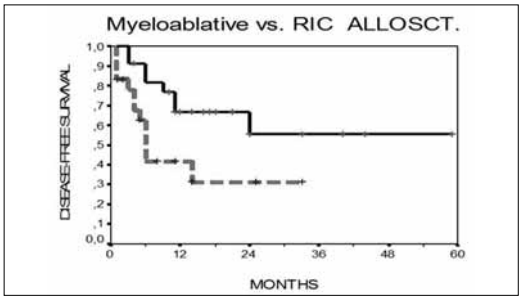

Figure 1. DFS in RIC vs M.Ablative ALLOSCT
DFS in RIC vs M.Ablative

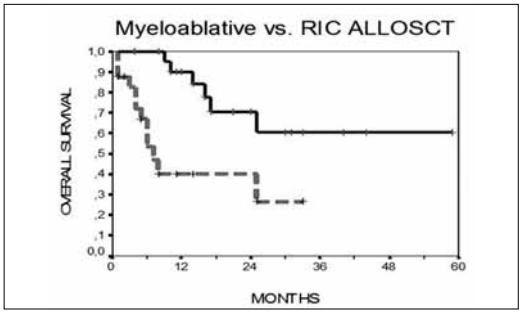

Figure 2. Survival in RIC vs M.Ablative ALLOSCT
OS in RIC vs M. Ablative

ADSOYAD	*YAÞ*	*TANI*	*DFS.AY*	*OS.AY*
COHORT 1				
NÖ.(Myeloablative)	26	2	33	33
ÝI. (M.Ablative)	44	2	14	25
HA.(M.Ablative)	27	1	6	6
VS.(M.Ablative)	31	2	25	25
ÞK.(M.Ablative)	33	1	6	8
ÜÞ.(M.Ablative)	22	2	5	6
HB.(M.Ablative)	46	2	1	1
AH.(M.Ablative)	18	1	4	5
AO.(M.Ablative)	18	2	3	4
ÝE.(M.Ablative)	20	2	14	14
MÞ.(M.Ablative	28	2	4	4
HD.(M.Ablative)	28	2	6	7
HK.(M.Ablative)	47	2	11	11
MÇ.(M.Ablative	17	1	8	8
MT.(M.Ablative)	46	1	1	1
TÞ.(M.Ablative)	16	1	1	1
KM.(M.Ablative)	26	1	5	5
HD.(M.Ablative)	52	2	5	5
OY.(M.Ablative)	53	2	5	5
AA.(M.Ablative	18	1	1	3
YV.(M.Ablative)	19	2	2	2
AÜ.(M.Ablative	37	1	2	2
FY.(M.Ablative)	38	2	1	1
DK.(M.Ablative)	43	2	1	1
COHORT 2				
AK.(RIC AlloSCT)	18	2	59	59
CC.(RIC)	31	2	6	10
EE.(RIC)	37	1	18	31
GTK.(RIC)	25	1	11	16
HY.(RIC)	40	2	9	9
HÞ.(RIC)	33	2	44	44
HT.(RIC)	41	2	6	14
MU.(RIC)	38	1	33	33
MT.(RIC)	23	1	3	30
ÖG.(RIC)	44	1	40	40
SL.(RIC)	40	1	11	17
SG.(RIC)	37	1	24	25
FP.(RIC)	17	1	24	24
SY.(RIC)	27	2	21	21
SK.(RIC)	21	1	17	17
MEG.(RIC)	53	2	16	16
NÖ.(RIC)	39	2	14	14
ÝA.(RIC)	25	2	12	12
MA.(RIC)	33	2	11	11
EE.(RIC)	26	2	10	10
MS.(RIC)	28	1	3	8

