Oral presentations

Friday, 05.09.03

First Session: The problems of coagulation and fibrinolysis.
Chairmen: Prof. Afanasyev B.V., Prof. Selivanov E.A., Prof. Wisloff F.
Sandset P.M. New trends in treatment of DVT/pulmonary embolism.

Second session. Problems in bone marrow transplantation.
Chairmen: Dr. Brinch L., Prof. Zander A., Dr. Ptushkin V.V.

Brinch L. Allogeneic haematopoietic stem cell transplantation in adult patients at the National University Hospital, Oslo, Norway.

Savchenko V.G. Strategies in bone marrow transplantation.

Afanasyev B.V. Hematopoietic recovery after bone marrow transplantation.

Kroger N. Reduced intensity conditioning for multiple myeloma and osteomyelofibrosis.

Vavilov V.N. Lymphocyte recovery after unrelated allogeneic hematopoietic stem cell transplantation.

Zaraisky M.I. Cytokine production during immunosuppressive therapy.


Ptushkin V.V. High-dose chemotherapy with autologous hematopoietic progenitor-cell transplantation in relapse and primarily resistant Hodgkin disease. Retrospective analysis of treatment of 178 patients in Russia, Belarus and Ukraine.

Novik A.A. The concept of cell therapy in autoimmune diseases.

Saturday, 06.09.03

First session: Pathogenesis and treatment of Multiple Myeloma.
Chairmen: Prof. Borset M., Prof. Waage A., Prof. Lisukov I.A.

Borset M. Life and death of myeloma cells.
Lisukov I.A. Immunological and molecular aspects of tumor progression in multiple myeloma.
Baikov V.V. Migration and adhesion of myeloma cells.
Mamaev N.N. A new approach to hematopoiesis evaluation under multiple myeloma.
Waage A. Thalidomide treatment of multiple myeloma.
Wisloff F. Quality of life and cost-value analysis in multiple myeloma.

Chairmen: Dr. Tangen J.-M., Prof. Savchenko V.G., Prof. Uss A.L.

Wergeland L. Molecular biology of acute leukemia.
Abdulkadyrov K.M. Problems in the treatment of hematological patients in Saint-Petersburg.
Parovichnikova E.N. Randomized multicenter studies on the acute leukemia treatment in Russia
Tangen J.-M. Norwegian patients <60 years with primary AML, treated with a common national programme which comprises repeated courses of high dose Ara-C as consolidation therapy, as well as allo-BMT. A report from the Norwegian Registry of acute leukemias and lymphoblastic lymphomas.
Mikhaylova N.B. Treatment results of patients under 60 years old with acute leukemia: summarized data from the Hospital Registries of St.-Petersburg, Leningrad region and Republic of Karelia. (Three year experience.)
1. NEW PERSPECTIVES ON VENOUS THROMBOEMBOLISM (VTE).

Sandset P.M.
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Venous thromboembolism (VTE) is relatively frequent with an overall incidence of 1/1000/year, is often disabling due to post-thrombotic syndrome and pulmonary hypertension, and is sometimes fatal. Diagnosis, prevention and treatment of VTE should therefore have high priority in the medical community. During recent years, great advances have been achieved in the field of mechanism, diagnosis and treatment of VTE. The importance of environmental risk factors, such as, age, cancer, surgery, and female hormones, acquired risk factors, such as lupus anticoagulants, anticardiolipin antibodies, and hyper-homocysteinemia, and genetic risk factors, such as antithrombin-, protein C- and protein S deficiency, and factor V Leiden- and prothrombin gene mutations, has been established. During the 1990ies, low molecular heparin(s) (LMWH) became established as the treatment of choice for the prophylaxis and treatment of VTE. LMWHs had superior pharmacokinetic properties compared with unfractionated heparin, including near complete bioavailability after sc injections and longer half-life in vivo. LMWHs could therefore be administered subcutaneously on a unit/kg basis essentially without monitoring and on an ambulatory basis. Numerous clinical studies have later demonstrated the superiority of LMWHs for the prophylaxis and treatment of acute VTE. In acute VTE, LMWH is given for 5-10 days followed by an oral vitamin K antagonist, e.g. warfarin. Clinical studies have demonstrated the optimal intensity (INR 2,0-3,0) and length of treatment. Due to high risk of recurrence and low rate of bleeding complications associated with low-intensity vitamin K antagonist treatment (INR <2,5), new guidelines tend to recommend prolonged treatment. Catheter based local thrombolytic therapy is a promising treatment modality which is currently used in many centers. Unfortunately, the efficacy and cost-effectiveness of this demanding therapy has not been documented in randomized clinical trials. Several new agents are currently in development for the prophylaxis and treatment of VTE, which includes direct thrombin inhibitors, factor Xa inhibitors, and inhibitors of factor VIIa/tissue factor. Two such compounds, i.e., a synthetic pentasaccharide (fondaparinux) and a synthetic thrombin inhibitor ((xi-)melagatran), have recently completed phase III clinical trials, which have demonstrated superiority as compared with LMWHs for the prophylaxis against postoperative thrombosis and non-inferiority with regard to treatment of venous thrombosis.
2. CORRECTION OF HEMOCOAGULATION AND FIBRINOLYSIS AT USE PLASMAPHERESIS AT THE PATIENTS WITH HEMOBLASTOSIS.


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Malignancies of hematopoietic and lymphatic systems - leukemias and lymphomas are characterized by features common to solid tumors and have similar mechanism of development, although they have some peculiarities (Nahimov E.I. 1987). In most cases they are accompanied by hemocoagulation and fibrinolysis defects, and overt clinical features of the disease.

The purpose of the work was to study hemocoagulations and fibrinolysis defects in patients with hemoblastosis and methods of correction of these conditions.

We carried out research of hemostasis system in 58 patients with hemoblastosis (Non-Hodgkins lymphoma, Hodgkins lymphoma, acute leukemias). The efficacy of plasmapheresis for correction of coagulation and fibrinolysis defects were studied.

In 94 % of studied patients defects revealed: decreased level of platelets, reduction of their adhesiveness, aggregation and circulating activity, reduction of fibrinogen level and total activity of curtailing plasma factors, and elevation of the degradation of fibrin and fibrinogen products with anticoagulant activity. The direct correlation between parameters of coagulation and clinical features of DIC-syndrome was the basis for use of plasmapheresis in a co-function with other kinds of treatment in the patients with hematological malignancies. The single volume of removed plasma was 400-500 ml and depended on the status of a patient and laboratory data researches, number of sessions have been 4-7. Exfused volumes of protein components of plasma were adequately corrected with donor fresh-frozen of plasma (60-70% from volume of the removed plasma), albumin (10-20 %), also was carried out infusion of colloid-crystalloidine solutions (10-20 %). The effect of plasmapheresis resulted in clinical improvement of the patients conditions and coagulation parameters.
1. ALLOGENEIC HAEMATOPOIETIC STEM CELL TRANSPLANTATION IN ADULT PATIENTS AT THE NATIONAL UNIVERSITY HOSPITAL, OSLO, NORWAY.

Brinch L.1, Egeland T.2, Albrechtsen D.3, Evensen S.A.1, Gedde-Dahl T.1, Tjonnfjord G.1,  
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Norway has a population of about 4.5 million people. Allogeneic haematopoietic stem cell transplantation is centralized to the National University Hospital, Oslo, which performs transplants in adults as well as in children. The first transplant was performed in 1976, between 1981 and 1985 a limited number of procedures were performed, mainly in patients with advanced stages of leukemia. From 1985 there has been a Norwegian programme for allotransplantation and between November 1985 and June 2003 350 transplantations have been performed in adult patients and about 150 in children.

The Norwegian allogeneic stem cell transplantation program is defined by a group of representatives from the departments of haematology of the five university hospitals, which gives advice to the Central Norwegian Health authorities on established indications, activities, new developments and prognosis for future requirements. We have distinguished quite clearly between established and developmental indications and have out of necessity given priority to established indications and methods due to the limited access to resources. This explains the relatively low number of transplantations compared to some other western European countries.

The activity was quite limited during the first few years, but has increased steadily from 1990 when the first transplantation with an unrelated donor was performed. It now has levelled off at about 35 procedures being performed per year in adults, and about 15 in children. During the past 2-3 years a limited number of transplantations with dose reduced conditioning have been performed.

The main indications have been CML in the first chronic phase, acute myeloid leukemia, acute lymphoblastic leukemia, myelodysplastic syndromes, severe aplastic anaemia, and a limited number of patients with multiple myeloma, primary myelofibrosis and PNH.

For most patients we have used and are still using the BuCy regimen as the conditioning regimen and for GVHD prophylaxis we have used cyclosporine with a short course of metotrexate. We have not used T-cell depletion.

The median age of the adult patient population (range 15-61 years) is around 40 years and the overall estimated survival at 10 years is about 55-60% for the total material with a treatment related mortality of about 25-30%. The overall survival in patients treated with a family donor is clearly better than in patients treated with an unrelated donor. This is probably explained by the high number of high-risk patients in the unrelated donor group which also had a higher treatment-related death rate than patients transplanted with a family donor.

More detailed information will be presented.
2. NEW APPROACHES IN HEMATOPOIETIC STEM CELL TRANSPLANTATION

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According to the data of the National Research Center for Hematology, Moscow, classical allogeneic BMT provided 58% of 12-years DFS in chronic phase CML patients (n=43) and 45% - in first CR AML patients (n=32). During the last 10 years new strategies in stem cell transplantation for high risk leukemia patients have been extensively developed and applied: 1) adoptive immunotherapy with donor lymphocytes (DLI); 2) non-myeloablative BMT (nm-BMT).

DLI in relapsed after allogeneic BMT patients are used in our clinic since 1993. 14 patients were treated: ALL – 2, AML – 6, CMML – 1, CML - 5. Donor lymphocytes were infused: 1) once in two weeks, 3 times, followed by one infusion in 2 months, 6 times; 2) once a week, 4 times. DLI were applied in acute leukemia relapsed patients without preceding chemotherapy, or after CR achievement, or while aplasia after reinduction chemotherapy. IL-2 was used in half of the cases (2-6 MUE). Chimerism was detected and monitored by PCR analysis (hypervariable regions of APO-B-gene, VWF-gene, DX S52, D17 S30, D1 S80, D1 S111, LP3, SRY) and by FISH-analysis for centromers of X and Y chromosomes.

None of ALL patients responded to DLI. CMML patient attained the second CR (+14 mo). CR was also achieved in 5 of 6 AML patients (83%). CR in two patients continued for 27 and 50 months, but both of patients died: one from relapse and the other - from B-cell lymphoma originated from ‘host’ hematopoiesis. Three other patients are well and alive after DLI for 3, 2,5 and 1,5 months. Mixed chimerism at relapse was registred in all patients and was converted to complete donor chimerism after effective DLI. 3 of 5 CML patients (60%) restored Ph-negative haematopoiesis with complete donor chimerism. Second CR duration is 6,5 years, 4 years and 4 months. CR in our patients was registred only in those who were transfused with higher lymphocytes dose: more than 1x10^8 cells/kg (1,2-1,4 x10^8 cells/kg) per infusion and total dose - 7,5 (4,7-10,5) x10^8 cells/kg. These counts exceeded 0,6-0,9 x10^8 cells/kg per infusion and total dose - 2,8 x10^8 cells/kg in patients with ineffective DLI.

Non-myeloablative regimens (Fludara + busulphan ± ATG or Cph) in hematopoietic stem cells transplantation are being used in our center since 1999 year. 15 patients with higher median age - 42 years (18-52 years), and second CR after AML relapse (n=4) or partial remission (n=2), MDS with poor risk cytogenetic aberrations and in transformation (n=9), poor somatic status (cardiac failure) were transplanted. In 5 cases of MDS (3 with monosomy 7) transplantation was the first line treatment (4 of them are alive and well). Totally 4 patients died due to disease progression at 1-3 months after transplantation (3 of them with acute GVHD, II-IV grade), 2 – due to graft failure (1 case with CMV infection). 9 patients are in complete CR and alive for 2-36 months. 5 of them developed acute GVHD (+17 - +66 days), grade II-III and 4 – chronic GVHD (+90 - +101 days). Event-free survival at 3 years in this extremely high risk group of patients is 42% that could be considered as a really good outcome. GVHD constitutes the major problem in nm-BMT.
3. THE FEATURES OF HEMATOPOESIS RECOVERY DURING POSTTRANSPLANT PERIOD.

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The hematopoetic stem cells transplantation (HSCT) is the efficacious method of treatment of different hematological, oncological, autoimmune and genetic diseases. Rather high level of the mortality, related to cytopenia period, limits more wide use of HSCT. The main factors, affecting on speed of hematopoetic reconstitution, are those linked with patients: age, diagnosis, disease stage, status at the moment of HSCT, malignant cells and fibrosis presence in bone marrow, characteristics of previous chemotherapy and irradiation, CMV infection. Quality and quantity characteristics of transplant also significantly determine hematopoetic recovery: source of HSC (bone marrow, blood, cord blood, autologous, allogeneic, related, unrelated, donor’s age, period duration between HSC collection and transplant infusion, features of HSC manipulations ex vivo, CMV +/-), grade of immunological compatibility, influencing GVHD development, transplant priming by CSF, nucleated cells, CD 34+, CFU-GEMM, CFU-GM, CFU-E quantity. Type of conditioning regimen (myeloablative and non myeloablative) and curative protocol in posttransplant period has also impact on hematopoetic recovery.

Allogeneic HSCT was performed in 79 patients of age from 2 to 65 years: allo-BMT in 47 patients, allo-PBSCT in 32 patients. There were 17 patients with AML, 3 patients with MDS, 30 patients with ALL, 15 patients with CML, 7 patients with severe AA, 1 patient with Hodgkin disease (HD), 2 patients with non-Hodgkin lymphoma (NHL), 3 patients with solid tumor (ST), 1 patient with hypereosinophilic syndrome. Autologous HSCT was performed in 180 patients of age from 4 to 65 year: auto-BMT – in 89 patients, auto-PBSCT +/- BMT in 91 patients. Among them were 15 patients with AML, 12 patients with ALL, 1 patient with CML, 30 patients with NHL, 31 patients with MM, 35 patients with HD, 48 patients with ST, 3 patients with multiple sclerosis. Syngeneic BMT was performed in 3 patients.

The predictive parameters for shorter post-transplant cytopenia duration were: young age of recipient and donor, complete remission and good status at the moment of HSCT, early terms of HSCT with low number of preceding chemotherapy and irradiation courses, absent of GVHD and CMV-infection, nonmyeloablative conditioning, HSC priming be CSF, high level of nucleated cells and CD 34+ in transplant. The SCF used in posttransplant period, source of allogeneic HSC (related, unrelated), donor-recipient sex mismatch didn’t influence significantly on hematopoetic reconstitution speed. ABO-system incompatibility may cause of postpone reconstitution of erythropoiesis.
4. REDUCED INTENSITY CONDITIONING FOR MULTIPLE MYELOMA AND OSTEOMYELOFIBROSIS.

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High dose therapy followed by allogeneic bone marrow transplantation from a matched donor allows for curative treatment of leukemia and other hematologic malignancies.

The exploration of allogeneic transplantation in these areas is limited by the transplant related mortality.

The introduction of reduced intensity conditioning which relies less on the power of cytoreduction and more on the immunotherapeutic aspect of the bone marrow transplantation allows for further exploration of these treatments in other hematologic malignancies.

We report the feasibility of the matched unrelated donor transplantation in 31 patients with a stage II/III multiple myeloma following a reduced intensity conditioning regimen with Fludarabine (90 – 180 mg/m²) and Melphalan (100 – 140 mg/m²) and Antithymocyteglobuline (ATG, 3 x 10-20 mg/kg). Complete donor chimerism was detected in all evaluable patients by day 40. Grade II to IV acute GvHD was seen in 17 (57 %) and grade III for GvHD observed in 7 patient (23 %). The estimated probability of TRM at 1 year was 27 %.

The two year estimated overall and progression free survival is 46 and 36 %.

Dose reduced intensity conditioning provides for durable engraftment and reduces significantly the risk of transplant related organ toxicity in the severely pretreated patient population.

10 patients with osteomyelofibrosis (OMF) were treated with Busulfan (10 mg/kg BW, Fludarabine 6 x 30 mg/m² and ATG 3 x 20 mg/kg BW) bought by allogeneic family or unrelated donor transplant. Engraftment occurred in all patients, chimerism was complete by day 100. Transplant related mortality was 10 %. The longest follow-up of the OMF patients is 2 S years in complete remission. Reduced intensity conditioning transplant can be successfully applied for treatment of multiple myeloma and OMF.
5. THE PREDICTING VALUE OF THE LYMPHOCYTE RECONSTITUTION AFTER UNRELATED HAEMATOPOIETIC CELL TRANSPLANTATION IN PROGNOSIS OF CLINICAL OUTCOME.

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The speed of the absolute lymphocyte count (ALC) recovery after autologous and allogeneic haematopoietic stem cells transplantation (HSCT) is known as one of the important prognostic factors, which allows predicting of outcome for patients with different hematological and non-hematological malignancies. We provided the analysis of 168 allogeneic HSCT from unrelated donor in 163 adult patients, who were transplanted in BMT Centers of St Petersburg and Hamburg in 1993-2003. 73 of them had CML, 44 – acute leukemia, 18 – multiple myeloma, 14 – MDS, and 13 – other hematologic malignancies. Median follow-up was 211 days (21-2593). We took a cutoff of ALC 500 cells/mkL at about 1 month after transplantation (mean point of study – 29 days, range 20-43). The mean ALC 4 weeks post transplant was 457 cells/mkL (range 0-1980 cells). We observed superior overall and progression free survival by those of patients who reached ALC 500 cells/mkL or more 1 month after transplantation (p < .005 and p < .01, respectively). The estimated 4-years overall and disease-free survival was 66,8% and 67,2% for patients with rapid lymphocyte reconstitution versus 41,6% and 32,6% for patients with delayed lymphocyte reconstitution respectively. The mean ALC was also significantly higher in patients with reduced conditioning regimen versus sequential regimen (576 cells/mkL versus 404 cells/mkL, p = .01) and in those patients, who received myeloablative preparative regimen with lower ATG dose (45 mg/kg) versus 90 mg/kg ATG (636 cells/mkL versus 309 cells/mkL with p-value of < .0005). The comparing of lymphocyte reconstitution of patients depending on their CMV-seropositivity shows the significantly higher ALC levels in CMV-positive (anti-CMV IgG+) versus CMV-negative (anti-CMV IgG-) patients: 521 cells/mkL versus 371 cells/mkL, p < .05. Absolute lymphocyte count at 4 weeks after unrelated allo-HSCT have a high prognostic value and depended on preparative regimen intensity, dose of ATG and CMV-status. The evaluation of ALC on day +28 allows predicting of the clinical outcome after transplantation.
The aim of this study was to evaluate of lineage-specific donor hemopoiesis reconstitution. We examined 6 patients with acute leukemia and chronic myelogenous leukemia after allogeneic BMT from HLA-matched sibling donors of the opposite sex. In 5 of 6 patients conventional preparative regimen (busulfan and cyclophosphamide) was used and one patient received nonmyeloablative conditioning regimen with fludarabine, busulfan and antithymocyte globulin. We used fluorescence in situ hybridization (FISH) with centromeric probes for X and Y chromosomes. FISH was performed on samples of bone marrow and cytological slides of lymphocytes peripheral blood. 600 interphase cells were examined, detecting level was 0.2%, no false positives were found. The studies were carried out 14 days, 1,2,3,6 and 12 months after transplantation.

5 patients after myeloablative conditioning had donor chimerism more than 90% (range 91.2% to 98.6%) in both myeloid and lymphoid cell lineages at one month after BMT. At 12 months after transplantation the quantity of donor cells in both cellular lineages of these patients increased to 98-100%. In one patient, who received nonmyeloablative preparative regimen, donor myeloid chimerism was achived 92% by month 1 after BMT, whereas the count of donor lymphoid cells was 54%. On month 2, the percentage of donor lymphocytes was 72%, on month 3, the count of donor lymphocytes increased to 82%, and only by month 6 posttransplant donor lymphoid chimerism was achived 94%.

Thus, allogeneic BMT after conditioning regimen with reduced-intensity is characterized by delayed donor lymphocytes recovery, while the kinetics of donor myeloid reconstitution does not change as compared with myeloablative regimen.
7. MONITORING OF IMMUNE RECONSTITUTION IN PATIENTS FOLLOWING ALLOGENIC TRANSPLANTATION BY FLOW CYTOMETRY

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Hematopoietic recovery after allogeneic transplantation and the success of the transplantation is depended on the number and quality of hemopoietic stem cells (HSC) as well as the immune system’s patient recovery. HSC can be found in blood collected from donors HSC treated with administration of cytokine colony stimulating factor. However, the quality of HSC in peripheral blood may not become obvious early after transplantation due to the large cell doses that are administrated, and long-term follow-up will be needed to reveal defects in the HSC pool after PB grafting.

28 patients were monitored after 29 allogenic transplants. The median age was 19.74±13.23. The median age of the patients before 21 was 13.0±5.0 and after 21 was 34.7±14.0. They suffered from ALL (n=14), AML (n=5), severe aplastic anemia (n=2), CML (n=4), non-Hodgkin’s lymphoma (n=1), Hodgkin’s disease (n=1), cancer (n=1). Donors were matched unrelated (n=17) and family HLA identical siblings (n=11).