Case: A thirty-nine year-old woman presented with headache, vertigo and jaundice was hospitalized in the gastroenterology clinic because of increased bilirubin and hepatic function tests. Corticosteroid therapy was given with the diagnosis of autoimmune hepatitis in the early period. Medical history did not reveal transfusion, any exposure of chemical substance and radiation, and ANA, anti-DNA, markers of HAV, HBV, HCV, CMV and HIV were negative. She had also had severe thrombocytopenia, mild anemia and leucopenia. Since liver biopsy findings were nonspecific and serologic tests for autoimmune hepatitis were negative, steroid therapy was planned to be decreased gradually and stopped. The hepatic function tests were decreased to normal levels and AA was diagnosed after two bone marrow biopsy and blood counts findings. Antithymocyte globulin (ATG) and cyclosporine were administrated, but was stopped because of supraventricular tachycardia. After antiarrhythmic treatment, ATG was completed and cyclosporine was continued with gradually decreased doses. Hypertension and hirsutismus observed were thought to be related with cyclosporine. In the follow up period, pancytopenia was continuing, but there was no need of erythrocyte transfusions, absolute neutropenia disappeared, but thrombocyte transfusions were required in longer periods. Because of problems in the support of the drug, cyclosporine was stopped and then restarted. Treatment of patient is continuing.

Discussion: AA associated with hepatitis is a rare disorder. The role of causes other than known infectious agents in the etiology is unclear. The responsiveness to immune suppressive therapy suggests that AA may be related with immune mechanisms triggered by infections. The duration between hepatitis and pancytopenia is 6-12 weeks, but there are also cases that both diseases occurred simultaneously or hepatitis developed after AA. AA associated with hepatitis is known to have a bad prognosis and has no specific therapy. ATG, cyclosporine and in proper cases bone marrow transplantation are still the most suitable treatment methods.

In the literature, there is a case report of deterioration of cytopenias with steroid therapy, as similar our case. This condition and cyclosporine associated supraventricular tachycardia in our patient was considered to need specific treatment for hepatitis associated AA.

Conclusion: AA associated with hepatitis is a rare condition which requires more knowledge for etiology and treatment. With increase in the number of cases, evaluation of clinical findings and treatment outcomes will be possible. Also the etiology may be clear by extension of serologic tests.

BSH076

SIX-YEAR EXPERIENCE OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA OF A SINGLE CENTER FROM TURKEY

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The aim of this study is to compare outcomes of modified BFM-95 ALL protokol of a single pediatric hematology center with original BFM-95. Kocaeli University Pediatric Hematology Department was established in May 2000 after Marmara earthquake. Until July 2006, 60 children with acute lymphoblastic leukemia were admitted. Two infant leukemia and one Burkitt leukemia (2 are long-term survivals) were not enrolled to evaluate a more homogenous group. Another two patients had to continue their therapy in other centers due to families move to another city. Fifty-five children (49.1% girls and 50.9 boys) with ALL were treated according to the TRALL-2000 protocol which is modified from BFM-95 ALL protocol. Modification was reducing methotrexate to 1 g/m2 in non-T ALL and adding 1200 cGy cranial prophilaxis to all medium risk patients. Mean age was 6.6± 3.5 years (median 5.4, range 2.5-15.1) years, mean initial leukocyte count was 47 074 ± 94 825 (range 700-630 000). 16 patients (29%) were in the standard, 31 (56%) medium and 8 (14.5%) in the high risk group. Immunophenotype revealed 20% T-cell and 80% prekursor B-cell ALL. At presentation 3.6% had central nervous system and 12.7% mediastinal involvement. Fifty-one patients (92%) were good responders to prednisolone, and remission rate at the end of induction was 96.4%. Mortality was 16% (9/55). There were two (3.6%) early deaths before remission (one typhlitis, one septicemia in a primary resistant leukemia), 3 deaths in remission (hepatotoxicity, infection, neurologic complication, respectively), 4 with resistant relapse. Four of the relapses were in the bone marrow, and 1 in the central nervous system. Six-year event free survival (EFS), relapse free survival (RFS), and overall survival rates (OS) were 81.8%, 84.9%, and 83.6% respectively. OS in patients with T-cell ALL was 72,7% and in patients with prekursor B-cell ALL was 84%. One of the relapsed patients is surviving after allogeneic stem cell transplantation from matched sibling donor. OS, EFS, RFS rates were 100 % respectively in standard risk group; OS, EFS, RFS rates were 83.9%, 80.6%, 80.6% respectively in medium risk group; OS, EFS and RFS rates were 50%, 50% and 66.7 % in high risk group respectively.