Peripheral blood mobilization was performed using G-CSF “Neupogen” (Hoffmann-La Roche), “Granocyte” (Aventis). Peripheral blood stem cells were collected from donors by leukapheresis using Spectra “Gambro” separator on the fifth day of stimulation. The percentage of CD34+ cells was determined directly after leukapheresis procedure on a DAKO Galaxy (15 mw argon Laser flow cytometer) (Denmark) according to the International Society for Hematotherapy and Graft Engineering (ISHAGE) guidelines. To avoid changes in marker expression by cell preparation, no purification or cryopreservation of CD34 cells was performed before analysis. The median number of infused CD34+ cells was 8.6±6.2 * 10^6 per kg weight. Blood was processed using a modification of lyse and wash procedure after staining with conjugated Mabs cocteil CD45/CD14 (2D1/MP9), CD3/CD19 (SK7/HD37), CD4/CD8 (SK3/SK1), CD3/CD(16+56), CD3/HLADR (L243) and negative control from Simultest Kit, Becton Dickinson [BD].

NK cells were the first lymphoid cells found in the blood. Most T cells were CD8+ and might be speculated as recipient origin. In most patients the appearance of peripheral B-cells was detected after D +28. The chronological order in which lymphocyte subtypes appeared in the circulation was nearly identical in all patients of PBSC and bone marrow groups.
8. EVALUATION OF CYTOKINE GENE EXPRESSION: A POTENTIALLY NEW APPROACH TO PREDICTION AND MONITORING OF POST-HSCT COMPLICATIONS.

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**Background.** Development of major complications of allogenic HSCT - acute graft-versus-host-disease (aGvHD), immune suppression and infections - is very closely depended on the activities of immune system. For this reason, a crucial role of certain cytokines in posttransplant immune disorders cannot be underestimated.

**General purpose.** Evaluation of IL-1beta, -2, -6, -10 mRNA expression levels as predictive markers for post-HSCT complications.

**Materials and methods.** We observed a group of thirteen hematological patients underwent allogeneic unrelated HSCT. Total RNA was extracted by phenol-chloroform protocol from peripheral blood leukocytes, bone marrow cells, or skin biopptates (in aGvHD) before HSCT and at different terms (up to d+50) during post-HSCT period. RT-PCR-based protocols were performed for each interleukin, using sequence-specific primer sets. The relative quantities of electrophoresis-separated PCR products that corresponded to specific cytokine mRNA have been assessed by Gel-Pro Analyzer 3.1 software.

**Results.** IL-1beta, -2, -6, -10 mRNAs were not detectable for, at least, 7-10 days post-HSCT, independent of conditioning regimens applied. Recovery of cytokine mRNA production was observed simultaneously with increase in peripheral leukocyte number. In general, elevated contents of IL-1beta, -2 and -6 mRNAs (5-7 times as compared to pre-HSCT levels) correlated significantly with aGvHD occurence and returned to basal levels upon immunosuppressive therapy (especially, with glucocorticoids). Interestingly, increased levels of IL-2, -6 and -10 correlated with emergence of viral infections (i.e., CMV and HSV type 1, 2). Upregulated cytokine gene expression was registered 3 to 5 days before the clinical manifestations of above mentioned complications.

**Conclusions.** Our preliminary findings indicate that monitoring of *in vivo* cytokine gene expression during post-HSCT period could be proposed as a new diagnostic approach to predict the early HSCT complications and, in particular, aGvHD.
9. POTENTIAL SIGNIFICANCE OF IL-6 GENE POLYMORPHISM AND
EXPRESSION AS A PROGNOSTIC TOOL IN HEMATOPOIETIC
STEM CELL TRANSPLANTATION (HSCT).

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Background. Interleukin-6 (IL-6) may be important for development of severe
HSCT complications, e.g., acute graft-versus-host disease (aGvHD) and post-
transplant infections. IL-6 production has been shown to correlate with a biallelic
C/G polymorphism at the position -174.

Methods. We investigated twenty-one donor/recipient pairs who underwent
HLA-matched HSCT. Genomic DNA was isolated from peripheral blood leuko-
cytes (PBL), and C/G-174 promoter variants for IL-6 gene were detected by
means of allele-specific PCR. Moreover, time-dependent shifts in IL-6 mRNA
expression were analysed in five HSCT cases (up to d+30) using common RT-
PCR, with total leukocyte RNA as starting material.

Results. The incidence of different IL-6 genotypes was 52%, 34% and 14% for
C/G, G/G and C/C, respectively. Hence, PCR-based technique for IL-6 allele
polymorphism detection proved to be a sensitive method for post-BMT chimerism
evaluation in cases of different allelotypes in donor and recipient. Thus marker of
chimerism was informative in 34% of HSCT pairs. Meanwhile, we have not
found strong correlations between high-producer IL-6 genotype (G/G) and clinical
signs of aGvHD. However, the levels of IL-6 mRNA dropped significantly during
conditioning regimen, especially if cyclosporine A was included. Significant in-
crease of IL-6 mRNA was observed in cases of aGvHD.

Conclusions: Our preliminary study showed that neither donor, nor recipient IL-
6 polymorphisms were associated with occurrence of acute GvHD. IL-6
genotyping could be used as a sensitive method for donor chimerism assessment
for, at least, one-third of HSCTs. Interestingly, semiquantitative detection of IL-6
mRNA may allow more accurate evaluation of conditioning efficiency, as well as
prediction of acute GVHD.
10. THE REVALUATION OF ABO-INCOMPATIBILITY AS A RISK FACTOR AFTER HEMOPOIETIC STEM CELL TRANSPLANTATIONS

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During last years ABO-incompatibility was revaluated as a risk factor of hemopoietic stem cell transplantation (HSCT). While ABO-barrier does not influence the survival and the incidence of relapses after HSCT, an increased number of immunohematological complications and transfusion problems can be expected.

**Material and methods.** The study included 63 patients (pts), who underwent HLA-matched HSCT. 32 pairs donor-pts were ABO matched (gr.I), 31 pairs were ABO mismatched (19 - major and 2-bidirectional - gr.II, 10 - minor - gr. III). The ratio of MUD/sibling transplants was 7/56, PBSC/ BMT – 17/46. The procedure of plasma removal was performed by marrow centrifugation for all cases with minor and bidirectional ABO-incompatibility, and red cells were removed by sedimentation with 10% dextrane or 6% HES for the cases of major and bi-directional ABO-incompatibility. Three groups had similar conditioning regimens and GVHD prophylaxis.

**Results.** We did not observe any cases of acute hemolysis after BMT or PBSC infusion. The onset time of erythropoiesis differed between gr.I and gr. II (the mean delay of 1‰ reticulocyte level was 10+ days). In gr.II there was a 1.5-fold increase of requirement of RBC transfusions compared to gr.I. We observed the delayed onset of immune hemolysis in 4 cases (2 pts in gr. II and 2 – in gr.III) that were successfully treated with i.v. fluids, corticosteroids, transfusions of 0-group RBC. A higher risk for the development of acute GvHD III-IV gr. in the pts of gr.I (10/31) and gr. III (3/10) compared to with gr.II (4/21) was revealed in our study.

**Conclusion.** ABO-incompatibility seems to be a additional risk factor of the HSCT. It may be the cause of delayed onset of erythropoiesis, the increased requirement the RBC transfusion, for some cases of immune hemolysis and higher incidence of acute GvHD.
11. INFLUENCE OF PLASMA EXCHANGE ON THE DONOR RED CELL ENGRAFTMENT AFTER MAJOR ABO-INCOMPATIBLE BONE MARROW TRANSPLANTATION

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Delayed donor red cell engraftment is observed after major ABO-incompatible bone marrow transplantation (BMT). It is accompanied with absence of red cell in bone marrow aspirates. One of possible mechanisms is the function of host plasma cells, which can produce isohemagglutinins for prolonged period. The aim of our work was to demonstrate the efficacy of plasma exchange in delayed donor red cell engraftment.

3 patients with allogeneic BMT have been included in our study. Procedure was performed for treatment of acute leukemia, chronic myeloid leukemia and myelodysplastic syndrome. All pairs “donor-recipient” had a major ABO-incompatibility, donor’s red cells had A(II) phenotype and recipient’s – 0(I). Myeloablative conditioning (busulfan+cyclophosphamide) was used in 2 cases and nonmyeloablative conditioning (cyclophosphamide + fludarabine + busulfan) was performed for patient with myelodysplastic syndrome. To decrease levels of isohemagglutinins plasma exchanges were performed from 1 to 3 times in all patients. Red cells were depleted from bone marrow graft. Prophylaxis of graft versus host disease was either cyclosporin A alone or combination of cyclosporin A and methotrexate. To estimate red cell chimerism we used differential agglutination and polymerase chain reaction was used to detect leukocyte chimerism. The recovery time of leukocyte and platelet levels didn’t differ compared to ABO-identical BMT. The engraftment of donor red cells was markedly delayed. Until +90 day erythroid cells were not defined in bone marrow aspirates and donor red cells were absent in weekly blood samples in 2 cases. One patient has mixed erythroid chimerism mostly with host red cells for prolonged period. In 2 cases the titer of isohemagglutinin are markedly increased and we proposed the function of substantial host plasma cells. Plasma exchange was performed at +104, +110, and +120 days. 4 procedures were performed in each patient with interval 2-3 days. There were no signs of graft versus host disease. Full donor leukocyte chimerism was detected on +100 day in all cases.

After plasma exchanges donor red cells were revealed in 2 patients who showed no erythroid cells previously. The level of donor erythrocytes increased to 80 % in case with mixed chimerism. Full donor erythropoiesis was sustained in 2 cases. Signs of chronic graft versus host disease were detected in 2 patients, but we can’t connect it with improvement of graft’s function. Conclusion: plasma exchanges can stimulate engraftment of donor erythroid cells in major ABO-incompatible BMT.
12. THE USE OF PREDNISOLONE IN THE PROPHYLAXIS OF ACUTE GRAFT VERSUS HOST DISEASE.

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Acute Graft versus Host Disease (aGVHD) is one of most serious complications of allogeneic bone marrow transplantation. (BMT). According to our date the early aGVHD related mortality is 12% in the patients with acute leukemia and 50% in the patients with CML receiving a standart prophylaxis consisting of CsA and short course MTX. There are publications about reduction of frequency of GVHD in patients received additional some medicines such as metronidazole or prednisolone (P). The aim of this work is the comparative analysis frequency of aGVHD in patients with CsA –MTX profylaxis and giving additional P on day +14 in dose 0.5 mg/kg b.w., on days + 21 - 34 in dose 1 mg/ kg, after day +34 - 0.5 mg/kg again and then the dose was reduced gradually. We observed 26 pts : 13 – with AL and CML, 11 F and 15 M, age 15-46 years, the duration of disease to BMT was median – 14 mo. We used before BMT BU+Cph in 24 cases and non- myeloablative regimen (Fludara+ BU+Cph or ATG) in 2 pts. Analysis was carried in two groups of patients receiving P (I) and without P (II). In group I aGVHD was observed in 5 of 13 pts (38%) and in group II – in 7 (53%).

In patients of the first group I-II stages aGVHD was observed in 4 of 5 pts (80%), but in the second group – in 71% cases. More severe stages of acute GVHD were noticed in 20% of patients in group I and 29% in group II. In the patients with P aGVHD occurred on day +36 (median) and without P on day +19. These preliminary results show that prednisolon administration promotes the reduction of the frequency and severity of aGVHD and the development of clinical symptoms in more late time.
13. HIGH DOSE CHEMOTHERAPY (HDC) WITH AUTOLOGOUS STEM CELL RESCUE (ASCR) IN PATIENTS WITH POOR PROGNOSIS HODGKIN’S DISEASE: RESULTS FROM 4 CENTERS IN BELARUS, RUSSIA AND UKRAINE.

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Between September 1990 and March 2003 a total of 184 poor prognosis Hodgkin’s disease patients treated with HDC with ASCR were included in the analysis. 4 transplant teams from Belarus (81 pts), R.F. (72 pts) and Ukraine (31 pts) reported dates. Median age of patients at transplant was 27 years (range 11 - 56), M-89/F-95. Stage at primary diagnosis was IV in 26,8%, III in 34,4%, II in 37,2% and I in 1,5% of patients, B symptoms were observed in 61% of patients. They received 2 - 34 courses of standard dose chemotherapy (mean 9 courses) before including to HDC treatment program. Primary resistance was the reason to perform HDC in 44,6% of the patients, early relapse in 27,2%, late or multiply relapses in 26,6% and 1,6% of patients received HDC as consolidation of first complete response. At the moment of including to HDC treatment program 43% of patients had stage IV disease, 26 % - stage III, 27% - stage II and 4% - stage I disease; 53% of patients had B symptoms. Before HDC 84,8% of patients received IInd line reinduction chemotherapy (54,5% dexa-BEAM, 16,9% DHAP-like regiments, 3,8% other regiments). Complete response (CR) or complete response uncertain (Cru) were achieved after IInd line chemotherapy in 25% of patients, partial response (PR) in 46,8%, stable disease (SD) in 12,8% and disease progression (PD) in 15,4% of patients. Most patients (81%) received BEAM as a HDC regimen, 11% received CBV±mitoxantrone, 6% other HDC regiments and 2% of patients received 2 HDC cycles. Autologous bone marrow was used as a rescue in 29% of patients, peripheral blood stem cells in 54% and combined transplant in 17% of patients. Median time till ANC >0,5*10^9/l and platelet >20*10^9/l recovery was 13 days, 100-days mortality was 5,4% (10 pts).
CR and CRu after HDC were achieved in 68.2% of patients; PR in 15.9% and 15.9% had SD or progressed. Median follow-up for survived patient was 30 months (range 2-139). Median overall survival (OS) and disease free survival (DFS) were not reached. The actuarial 5-years OS and DFS for the entire group of 184 patients were 60% and 69.7%, respectively; 10-years OS and DFS were 56% 69.7%, respectively.

In univariate analysis for OS (log-rank test), sex, age, transplant center, primary, multiple previous chemotherapy, disease status (primary resistant vs early relapse vs multiple relapses), relapse sensitivity, reinduction II\textsuperscript{nd} line regimen, and transplant source had no predictive value. With the respect for OS predictive value had B symptoms at diagnosis (p=0.02) and before including to HDC treatment program (p=0.0017), histology (p=0.028), stage (p=0.003), response after induction (p=0.0013), status at transplant (p=0.0016) and HDC regiment (0.015). For DFS B symptoms before including to HDC treatment program (p=0.032) and age (p=0.0075) had prognostic value. In multivariate analysis only B symptoms at diagnosis saved it’s prognostic value with respect for OS and DFS.

We conclude that HDC and ASCR is an effective strategy for patients with refractory HD or relapsed after conventional dose chemotherapy.

14. RESULTS OF STANDARD AND INTENSIVE POLYCHEMOTHERAPY (PCT) OF LYMPHOGRANULOMATOSIS (LGM)

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78 patients with histologically distinctive LGM were examined. Additional immuno-histochemical analysis (CD45-, CD30+, CD15\pm) was needed in 19 (25%) cases in order to prove the diagnosis of LGM. Staging of patients included compulsory use of computer-aided tomography. Stage distribution of patients was as follows: stage I-II – 52%, stage III – 26%, stage IV – 22%. Extranodal foci were detected in 36% of patients; tumor nodes bigger than 5 cm across diameter were detected in 43% of patients. Patients’ age ranged from 15 to 65 years, mean age was 33±4 years. Male/female ratio was 1:1.
First-line treatment (ABVD, COPP-ABV) (treatment duration from 4 to 8 courses) supplemented in most cases with radiotherapy resulted in partial or complete remission in 82% of patients. 15 patients (8 of them initially had stage II; other initially had stage IIIB-IV; all of them had 2 and more factors of poor prognosis) were resistant to standard therapy and underwent 2-4 courses of intensive PCT (Dexa-BEAM, DHAP). Treatment intensification was unsuccessful in 5 patients: disease progressed during the first 4 months after the termination of course of treatment.

10 patients had positive results after the course of second-line treatment: all of them had partial or complete remission. Here, the most pronounced effect was noted regarding tumor infiltration of pulmonary tissue, spleen and peripheral lymph nodes. In most cases mediastinal tumor masses didn’t change their volume. 5 of these patients underwent high-dose PCT (BEAM) followed by autologous bone marrow transplantation and/or peripheral stem cells (PSC) transplantation to consolidate the remission. At a later time, two of these patients had a relapse of LGM after 4 and 18 months respectively, and three of them remained in remission for 15, 18 and 25 months respectively. 6 patients still had mediastinal tumor masses from 17x30 mm to 60x90 mm after treatment intensification up to high-dose PCT with autotransplantation of PSC (3 patients). Gamma-therapy (gamma-radiation dose 36-40 Gy) didn’t change the volume of these tumor masses. Control computer-aided tomography with bolus injection of contrast agent or positron emission tomography didn’t reveal any vascularization of these masses. So, the latter were considered as fibrous tissue. Further follow-up showed their constant volume during 9-23 months, and these patients didn’t show any other signs of LGM.

Best treatment results using intensive protocols of PCT were achieved in three other patients with LGM stage III-IV who initially had factors of poor prognosis. Here, the main portion of their tumor mass was reduced by first-line treatment followed by high-dose PCT with auto-PSC transplantation during partial remission. Complete remission remains for 15, 16 and 19 months respectively. Hence, treatment intensification overcomes resistance to standard chemotherapy in part of LGM patients. Consolidating course of high-dose PCT in patients with initially advanced stages and factors of poor prognosis allows achieving complete remission for more than a year.

Further co-operative research is needed to make final conclusion about the role and place of intensive PCT in the treatment of LGM.
15. AGGRESSIVE T-CELL LYMPHOMAS: CLINICAL FEATURES AND TREATMENT RESPONSE.

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Since 1998 diagnosis of aggressive peripheral T-cell lymphoma was verified in 26 patients (19 men and 7 women) aged 18-70; mean age – 43. Lymphoma’s immunochemical variants were as follows: peripheral unspecified T-cell lymphoma – 11 patients; anaplastic giant cell lymphoma – 9 patients; angioimmunoblastic lymphoma – 5 patients; panniculitis-associated lymphoma – 1 patient. Skin T-cell lymphomas were not analyzed. Despite short case history, most part of patients (n=24) had disease stage III-IV. Neck and axillary lymph nodes were involved in tumor process most frequently (80% of patients); mediastinal involvement was verified in 48% of patients; subphrenic lymph nodes involvement was found in 44% of patients; spleen involvement – in 20%, skin involvement – in 16%, liver involvement – in 12%, bone marrow involvement – in 12% of patients. Less common tumor localizations were as follows: pancreas (n=2), central nervous system (n=2), paranasal sinus and nasopharynx (n=2), stomach (n=1), small intestine (n=1), bones (n=1), subcutaneous fat (n=1). 85% of patients had B symptoms.

First line therapy included standard ÑÍÎÐ program (n=23). Complete remission was achieved in 11 cases (48%), two patients were in partial remission. Three patients (two patients with analastic giant cell lymphoma in complete remission; one patient with angioimmunoblastic lymphoma in partial remission) who had high-risk factors of relapse underwent high-dose chemotherapy (BEAM regimen) during standard chemotherapy followed by autologic peripheral stem blood cell transplantation in order to consolidate remission. In two cases DHAP program of intensification preceded high-dose chemotherapy during consolidation stage. As a result of high-dose consolidation, one patient with partial remission passed into completed remission. One patient with anaplastic lymphoma showed tumor relapse that was sensible to chemotherapy. 10 patients (43,5%) were resistant to CHOP regiment, and 8 of them died due to tumor progression. In two cases resistance was overcome by intensive DHAP chemotherapy (complete remission). One of these patients underwent consolidation with high-dose BEAM program followed by autologic peripheral stem blood cell reinfusion.

As a whole, 13 patients in this sample of peripheral T-cell lymphoma patients are in complete remission (remission duration from 3 to 40 months), 1 patient is in partial remission (42 months of follow-up). Low response rate to first-line treatment in CHOP regiment in patients with peripheral T-cell lymphomas gives grounds for early chemotherapy intensification.
16. PRELIMINARY RESULTS OF HIGH – DOSE CHEMOTHERAPY WITH AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN ONCOHEMATOLOGIC PATIENTS.

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Since May 2001 to May 2003 10 patients received high – dose chemotherapy (HDCT) with autologous peripheral blood stem cells transplantation (autoPBCT) in hemotologic department of Republican Hospital. They were: 7 patients with Hodgkin’s disease (HD) (2 with primary refractory HD, 5 with relapsing HD), 2 patients with multiple myeloma, 1 patient with anaplastic large cell early relapsing lymphoma. Age of patients was 25 – 48 years. In all patients procedures of harvesting of PBSC were performed using a COBE “Spectra” blood cell separator. In 2 patients (1 with primary refractory HD and 1 with multiple myeloma) autoPBCT was made 72 hours after procedure of harvesting of peripheral blood stem cells without cryoconservation, in remainder (8 patients) peripheral blood stem cells were cryoconserved with temperature – 85° C. The conditioning regimen in 4 patients with relapsing HD and 1 patient with anaplastic large cell lymhoma was BEAM, in 2 patients with primary refractory HD – modified CBV, in 1 patient with HD - miniBEAM. In all patients with multiple myeloma the conditioning regimen was melphalan 140 mg/m². During infusion of peripheral blood stem cells there were different kinds of adverse reactions in all patients: nausea and vomiting (8 patients), hyperemia of skin (10 patients), chest pain (3 patients), cerebral transitory ischemic attack with complete resolution (1 patient). Duration of aplasia was 3 – 11 days with conventional complications: candidosis and herpetic infection of oral cavity (all patients), febrile neutropenia (1 patient), pneumonia (1 patient), and hemorrhagic syndrome (5 patients).