Standards of the hospital rooms improved and number of beds increased in the last year. Although standards of the hospital rooms were poor in the early years, it seems that survival rates with modified BFM-95 protocol are comparable to original protocol.

BSH077

THE DIFFERENT BCR/ABL TRANSCRIPTS IN CML

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Philadelphia chromosome t(9:22) is the most common type of cytogenetic anomaly in human leukemias. It is present in 90% of chronic myeloid leukemias (CML) and 3-40% of acute lymphoblastic leukemias (ALL). It arises from a reciprocal translocation of ABL sequences from chromosome 9 to BCR sequences on chromosome 22. There are two

major forms of BCR/ABL fusion genes. In CML, the breakpoint in the BCR gene occurs in the major breakpoint cluster region (M-bcr). This BCR-ABL fusion gene in CML is transcribed into one of two 8.5kb mRNAs called as b2a2 and b3a2 respectively. They encode p210 proteins. The second forms, e1a2 and e1a3 occur in minor breakpoint cluster region (m-bcr). They are transcribed into 7.0 kb mRNAs and present mostly in ALL (p190). Breakpoint in micro breakpoint cluster region encodes e19a2 fusion gene and produces p230 protein. This fusion gene is present in chronic neutrophilic leukemias. All these proteins enhance protein tyrosine kinase activity and they have been thought to play a main function in leukemic transformation. In this study, we detected the ratio of different type of BCR/ABL gene transcriptions. Sixty-six t(9:22) positive CML patients from two different centres were included in to study. Sokal scores of patients were calculated. All of the patients had active disease when the samples were collected. Twelve patients were received treatment with interferon (IFN)/IFN-ARA-C. Each patients were searched for p210 and p190 by nested reverse transcriptase PCR according to Biomed I protocol. P230 fusion gene was not searched in this study. Results are given on table 1. The most frequent type of transcriptions was p210 (n=50, 76%), p190 consisted of 16.5% (n=11). Five patients had both p190 and p210 transcriptions (7.5%). The most common type of p210 was b3a2 transcription (n=27, 54%), p190 was e1a3+e1a2 (n=4, 36%), p210+p190 was b3a2+e1a3 (n=2, 40%) and b3a2+e1a3+e1a2(n=2,40%). The clinical differences and effects to prognosis of different types of transcriptions aren't known in CML yet. In this study there was no significant relation between Sokal scores and transcriptions. In recent studies, p210 was detected to be the unique transcription in 90% of CML patients. P190 transcription in CML was reported as sporadic cases. In our study, ratio of p210 was low. In one study, it was shown that 88% of chronic phase CML and 100% of blastic phase CML patients had p190 expression but in low levels. And it was explained that co-expression of p190 with p210 could occur by "alternative" or "missplicing". According to our findings co-expression of p190 with p210 was 7.5% by nested PCR, a sensitive protocol. Co-expression of p190 with p210 can exist in accelerated or blastic phase, however this suggestion can not explain the p190 expression alone. In the literature, it was shown that p210 disappeared after the imatinib mesylate (IM) treatment in a patient with co-expressions of p190 and p210. P190 survived. In our study we didn't aim to detect transcriptions after treatment. The samples were collected before treatment with IM. But there was no significant difference between the patients who received IFN and/or IFN-ARA-C and who did not according p190 expression. In another study which was performed to detect the sensitivity of 10-8, it was shown that healthy subjects could also be express BCR/ABL transcript. In conclusion, the ratios of different BCR/ABL fusion genes can be changed according to study protocol.

	p190 (n=11, %16.5)			*p210 (n=50, %76)*			*p210+p190 (n=5, %7.5)*		
transkript	e1a3	e1a2	e1a3 + e1a2	b2a2	b3a2	b3a2 + b2a2	b3a2 + e1a3	b3a2 + e1a2	b3a2 + e1a2+e1a3
n	2	5	4	15	27	8	2	1	2
%	%3	%7,5	%6	%23	%41	%12	%3	%1.5	%3

BSH078

PROGNOSTIC SIGNIFICANCE OF CELLULAR VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION IN CHRONIC MYELOID LEUKEMIA

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Background: The chronic phase (CP) of chronic myelogenous leukemia (CML) is characterised by the presence of chimeric BCR/ABL gene and a profligate growth of mature polymorphonuclears. The accelerated phase and blast crisis (AP and BC) of CML may show additional oncogenic aberrations and pronounced anaplasia manifested by an increase in organomegaly and blast count. Increased angiogenesis in bone marrow is one of the characteristics of chronic myeloid leukemia (CML). Vascular endothelial growth factor (VEGF) is one of the most potent and specific regulators of angiogenesis which principally targets endothelial cells and regulates several of their functions, including mitogenesis, permeability and migration. The impact of elevated VEGF expression on the course of chronic myeloid leukemia is unknown.

Aim: The follow-up of VEGF expression during the course of CML.

Methods: We studied 85 patients (pts.) with the median age opf 50 (range 16-75 years). At the commencement of the study, 29 pts.were in CP, 25 in an AP, and 31 in the BC. The temporal expression (percentage positivity per 1000 analysed cells) VEGF proto-oncogene proteins over the course of CML was studied using the immunohistochemical tehnique which utilizes relevant monoclonal antibodies. It was correlated with the laboratory (Hb, WBC and platelet counts, and the percentage of blasts) and clinical parameters (organomegaly, duration of CP, AP, and BC) of disease progression.

Results: The expression of VEGF protein was most pronounced in an AP (ANOVA, p=0,033). The level of VEGF expression correlated inversely with degree of organomegaly (Pearson, r=-0,400, p=0,011). High expression of VEGF correlated with a longer duration of CP (log rank, p=0,0304) and with a longer overal survival (log rank, p=0,042).

Conclusion: The significance of changes in VEGF expression, estimated by a histochemical approach over the course of CML, may be of clinical importance in deciding on and timing of therapy. These data suggest that VEGF plays a role in the biology of CML and that VEGF inhibitors should be investigated in CML.

BSH079

IMMUNOPHENOTYPIC PROFILE AND CLINICAL CHARACTERISTICS IN PATIENTS WITH ADVANCED STAGE MANTLE CELL LYMPHOMA: SINGLE CENTER STUDY

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The objective of the study was to evaluate immunophenotypic profile and clinical characteristics in patients (pts) with advanced stage mantle cell lymphoma (MCL) and their possible influence on overal survival (OS). Forty pts with advanced stage: (CS IV) and leukemic phase (CS V) of MCL were examined. Diagnosis was based on morphology, immunophenotype, histology and cyclin D1 expression. Bone marrow cell and/or peripheral blood mononuclear cell flow cytometric analyses of the following antigens performed: HLA-DR, CD19, CD20, CD22, CD23, CD25, CD10, SmIg, kappa, lambda, CD79b, CD38, FMC7, CD3, CD2, CD5. Among 40 pts, there was 14 pts in IV CS, and 26 pts in CS V. All pts were treated with CHOP. The median age was 63 years and male:female 1.8:1. Presented features included splenomegaly (80%), lymphadenopathy (52%), hepatomegaly (50%) and extranodal localisation (22.5%). Median peripheral blood lymphocyte count was 72x109/l, 50% pts had anemia and 57% thrombocytopenia. Morphology of peripheral blood showed blastoid variant in 17.5%, and typical MCLand small cells in rest of cases. Immunological markers showed a typical phenotype (CD5+ CD23-, Cyclin D1) in all cases. Pathohystological type of bone marrow (BM) infiltration was predominant diffuse (72.5%), and in rest of pts was nodular. Comparing pts with leukemic phase of MCL with CSIV(BM), we found significant higher expression of CD19(76.2%vs.64.4%, p=0.05), CD20 (78.3%vs.67.1%, p=0.04), and CD23(9.5%vs.3.9%, p=0.02), but expression of CD23 was always negative(<30%). Also, in pts with blastic variant of MCL, there was significant higher expression of CD23,comparing with typical MCL (18.3%vs.5.3%, p=0.000004). Median OS was 20 months.There were no significant differences in OS between pts with CS IV and leukemic phase (p>0.05). Survival analyses showed that negative prognostic influence had high IPI (log rank, p<0.01), presence of extranodal localisation (p<0.01), and diffuse type of BM involvement (p<0.01). The influence of CD38 expression was of limited value (p=0.08).But, using Cox regression according to OS, only IPI was factor with indipendent prognostic value (p=0.000225). Our results demonstrated that in this group of advanced MCL pts treated equally, the most powerfull prognostic factor is IPI, while extranodal localisation and type of BM infiltration were of limited value.