Results of high – dose chemotherapy with autologous peripheral blood stem cells transplantation were following: 4 patients achieved complete remission; duration of remission 3 – 10 months. 1 patient with multiple myeloma achieved complete remission of 19 months duration. In 1 patient with HD we did not achieve effect and 1 patient with anaplastic large cell lymphoma there was relapse after 8,5 months after autoPBCT. 2 patients with primary refractory HD died because of disease progression in 1 – 3 months after autoPBCT. In 1 patient with multiple myeloma autoPBCT has been made just in May 2003, thus it is too early to estimate the effect.

Conclusions: high – dose chemotherapy with autologous peripheral blood stem cells transplantation was effective in 80% patients with relapsing HD and multiple myeloma but completely unsuccessful in patients with primary refractory HD. In spite of different complications during procedure of infusion of peripheral blood stem cells and after high – dose chemotherapy the safety of this kind of therapy was rather high (90%).
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Effective methods of mobilization and harvesting of peripheral blood stem cell (PBSC) are the basis of successful autotransplantation. There are several methods of stem cell mobilization. We analyzed our own experience of this procedure.

There were 15 procedures of mobilization and harvesting of PBSC. In 9 patients we used combined regimen of mobilization with both cytostatic chemotherapy and G-CSF and in 6 patients with G-CSF only. We assessed the amounts of mononuclear cells and CD34+ cells in the ultimate product of procedure. The amounts of CD34+ cells assessed by means of Becton Dickinson cytoflowmeter in the laboratory of immunology of St. Petersburg Pavlov State Medical University, St. Petersburg. Procedures of harvesting of PBSC were performed using a COBE “Spectra” blood cell separator. In all patients we used the single procedure of large volume leukapheresis with an average of 5 – 6 total blood volumes processed.

Among 9 patients with combined regimen of mobilization the cytostatic chemotherapeutic regimen were DEXA-BEAM (6 patients), DHAP (1 patient), ESAP (1 patient) and C-DEA (1 patient). Colony- stimulating factor (Neupogen or Granocyte) used in dose 5 mcg/kg/day during 5 days (8 patients) or 11 days (1 patient). In all patients harvesting procedure were performed next day after completion of mobilization (in 8 cases – 6th day, in 1 case – 12th day). Start of stimulation with G-CSF began the day, when amount of leukocytes and granulocytes was minimal (4 – 7th day after finishing DEXA-BEAM regimen, 13th day after finishing DHAP regimen and 11th day after finishing ESAP and C-DEA regimens). In all 8 patients with 6th day harvesting of PBSC the mean yield of mononuclear cells per kg (MNC/kg) was 10,816 x 10^8/kg (3,312 – 18,32 x 10^8/kg), CD34+ cells/kg - 11,873 x 10^6/kg (3,67 x 10^6/kg - 19.16x10^6/kg). The level of CD34+ cells among mononuclear cells was 1,1 – 4,57%. In one case the mobilization with G-CSF continued 11 days, but result was unsatisfactory (MNC/kg - 0,85 x 10^5/kg, CD34+cells/kg - 0,71 x10^6/kg or 0,84%), presumably because of massive previous chemotherapy and elderly of the patient.

Among 6 patients with regimen of mobilization by G-CSF only, in 5 cases we did not receive the yield of adequate amounts of PBSC (CD34+ cells/kg - 0.28 - 1.78x10^6/kg or 0.01-0.4%). The possible causes were inadequate dose (7 mcg/kg/day 1-5 days – 2 patients), short duration of stimulation (10 mcg/kg/day 1-3 days – 1 patients) and previous x-ray therapy on vertebral column and pelvis (2 patients). Only in one patient in this group in spite of low dose (6 mcg/kg/day) and short duration of stimulation (3 days) there was the yield of adequate amounts of PBSC (CD34+ cells/kg - 8.21 x 10^6/kg). We consider that use of combined regimen of mobilization with both cytostatic chemotherapy and G-CSF and large volume leukapheresis provides effective means of successive PBSC harvesting and has priority over regimen of mobilization by G-CSF only.
18. HIGH-DOSE CHEMOTHERAPY IN BREAST CANCER. STATE OF ART AND PROSPECTIVES.

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Breast cancer (BC) is one of the most frequently diagnosed cancers, with a lifetime risk in the more developed countries of one in eight women presenting with breast cancer. In the world, there are more than 1,000,000 cases each year. Most patients with high-risk breast cancer (HRBC) are defined by extension of axillary node involvement. Analysis of treatment outcomes in patients with 10 or more positive lymph nodes indicates that up to 87% will relapse by 5 years. More than 20 randomized trials have compared standard chemotherapy with dose-intensified regimens for BC patients. Taken together, these trials support the value of dose intensity. The role of high-dose chemotherapy (HDCT) and autologous hematopoietic stem cell rescue in breast cancer is still controversial. Lloyd E.Damon et al. analyzed the outcomes of 1111 consecutive patients with histologically proven breast cancer who underwent HDCT at 5 major California medical centers. With a median follow-up of 2.8 years (range, 0.1-8.2 years) after HDCT and autologous hematopoietic stem cell rescue, the estimated 5-year event-free survival (EFS) and overall survival (OS) for stage II/IIIA patients with >=10 involved axillary lymph nodes were 67% and 76%, respectively.

According data of different authors the 5 year relapse free survival was estimated at the level of 17% to 56%(weighted mean, 38%) in patients received conventional-dose adjuvant chemotherapy with follow-ups ranged from 3.3 to 10.3 years. The Scandinavian study compared a single high-dose treatment with 6 additional cycles of moderately intensive (individually tailored) FEC following induction FEC chemotherapy. No difference in EFS or OS has been reported to date; however, a significant increase in drug-induced leukemia (3%) has been seen. The CALGB 9082 study evaluating consolidation with high vs moderate doses of cyclophosphamide, carmustine, and cisplatin showed no difference in EFS (61% vs 60%) or OS (72% vs 70%) between the 2 groups, although there was a lower relapse rate in the subgroup of patients under the age of 50. Dutch randomized trial, which accrued from 1992 to 1999 a total of 885 patients with HRPBC. The analysis for a median 4.5 years, shows statistically significant differences in favor of the transplantation arm in EFS (77% versus 62%; P = .009) and OS (89% versus 79%; P = .04).

Experience of Belorussian Hematology and BMT Centre confirm that HDCT with autologous blood stem cell rescue can be safely administered to patients with HRBC (n=56). The 100 days treatment-related mortality was 0%, 5-year EFS and OS were 61% and 79% respectively. 5-year OS of 52% in similar cohort of patients receiving conventional-dose adjuvant chemotherapy in Belorussian Research Oncology Institute (prof. L.Putirsky) is 52%. Further studies are necessary to clarify the role of HDCT in HRBC.
19. THE CONCEPT OF CELLULAR THERAPY IN AUTOIMMUNE DISEASES.

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Autoimmune diseases (AID) include a large number of entities with different clinical features and common mechanisms of pathogenesis and principles of treatment with immunosuppressive therapy as its basis. Conventional methods of treatment allow to manage pathological process to some extent but they do not cure AUD. High dose therapy (HDT) with transplantation of blood stem cells (SCT) is a principally new approach which may result in curation of some AID.

The concept of high dose therapy with transplantation of blood stem cells in AID has been developed by the experts of Russian Cooperative Group for Cellular Therapy (RCGCT).

The major components of the concept are as follows:

- Determination of the strategy of HDT+SCT
- Contents of the method of HDT+SCT
- Main indications to the use of the method of HDT+SCT
- Methodology of conducting HDT+SCT
- Algorithm of conducting HDT+SCT
- Trends of further research

In accordance with the first item of the concept the final goal of HDT+SCT in AID is to improve PATIENT’S QUALITY OF LIFE as much as possible. Postulation of such paradigm allows to determine the choice of the key outcomes of HDT+SCT in AID. Along with traditional laboratory and instrumental tests which point to changes in pathological process during treatment the important outcomes are quality of life parameters of a patient which focus to the complex of physical, psychological and social functioning of an individual.

The main task of HDT+SCT in AID is eradication of a pool of autoreactive lymphocytes and their precursors and it might be achieved in different ways which is dependant of the therapy program. The major programs of HDT+SCT in AID within this concept are as follows:

- Myelo/immunoablative therapy +AlSCT
- Myelo/immunoablative therapy +AutoSCT
- Non-myelo/immunoablative therapy +AlSCT
- Non-myelo/immunoablative therapy +AutoSCT
- Non-myelo/immunosupressive therapy + storage of SCT
In accordance with indications and methodology of conducting HDT+SCT within the concept there is chosen 4 types of operations:

- Early transplantation
- Regular transplantation
- Salvage transplantation
- Delayed transplantation

The algorithm of conducting HDT+SCT includes 3 stages:

- HDT+SCT itself
- The program of early post-transplantation rehabilitation
- The program of long-term post-transplantation rehabilitation

The method of HDT+SCT is at the stage of clinical trials. The preliminary results of both domestic and foreign (EBMT) studies demonstrate the efficacy of the present approach in systemic AID.

*AlSCT* – *allogenic SCT*

*AutoSCT* – *autologous SCT*

*EBMT* – *European Group for Bone Marrow Transplantation*
20. AUTOLOGOUS HEMATOPOETIC STEM CELL TRANSPLANTATION (AUTO-HSCT) IN MULTIPLE SCLEROSIS (MS): TWO YEARS FOLLOW-UP.

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Autoreactive T-lymphocytes in MS promotes autoaggression relapse in MM. They are the largest for high-dose immunosuppressive disorder. The aim of the study was the evaluation of the dynamics of clinical and MRI patterns of the disease after HDISP and auto-HSCT (two years follow-up). 17 patients with predominantly secondary progressive MS were included (age 18-45 years old). Duration of the disease before the HDISP and auto-HSCT was 3-15 years, EDSS-6,1+/−0.2. The indications for HDISP were mainly low efficacy of conventional therapy that included predisposing progressive neurological features of the disease. In a month after auto-HSCT, the majority of patients reduced signs of pyramidal insufficiency (p<0,02) as revealed by muscle force increase. Regress of cerebellar and disuric disorder was also observed. The parameter of total neurological deficiency reduced from 12,6+/−1,6 to 8,8+/−2,5. After 20-24 months, the disability status (EDSS) was stable (5,85+/−0,21). Moreover, improvement of pyramidal function was observed (p<0,043). It allowed patients to keep self-service for long time. The index of progressing of disease reduced from 0,19+/−0,15 to 0,79+/−0,06 (p<0,03) whereas in control group (not undergone auto HSCT) this index increased by 20%. In 12 patients, stabilization of neurological status was achieved. Meanwhile 4 patients had insignificant worsening of cerebellar function and dysarthring. In general, according to FS scale cerebellar dysfunction was less severe after treatment than before it. MRI (with gadolinium) was performed in 6 patients. 4 patients showed reduction of the quantity of lesions (not less than 50%) and/or reduction of their size. These patients had regress of neurological signs (almost in all groups of FS scale) with tendency in increase in implement activity. Insufficient positive MRI dynamic was revealed in 1 patient, who showed transient worsening of dysarthria and ataxia in 1 patient with progressing worsening of neurological symptoms negative MRI dynamics (increase of the number of lesions and their size) was revealed. The analysis of the data have shown that stable positive clinical and MRI dynamics can be obtained in patients with disease duration up to 8 years, EDSS <=6,5 and neurological deficiency not higher than 13. Age, duration of the disease and the speed of progression are prognostic factors for auto-HSCT. Thus, our data claims that auto-HST seems to be the pathogenetic method for MS treatment.
21. THE MECHANISM OF G-CSF INDUCED CD34+CELLS MOBILIZATION.

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Background: It is known, that the level of G-CSF-receptor expression is the greatest on mature granulocytes, and this cells can be target for G-CSF-induced CD34+cells mobilization. Granulocytes and monocytes as the cells-targets for the action of G-CSF, probably, can produce other messengers. IL-8 can be one of them.

The aim: We evaluate efficacy of CD34+cells mobilization and CFU-GM count in PB and BM in dependence on IL-8 serum level dynamics during G-CSF course and according to granulocytes, lymphocytes and monocytes blood count before G-CSF course in children with various oncological and oncohaematological diseases and in apparently healthy donors.

Methods: For evaluation of CD34+cells number we used flow cytometry, for evaluation of CFU-GM number in blood (PB) and bone marrow (BM) we used clonogenic cultivation, the cell composition of PB and BM was analyzed by automatic counter and morphological analysis. The serum level of IL-8 was analyzed by ELISA-assay.

Results: We revealed a reliable direct correlation between the number of granulocytes prior to the beginning of administration of G-CSF and the number of CD34+cells obtained other mobilization, meanwhile the efficacy of CD34 mobilization not depending upon the baseline level of lymphocytes and monocytes. The efficacy of stem cells mobilization depends neither upon the main diagnosis and the initial lesion of the bone marrow. It was determined that reaching the necessary number of the CD34+ cells required less procedures of cytapheresis and shorter duration of using G-CSF in groups with a greater number of granulocytes prior to the beginning of mobilization. There were no increase of CFU-GM count in BM during G-CSF-induced CD34+cells mobilization, and the percentage of CFU-GM in PB and BM was the same before and after using G-CSF. The serum level of IL-8 during G-CSF stimulation was increased 6,5 fold. There was correlation between increased degree of serum IL-8 level and CD34+cells and CFU-GM count after G-CSF mobilization.

Conclusion: The number of granulocytes at the moment of prescription of G-CSF turned out the most important criterion for predicting the efficacy of peripheral stem cells mobilization. It seems, IL-8 can be messenger for the G-CSF action on bone marrow CD34+cells, stimulating they migration from bone marrow to peripheral blood.
22. THE FREQUENCY OF EARLY COMPLICATIONS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) WITH MYELOABLATIVE (MA) AND NON-MYELOABLATIVE (NMA) CONDITIONING REGIMEN.

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The frequency of early complications (before 100 days) of HSCT was measured on 97 patients with hemoblastosis (median age 24±1,2), underwent allogeneic HSCT (allo HSCT) (n=33, median age 24±2,0) and autologous HSCT (auto HSCT) (n=64, median age 25,5±1,6).

Conditioning regimens in alloHSCT were myeloablative in 26 patients (78,8%, median age 21±2,0) and consisted of Busulfan (Bu) and Cyclophosphamide (Cy), and non-myeloablative in 7 patients (21,2%, median age 35±4,0), consisted of Fludarabine (Flu)+Ms+anti-T-lymphocyte globulin (ALG) or Flu+Bu+Alkeran (Alk). In group of MA HSCT 11 patients had CML, 8 – ALL, 5 – AML, 2 – MDS. In group of NMA HSCT 4 patients had CML, 2 – ALL, 1 – AML. HLA matched related MA HSCT were done in 26 cases (100%) and in group of NMA HSCT 6 cases were HLA matched related (85,7%) and 1 HLA-matched unrelated (14,3%). The frequency of infection complications (bacterial and viral) in NMA HSCT group was less than in MA HSCT group: 57,1% è 88,4% respectively. The median duration of febrile neutropenia was significantly lower in NMA HSCT group: 7±1,5 и 12±1,7 days (p<0,05). Hemorrhagic complications were observed in 9 patients with MA HSCT (34,6%) and in 3 with NMA HSCT (42.8%) (p>0,05). Acute graft versus host disease (GVHD) grade 2-4 was observed in 5 patients with MA HSCT (19,2%) and was not found in group of NMA HSCT (p>0,05). The toxicity of conditioning regimens for cardiovascular system, respiratory system, digestive and urogenital system (only grade 3 or more toxicities were considered) were 38.4% in MA HSCT group and 28% in NMA HSCT. The engraftment of stem cells was achieved in 100% cases as in MA as NMA HSCT groups and was significant faster in NMA HSCT (18±4,1 and 24±2,5 days respectively, p<0,05).

Conditioning regimen in autologous HSCT was in MA (Bu+Cy) 25 cases (39%) and NMA 39 cases (61%) (TACC, LACE, BEAM). In group of MA HSCT 15 patients had AML, 10 – ALL; in group of NMA 13 had AML, 16 - ALL, 10 – multiple myelomas. The number of infection complications were smaller in NMA HSCT (87,1% and 92%, respectively, p>0,05). Toxicity complications grade 3-4 were some frequently in MA HSCT, than in NMA HSCT (40% and 30,7% respectively, p>0,05). Hemorrhagic complications were in 10 cases of auto HSCT (15,6%) and there were no significant differences between them after MA and NMA HSCT (MA-16%, NMA-15,3%). The recovery of absolute neutrophil count and platelets was significant faster in NMA HSCT (22±2,0 and 31±2,5, p<0,05; 17±1,7 and 29±3,0, p<0,05, respectively).

In conclusion, the use of NMA conditioning regimens both in allo HSCT, and in auto HSCT accompany more faster engraftment; the frequency of hemorrhagic complications were significantly lower, while the frequency of infections and toxicity complications had no significant differences.
Pathogenesis and treatment of Multiple Myeloma.

1. LIFE AND DEATH OF MYELOMA CELLS.

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Multiple myeloma (MM) is a clonal growth of slowly proliferating malignant plasma cells. The disease is usually characterized by production of monoclonal immunoglobulin and degradation of bone. Despite advances in treatment in recent years, MM is still an incurable disease.

The growth of MM cells in a patient is usually restricted to one characteristic location, namely the bone marrow. Even though the transformation of a B cell into a malignant myeloma precursor cell likely occurs in a lymph node, the progeny of this cell is found almost exclusively in the bone marrow. This particular tropism of MM cells does not merely reflect the growth requirements of its normal counterpart, the plasma cell, since plasma cells are also found in other locations like tonsils and Payer patches of the gut, sites that are not favored growth places for MM cells. Furthermore, human MM cells usually do not grow in immunocompromised mice, but are able to grow inside human bone fragments implanted in such mice. A reasonable explanation for this tropism is that MM cells are critically dependent on factors that are present only within the human marrow, for their prolonged survival and growth. The dependence of MM cells on the fertile soil of the bone marrow is a potential Achilles’ heel for the myeloma cell clone. If we knew the identity of the required factors, therapy could be directed to inhibit the supporting role of these factors. Downstream intracellular signaling events evoked by the critical factor(s) are also possible targets for therapy. Cytokines like interleukin (IL) -6, -10, -15, -21, tumor necrosis factor, insulin-like growth factor-I and hepatocyte growth factor are known to stimulate growth and inhibit apoptosis in MM cells. Nevertheless, none of these cytokines, alone or in combination, are able to sustain the proliferation of primary MM cells in vitro, indicating that critical stimulating factors are yet to be identified.

Other bioactive proteins inhibit the growth and/or induce apoptosis in MM cells. These factors include members of the TNF cytokine family (TRAIL and Fas-ligand) as well as members of the TGFβ family (activin and bone morphogenetic proteins (BMP) -2, -4, -5, -6 and -7).
Several genetic aberrations have been found in MM cells, but none of them are present in a majority of cases. Translocations involving the immunoglobulin heavy chain locus on chromosome 14q32 can be found in roughly 50% of patients, and are believed to be the initial oncogenic event in these cases. However, the partner chromosome in heavy chain translocations varies. The most frequent translocations are t(4;14), t(11;14) and t(14;16), resulting in dysregulation of the genes for fibroblast growth factor receptor 3, cyclin D1 and c-Maf, respectively. The variation in genetic aberrations between cases of MM indicates that MM is a heterogeneous disease. Recent reports showing that t(11;14) and t(4;14) imply a favorable and an adverse prognosis, respectively, support the view that MM can be divided into several entities.