BSH080

MULTIPLE MYELOMA: DELINEATION OF DISTINCT SUBGROUPS AND THEIR CLONAL EVOLUTION BY INTERPHASE CYTOGENETICS

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Two partially overlapping pathogenetic pathways in multiple myeloma (MM) have been proposed: non-hyperdiploid and hyperdiploid MM. Reccurent IgH-translocations have been proposed as seminal event associated with non-hyperdiploid MM. In view of the limitations of conventional cytogenetics in MM, interphase fluorescence in-situ hybridization (iFISH) was applied to delineate MM subgroups and their clonal evolution as a basis for further biological and prognostic evaluation. The study included 81 newly diagnosed MM patients (pts). Mean age was 61 years (range, 38-83). The distribution according to the clinical stage of disease (Salmon&Durie) was as follows: I 8pts; II 12pts; III 61pts. There were 48pts with IgG monoclonal protein, 20 with IgA, and 12 with secretion of light chains. Non-secretory MM was diagnosed in 1pts. Application of the ISS score revealed the following distribution: (1) 25pts; (2) 37pts; (3) 19pts. iFISH was applied on CD138-selected bone marrow cells (median purity 95%) with 10 specific probes for chromosomes 1q21, 6q21, 8p12, 9q34, 11q23, 13q14.3, 17p13, 22q11 and 2 commercial dual-color fusion probe sets for the translocations t(11;14)(q13;q32.3) and t(4;14)(p16;q32.3). A copy number score (CS) was calculated for each patient by subtracting the number of probes expressing losses from the number indicating gains. Clustering analysis based on Kendall's τ coefficient and statistical modeling of oncogenetic tree based on maximum likelihood estimation were applied to elaborate the associations and biological order of cytogenetic aberrations. Per patient, a median of 5 probes (range, 1-10) displayed aberrant signal numbers. Additional copies were most frequently found for chromosomes 15q22, 19q13, 9q34, 11q23, 1q21. Common losses were observed for 13q14.3, 17p13, and 22q11. Predominance of gains or losses was quantified for each patient, by a CS value. Two peaks (CS= +3, and CS= 0) were found by plotting patient numbers over CS values, corresponding to hyperdiploid and non-hyperdiploid MM. Cluster analysis revealed four major branches: (i) gain of 9q, 15q, 19q, and/or 11q; (ii) deletion 13q and t(4;14); (iii) t(11;14); and (iv) gain of 1q. Statistical modeling of oncogenetic tree indicated as early independent events: (i) t(11;14); (ii) gain of 15q/9q and/or 11q as a pathway putatively leading to the hyperdiploidy; (iii) deletion 13q followed by t(4;14) as a pathway leading to the non-hyperdiploidy; and (iv) in agreement with the clustering analysis, gain of chromosome 1q21 separated early from the pathway leading to the non-hyperdiploidy. Aberrations of 17p13, 22q11, 8p12, and 6q21 were found as a subsequent events. MM with gain of 1q was found as a subentity with significantly higher β2 microglobulin and lower hemoglobin levels, indicating a poor prognosis. Clustering and statistical modeling of MM oncogenetic tree model based on iFISH results, give evidence of distinct myeloma subgroups with possible different clinical outcome.