2. IMMUNOLOGIC ASPECTS OF MULTIPLE MYELOMA PROGRESSION.

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Multiple myeloma (MM) progression is characterized by normal haemo- and immunopoiesis suppression, malignant clone selection with increased ability for cell proliferation and metastasizing. Suppression of normal B-lymphopoiesis is often found in the cases of MM but the exact mechanisms are still unclear. Recently it has been shown that pre-B cells significantly decreased dependently when the numbers of plasma cells in the bone marrow (BM) in myeloma patients are increased. Moreover, plasma cells induce apoptosis of pre-B cells in vitro, but only in the presence of BM stromal cells. In order to clarify the mechanism of this suppression by plasma cells, we studied how the function of BM stromal cells could be modulated by attachment of plasma cells. We performed co-culture of human BM stromal cell line KM-102 or primary BM stromal cells with human myeloma cell line KMS-5. Using RT-PCR method we examined mRNA expression of the cytokines that possible affect survival of pre-B cells (IL-7, MIP-1α, MIP-1β, TGFβ1) in BM stromal cells. We found that myeloma cell line KMS-5 negatively modified survival of pre-B cells through decreased expression of IL-7 and increased expression of MIP-1β and TGFβ1 mRNA in both BM stromal cell line and primary BM stromal cells. KMS-5 cells themselves did not express MIP-1β and IL-7 mRNA and were weak positive for TGFβ1 mRNA. Furthermore, we confirmed that both MIP-1β and TGFβ1 suppressed survival of sorted pre-B cells directly in vitro. We also showed that attachment of myeloma cells increased expression of IL-6 and IL-11 mRNA (paracrine stimulators of MM cells growth) in BM stromal cells.
Recently MM cells were shown to have heterogeneity with regard to the expression of CD49e (VLA-5, one of β1 integrin) and MPC-1 adhesion molecule: immature and mature myeloma cells. We examined mRNA expression of cyclin D1 and CDK inhibitor p16 in MM cells. Mature myeloma cells (VLA-5+, MPC-1+) did not express cyclin D1 mRNA, but were positive for p16 mRNA. Immature myeloma cells (VLA-5-; MPC-1-) were negative for p16 mRNA and positive for cyclin D1. It was shown that high proportions of VLA-5- immature myeloma cells correlated well with poor response to treatment in MM and characterized by increased ability for proliferation. Further studies would be required to clarify how expression of cytokine genes is modified in BM stromal cells and how cell cycle is modulated in malignant cells in case of MM progression.

3. ADHESION AND MIGRATION OF MYELOMA CELLS.

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Progression of multiple myeloma, as well as of every other malignancy, depends considerably on abilities of tumor cells to attach and migrate throughout tissues. Adhesion and migration are elements of a complex interaction between tumor cells and their microenvironment and are mainly based on ligand-receptor model. Most important receptors are transmembrane α-β heterodimers of integrin family. Binding of ligand to the integrin molecule initiates signaling cascades, which result in upregulation of a number of transcription factors entering the nucleus with effects on proliferation and apoptosis. Intensity of adhesion and migration is defined by abundance and state of cell surface integrins. Integrins can be regulated by cytokines in inside-out fashion. There is no doubt that deeper insight into the mechanisms of adhesion and migration of the tumor cells will specify additional targets in anticancer therapy, fighting this component of tumor progression. These mechanisms are not well understood so far.
We (R.Holt, V.Baykov, T.Ro, S.Brabrand, A.Sundan, A.Waage, M.Borset – on behalf of The Nordic myeloma study group) studied different myeloma cell lines, assuming that each of them may represent certain type of molecular organization of myeloma cells. 96-well plates precoated with ligands (fibronectin, VCAM, collagen I and IV, laminin and vitronectin) were used for adhesion assay. Migration assay was carried out in «Transwell» plates. The cytokines used were BMP-7, EGF, FGF2, HGF, IFN-α, IGF-1, IL-1β, IL-6, IL-15, IL-21, MIP-1α, SDM-1α, TNF and VEGF. Integrin content (β1, β2, β7, α4, α5, αv, αl, αx) was assessed by flow cytometry. Inhibitors of PI3-kinase (LY294002 and wortmannin), of MAP-kinase (U-0126 and PD-98059) as well as c-Met receptor inhibitor, pertussis toxin and neutralizing antibodies to α4 and β1 were used to study extra- and intracellular signaling pathways.

INA-6 and ANBL-6 cells were able to adhere to fibronectin and VCAM-1. Adhesion was enhanced upon stimulation with HGF (INA-6 only), IGF-1 and SDF-1α. These cytokines led to increased migration as well, and it was found that HGF and SDF-1α-stimulated migration was directional, suggesting the role of these cytokines as chemoattractants.

α4β1 (VLA-4) was by far the most abundant integrin on the cell surface among eight cell lines tested. However adhesion was not entirely dependent on integrin content, as INA-6 cells, with less than half the amount of β4 and α1 integrins on their surface compared to IH-1 cells, adhered much better to fibronectin and VCAM-1 than IH-1 cells. Neutralizing antibodies to α4 abrogated both adhesion and migration, whereas antibodies to β1 inhibited adhesion but led to only 50% decrease in migration.

Inhibitors of signal transduction were applied to study inside-out integrin activation pathways. Cytokine-stimulated adhesion was critically dependent on PI3-kinase and independent on MAP-kinase activity. HGF-receptor inhibitor blocked only HGF-mediated adhesion and migration, and pertussis-toxin had impact only on SDF-1α-mediated adhesion and migration, suggesting that IGF-1 and HGF-induced α4β1-integrin activation doesn’t act through G protein-linked receptors. Similarly, IGF-1 or SDF-1α are not upstream of the signal from the HGF-receptor.

In conclusion VLA-4-dependent adhesion and migration of myeloma cells are strongly activated by HGF, IGF-1 and SDF-1α. HGF and SDF-1α act as powerful chemoattractants. Initial steps of intracellular signaling cascades mediated by these cytokines are independent of each other, and PI3-kinase activity is crucial for carrying out cell adhesion and migration.
4. TREATMENT OF MULTIPLE MYELOMA: GUIDELINES OF THE NORDIC MYELOMA STUDY GROUP (NMSG).

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Myeloma is an incurable B-cell malignancy characterized by accumulation of plasma cells with low proliferative activity. Although a few per cent of patients present with localized plasmocytoma, most patients have wide spread plasma cell infiltration of the bone marrow, i.e. myelomatosis (MM).

Bone pain, hypercalcaemia and pathologic fractures due to multiple osteolytic lesions are the most common clinical presentation. However, up to 30% are diagnosed incidentally while being evaluated for unrelated problems. The MM cells produce monoclonal immunoglobulins (M-component). MM is differentiated from other causes of monoclonal gammopathy by marrow plasmacytosis > 10% and serum M-component > 25 g/l. Skeletal evidence for the existence of MM has been reported in archaeological material but the clinical features were first described around 1850. MM does not occur equally among races. The annual incidence is about 9 for black men, compared with 3 for white women. In the Nordic countries the incidence of MM is 5-6 with a male:female ratio of 3:2.

Solitary plasmacytoma is treated by local radiotherapy. In such patients as well as in patients classified as having smouldering, asymptomatic stage 1, or indolent MM, systemic chemotherapy should be deferred until there is evidence of disease progression. Critical disease features associated with a poor prognosis include high serum values of C-reactive protein and beta-2 microglobulin and deletion of chromosome #13.

Standard or high dose (HD) chemotherapy, like Melphalan / Prednisone (MP) and Vincristin / Doxorubicin / Dexamethason (VAD), has improved the median survival from 7 months in the prechemotherapy era to ~ 3 years. Nowadays patients with symptomatic MM and age < 65 years are offered aggressive HD chemotherapy, stem cell transplantation and intensive supportive care which may further improve survival up to 6 years. MM, however, remains an incurable disease, which eventually relapses and becomes resistant to chemotherapy.

New insights into the role of the microenvironment in MM provide multiple targets for novel therapeutic modalities (such as thalidomide) to induce apoptosis and inhibit angiogenesis. Supportive care measures also have improved, including bisphosphonate to reduce the incidence of bone-related disease, and erythropoietin to reduce the need of transfusions.

Clinical studies evaluating quality of life and economy of treatment modalities are crucial for patients as well as for society. Population and evidence based guidelines for all aspects of the diagnosis and care of MM patients are needed for optimal treatment of patients and for helping community hematologists practice the state of the art. Guidelines of Nordic Myeloma Study Group are available on the Internet address: http://www.nordic-myeloma.org.
5. A NEW APPROACH TO HEMATOPOIESIS EVALUATION UNDER MULTIPLE MYELOMA.

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To date a functional status of blood forming cells may be evaluated successfully by a light microscopy at the level of pre-ribosomal RNA (pre-rRNA) transcription/processing by means of selective nucleolar organizer silver staining. Using this approach we have studied the functional status of different classes of erythroid, granulocytic, megakaryocytic, and lymphoid elements in bone marrow smears from 50 patients with multiple myeloma (MM). The investigation showed the level of pre-rRNA transcription/processing in low-proliferating plasma cells (Pc) to take intermediate position between such highly-proliferating cells as promyelocytes (Pm) and polychromatic normoblasts (Er-II) that may be associated with their participation in elevated synthesis of both pathological proteins and interleukines.

Furthermore, the mean number of Ag-grains per nucleus in PC was closely correlated with those of lymphocytes (Lf, r=0.64; p=0.001), pronormoblasts and basophilic normoblasts (Er-I, r=0.64; 0.001), Er-II (r=0.63; p=0.01), Pm (r=0.42; p=0.003), megakaryocytes (Mg, r=0.72; p=0.01), and Interleukine-6 (IL-6, r=0.5; p=0.011). In turn, the content of IL-6 under MM was closely associated with levels of pre-rRNA transcription/processing in Er-I (r=0.47; p=0.016), Er-II (r=0.87; p=0.001), Pm (r=0.47; p=0.016), and Lf (r=0.43; p=0.027) while its association was negative with the mean number of Ag-grains in Mg (r=-0.61; p=0.022). In our opinion, the revealed stimulation of protein-synthesizing machine in erythroid and granulocytic elements under MM might be hypothetically explained by a elevated production of vascular-endothelial growth factor (VEGF) and the following stimulation of erythroid/granulocytic cells through their receptors to VEGF (J Clin Pathol 2002, 55:530-4). As for a reciprocal depression of the pre-rRNA transcription/processing and differentiation in Mg it may be related to a modification of protein kinase Ca which appears to be a key moment for chooses of the pathway of differentiation for hematopoietic progenitor either along the erythroid or megakaryocytic lineages.
6. THE EFFICACY OF INDUCTION, INTENSIFICATION REGIMES AND AUTO-SCT IN MULTIPLE MYELOMA (MM).

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39 MM patients (pts) were included in the study follow-up. Stages I A-4 pts (7,2%), II A-21 pts (38,1%), II B-3 pts (5,4%), III A- 8 pts (14,5%), III B-4 pts (7,2 %).
Induction therapy was: VAD-D-D (96 h continuous infusion of Anthracyclines and Vincristin + Dexamethazone p/o) - 26 pts, Idarubicine + Dexamethazone- 5 pts, others ( Cph+P, M+P)- 8 patients.
VAD-D-D resulted in responses in 21 patients (CR-10 pts (38,4%), PR-11 pts (42,3%) and progression in 5 pts (19,2%). Ida+D resulted in partial remission in 5 pts - very good response - RR and nCR. 19 resistant patients were undergone intensification : 13 pts-DexaBEAM ( 7 pts- one cycle, 6 pts - two cycles), 4 pts-high dose Cph ( 1,5-3,0 g/m²), 1 – Melphalan (50 mg/ m²), 1- IEV (Ifosfamid, Etoposid, Epirubicin ). One cycle of DexaBeam resulted in no CR, 4/7 PR, 1/7 SD, 1/7 progression. Two cycles of DexaBEAM was more efficacious – 4/ 6 CR, 2/6 PR. High dose cyclophosphamid resulted in CR in all included patients (4/4).
16 patients were undergone auto-SCT (PBSCT-10, BMSCT-6), included 2 patients with double transplantation. 9 patients were transplanted in CR, while others (7) – in PR. 11 patients are alive, 5- died (1-in CR), transplantation related mortality 0%. Auto-SCT resulted in 30,3% EFS, whereas EFS in patients without auto-SCT was 21,2% (p=0,55).
Conclusion: CR rate in VAD-D-D is higher in comparison to Idarubicin – Dexamethazone regimen (p< 0,05). DexaBEAM is efficacions intensification regimen in resistant MM, two cycles resulted in higher CR (p< 0,05). High dose Cyclophosphamid (one cycle) is comparable the efficacy of to two cycles DexaBEAM, although the former regimen seems to be less toxic and expensive. Auto-SCT results in higher EFS in comparison to patients without this procedure (30,3%/21,1%, p= 0,55).
7. THALIDOMIDE IN MULTIPLE MYELOMA.