BSH081

BIPHENOTYPIC AND BILINEAL ACUTE LEUKEMIA: REPORT OF 11 CASES

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Background: Biphenotypic acute leukemias are rare leukemias and account for less than 4% of acute leukemias. They are characterized by co expression of lymphoid and myeloid markers on the same leukemic cells. Bilineal acute leukemias comprise less than 1% of acute leukemias, basically characterized by two separate clones of myeloid and lymphoid leukemic blasts. The clinical behavior and treatment response of biphenotypic and bilineal acute leukemias are not clearly definite and there is no agreement about the type of treatment required.

Aims: to describe the biological and clinical features as well as treatment response in 9 cases of biphenotypic and 2 cases of bilineal acute leukemias.

Methods: We describe the clinical features, morphology and cytochemistry, immunological markers and cytogenetics in 9 biphenotypic and in 2 bilineal acute leukemias.

Results: Between January 1999 and December 2005, 862 cases of acute leukemias were diagnosed in Institute of Haematology in Belgrade, of these 9 cases were observed who fulfilled the EGIL (European Group for Immunological Classification of Leukemias) criteria for acute biphenotypic leukemias and 2 cases which defined as bilineal acute leukemias; 5 biphenotypic leukemias showed co expression of myeloid and T lymphoid markers, and 4 myeloid and B lymphoid markers. Both of bilineal leukemias showed two separate cell lines, myeloid and T lymphoid. Following features were observed at diagnosis: M/F: 5/4, median age 37 years (18-64), haemoglobin 9.3 g/dl (6.8-12.7), WBC 70x109/L (9-270), platelets 97x109/L (22-358), blasts in per. blood 57% (20-91), bone marrow blasts 86% (73-98). Both bilineal leukemias were female, 27 and 17 years old, with hemoglobin 11.1 g/dl and 11.5 g/dl, WBC 132x109/L and 119x109/L, platelets 49x109/L and 83x109/L, blasts in per. blood 75% and 55%, and bone marrow blasts 75% and 60%. Cytogenetics were available in 8 patients with biphenotypic leukemias: 2 (25%) showed t(9;22), in 1 (12.5%) case associated to complex karyotypic changes, 1(12.5%) cases showed complex chromosome abnormalities; 1 (12.5%) monosomy 7; 1 (12.5%) monosomy 8; and 3 (37.5%) cases had a normal karyotipe. PCR analyses was done in one Ph+ patients and showed BCR/ABL rearrangement with p190 expression. One of bilineal leukemias showed trisomy 4, and other del(8)q(22). Three (33.3%) patients received lymphoid like therapy: one obtained CR and still surviving; one relapsed after 3 months and died of disease progression; and one obtained CR with second line treatment and MUD PBSCT and still surviving. Six (66.6%) patients received myeloid like therapy: 4 (44.4%) died after induction therapy; 2 (22.2%) were resistant and died of disease progression. The overall median survival was 12 months, with 2 (22.2%) still surviving with a median follow up of 42 months. One patient with bilineal leukemia received myeloid like therapy and died after induction therapy; second received high dose therapy for AML followed by BMT, obtained CR and still surviving (6 years).

Conclusions: Our results confirm a severe prognosis of biphenotypic and bilineal acute leukemias, especially after myeloid like treatments. Although there are no uniform criteria about whether to treat these patients, intensive approach with high dose therapy followed by bone marrow transplantation, will be required to eradicate the disease.

BSH082

HEREDITARY THROMBOPHILIA IN YOUNG PATIENTS WITH MYOCARDIAL INFARCTION

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Background: Association between hereditary thrombophilic mutations and venous thromboembolism is well established. On the other hand, involvement of inherited prothrombotic anomalies in occurrence of coronary thrombosis and myocardial infarction (MI) is still a matter of debate. Whether prothrombotic mutations influence long-term prognosis in patients with myocardial infarction is not clear, too.

Aims: To investigate prevalence of hereditary thrombophilia in patients with MI younger than 40 years of age, and without traditional risk factors for atherosclerosis. To evaluate influence of inherited thrombophilia on long-term prognosis in those patients.

Methods:In this study we investigated prevalence of antithrombin (AT III), protein C (PC), protein S (PS) deficiencies, and factor V Leiden (FV Leiden) mutation in 33 (5F and 28M) selected patients who suffered first myocardial infarction before 40 years of age. In all investigated patients traditional risk factors for atherosclerosis, except smoking, were absent. Prothrombin fragment F 1+2, and D-dimer as markers of activated coagulation and fibrinolysis have also been measured. All laboratory investigations have been performed at least 3 months after acute coronary event. Patients have been followed in average next 8 years after first episode of MI, and all cases of coronary death, reinfarction and myocardial revascularization have been recorded.

Results: Presence of inherited thrombophilia has been found in 8/33 (24%) patients (AT deficiency in 2, PC deficiency in 2, PS deficiency in 1 and FV Leiden in 3 patients), and in 1/20 (5%) healthy controls (FV Leiden in 1 control person) matched by sex and age ($p<0.01$). Among patients with MI and inherited thrombophilia proportion of women was higher (38%) than among patients with MI but without thrombophilia (8%). Mean values of F1+2 and D-dimer were higher in MI patients with thrombophilia than in patients without thrombophilia (1.39±0.28 vs.1.04±0.43 nM, $p<0.05$; 69±25 vs.42±26 ug/l, $p<0.05$, respectively). During follow-up one patient was lost, and 10 patients (31%) suffered recurrent coronary event. Interestingly, 3 out of 8 (38%) patients with MI and hereditary thrombophilia died during follow-up while in the gruop without hereditary thrombophilia none of patients have died in the same period.

Conclusions: According to our results hereditary thrombophilia is not rare condition in selected young patients with myocardial infarction. More important, the presence of hereditary thrombophilia strongly influenced the long-term prognosis of young patients with myocardial infarction.

1ST BALKAN HEMATOLOGY DAYS

Author Index

TURKISH JOURNAL OF HEMATOLOGY
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The Turkish Journal of Hematology accepts invited review articles, research articles, brief reports, case reports, letters to the editor, and images in Hematology on subjects within the scope of hematology, on the condition that they have not been previously published elsewhere. All papers are subject to editorial revision for purpose of conformity to the style adopted by the Journal. Manuscripts should in general not exceed 20 typewritten pages for original articles or reviews and 7 typewritten pages for case reports, including all tables, figures and illustrations.

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Each of the following sections of the manuscript should be typed on separate pages. Title Page should include (in Turkish when possible): (a) title of the article in a concise but informative style, (b) first name, middle initial, last name of each author, (c) name of department(s) and institution(s) to which the work should be attributed, (d) name and address of author responsible for correspondence for the manuscript, (e) name and address of author to whom requests for reprints should be addressed, (f) source(s) of support in the form of grants, equipments, drugs, etc., and (h) short running title of no more than 40 characters.

Authorship: Each author should have participated sufficiently in the work to take public responsibility for the content. Any part of an article critical to its main conclusions must be the responsibility of at least one author.

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Discussion: Emphasize the new and important aspects of the study and the conclusions that follow them. Link the conclusions with the goals of the study but avoid unqualified statements and conclusions not completely supported by your data.

Acknowledgements: On a separate page after the completion of the Discussion section, one or more statements should mention, if necessary, (a) contributions that require acknowledgement but do not justify authorship, (b) any technical help, and (c) financial and material support, specifying the nature of the support.

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