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Thalidomide was withdrawn from the market in 1960 and a scandal in modern history of pharmacotherapy was a fact. Approximately 10 000 babies have been born with severe limb defects. In spite of the bad reputation, thalidomide has been extensively studied and interesting immunomodulatory, anti-inflammatory and anti-angiogenic effects have been demonstrated. In 1998 it was discovered that thalidomide is active against multiple myeloma and the results have later been confirmed by a number of studies. Patients with advanced multiple myeloma resistant to melfalan have mostly been studied. The studies have included from 10 to 169 patients and are all non-randomized. Evaluation of response has in most studies been based on decline of monoclonal protein, some of the studies have also evaluated the clinical response. The first study by Barlogie et al included 169 patients which have been followed for 2 years. The Nordic Myeloma Study Group conducted a study 1999-2000 including 65 patients which have been followed for 3 years. Overall, the studies on thalidomide in multiple myeloma can be summarized as follows. 1) Approximately 30 % of patients with advanced or resistant disease respond to thalidomide. 5-10 % have an excellent (complete) clinical response, 2) thalidomide can be combined with steroids which enhances the response rate as well as the rate of side effects, 3) the effect of thalidomide can be detected after 1-3 weeks in responding patients, 4) duration of response is highly variable, in the NMSG study 32 % of the patients were alive after 2 years, 5) thalidomide is apparently active also in previously untreated multiple myeloma. However, randomized studies including many patients are required to make a comparison with melphalan, 6) The side effects of thalidomide are considerable, approximately 30 % of patients have to stop taking the drug due to constipation, drowsiness, neuropathy or general discomfort, 6) Doses of 50-800 mg per day have been used in multiple myeloma. Optimal dose is not known, but doses above 200 mg seems not to be necessary.
Recommendation: Patients with multiple myeloma should try thalidomide as 2 line treatment or later. If there is no decline in monoclonal protein within 4 weeks, they will probably not respond and thalidomide can be stopped.
8. QUALITY OF LIFE AND COST-VALUE ANALYSIS IN MULTIPLE MYELOMA.

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Multiple myeloma is a disease with pronounced symptoms. The most distressing are skeletal pain, fatigue and reduced physical and role functioning. Altogether, these symptoms cause a substantial reduction of the patients’ quality of life. At the same time, some of the treatment modalities that have been tested and implemented in this disease may themselves cause significant side effects with a bearing on quality of life. Examples are interferon, high dose chemotherapy with autologous stem cell support, and thalidomide. For these reasons, the endpoints of clinical trials should not be restricted to response, response duration and overall survival. It is important to include quality of life as reported by the patients on specially designed questionnaires.

The Nordic Myeloma Study Group has integrated the measurement of quality of life into several phase II and III trials since 1990. A quality of life secretariat has been developed in Oslo. The questionnaire developed by the European Organization for Research and Treatment of Cancer (EORTC QLQ-C30) is used. A data program has been developed that registers patients at diagnosis and issues questionnaires at regular intervals during follow-up. Incoming questionnaires are scanned and automatically entered into data bases. The NMSG has run therapeutic trials with integrated QoL studies in Denmark, Finland, Norway and Sweden with up to 600 patients and more than 90% compliance. A close collaboration with the EORTC Quality of Life Group has been established.

In these studies, prospective data on resource consumption have been collected. In cooperation with experts on health economy, cost-value analysis has been performed for several treatment modalities including interferon and intensive therapy with autologous stem cell support. In this way, new treatment principles may be quickly evaluated not only for the impact on traditional endpoint such as response, response duration and overall survival, but also for the effect on patients’ quality and life and resource consumption.

The main results of these studies are:

1. The EORTC QLQ-C30 questionnaire is a reliable and valid instrument for the measurement of quality of life in multiple myeloma (1).

2. In a randomised trial, interferon α-2b used in addition to melphalan and prednisone during induction and maintenance treatment caused a significant QoL reduction during the first year (2). After the first year, there was no difference in any QoL score; however, only 60% of the patients remained on the drug after 24 months. There was no QoL benefit associated with the 5-6 plateau phase prolongation in the interferon arm.

3. A cost-utility analysis of the 3-months (not statistically significant) prolongation of overall survival in the interferon arm was performed (3). Cost per Quality Adjusted Life Year (QALY) was conservatively estimated at USD 110,000, which was not considered cost effective. This paper also described a method for transforming QoL profiles into a single value on the conventional 0-1 utility scale.
4. Quality of life (physical functioning) assessed before and during chemotherapy was an independent predictor for survival in multiple myeloma (4).

5. In a population based study, the NMSG found a survival advantage for high-dose treatment with autologous stem cell support, compared to conventional chemotherapy. During the first 6 months of therapy, patients in the intensive treatment arm had moderately lower scores for global QoL and role and social functioning, and there was also a significantly higher score for appetite loss (5). At 12 and 24 months the QoL was similar to the control patients, and at 36 months, there was a trend towards less fatigue, pain, nausea and appetite loss in the intensive treatment arm. Thus, the 18 months of prolonged survival seem to be associated with a good QoL.

6. Cost per QALY (total gain: 1.2 QALYs) gained by this intensive treatment was estimated at NOK 249,000 (USD 27,000) which is well within the frame of treatment modalities considered to be cost effective (6).

The effect on QoL of therapy with bisphosphonates and/or thalidomide is currently under investigation.

References:


9. THALIDOMIDE AND DENDRITIC CELL BASED IMMUNOTHERAPY IN THE TREATMENT OF PATIENT WITH RELAPSE OF MULTIPLE MYELOMA AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION.

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Patients with multiple myeloma (MM) who relapse after high-dose therapy and autologous stem cell transplantation (ASCT) have limited treatment options. These patients are usually resistant to conventional therapy. Survival remains low in this group of very heavily pretreated patients and alternative strategies to improve outcome have being investigated.

We report the case of a 38-year old man with refractory MM who responded to thalidomide and dendritic cell based immunotherapy.

MM was diagnosed in September 2000. An examination revealed bone marrow plasmocytosis (47.5%), Bence Jones proteinuria 5g/day, severe anemia, multiple destructions of bones, hypercalcemia, acute renal failure. The patient received 4 courses combination chemotherapy (VMmPx2, VAmPx2) including a high dose of Methylprednisolone (total dose 67.5 mg/kg), Vincristin 0.5 mg/day, Melphalan or Adriablastin 10 mg/m² daily as continue infusion for 1-4 days. Complete remission (CR) was achieved after the 3-rd course of chemotherapy. As consolidation patient underwent Melphalan 200mg/m² followed ASCT (26.01.01). After ASCT the patient had been treated with an interferon - alpha (INF-α) (Roferon A 3 million ME 3 times per week) within 6 months. Also patient has received a regular therapy with biphosphonate (Bondronat 2 mg every 6 weeks).

The duration of the first CR was 17 months. The relapse of disease (bone marrow plasmocytosis 89%, Bence Jones proteinuria 2g/day) was diagnosed in April 2002. The patient has received 2 courses chemotherapy (Melphalan 18 mg and Prednisolon 120 mg daily for 4 days) without effect. There were noted progressive anemia (hemoglobin below 60 g/l), decrease of platelet count below 20x10⁹/l, repeated hemorrhage. The patient was a heavy transfusion depended. Re-examination of bone marrow (BM) had revealed plasmoblastic transformation of MM (85% plasmoblastic cells). The therapy with a Thalidomide 200mg/day and dendritic cells (DC) based immunotherapy was started.
Autologous DC was generated from adherent peripheral blood mononuclear cells in presence of granulocyte macrophage colony-stimulating factor (GM-CSF) and INF-α. Thereafter DC were primed with lysate of patient’s BM plasmoblastic cells and cryopreserved. Six DC vaccinations (6-10 millions cells s.c.) were administered every two weeks, followed by DC vaccinations were administered every four weeks. After 2 months the patient had responded to treatment. There was observed an increase of hemoglobin (> 80g/l) and platelet numbers (>50x10⁹/l). After the 4 months therapy hemoglobin level reached 120g/l, platelet count reached 100x10⁹/l. Response in clinical symptoms correlated with reduction of bone marrow plasmoblastic cells (40%). Side effects associating with Thalidomide and DC vaccinations are not marked. The patient has got the treatment in outpatient setting.

At present patient continues to receive a Thalidomide and Bondronat. Now the patient’s condition is satisfactory. The patient is transfusion independent. In the conclusion, it is necessary to emphasize, that new approaches have resulted not only in partial response, but also have ensured adequate quality of life of the patient within 8 months, that is most important goal of treatment of the patients with refractory MM.
A number of infusional chemotherapy regimens are used in the treatment of patients with multiple myeloma (MM). However combination of Melphalan and Prednisolon (MP) remains a gold standard as the first line therapy. The efficacy of other combinations is estimated in comparison with MP therapy. In our center we use VMmP chemotherapy including a high dose of Methylprednisolone 67.5 mg/kg/course, Vincristin 2 mg/course as continues infusion and Melphalan 40 mg/m²/course per os for 1-4 days. 16 patients were enrolled in this study. Clinical characteristics were: 11 male, 5 female; median age 56 years (range 36-68); 4 patients - progressive stage II, 12 patients - stage III; IgG - 10, IgA - 4, BJ –2. All patients had multiple destructions of bones, 4 patients had also acute renal failure. Every patient received at least 2 courses VMmP followed maintenance therapy with MP schedule. Regression of clinical symptoms (reduction of bone pain, correction of hypercalcemia, decrease of creatinine level, improvement in Performance Status) was observed after 1st course of chemotherapy. Response to treatment was evaluated by SWOG criteria after 2 courses of therapy. 25% or greater reduction in M protein was observed in 14/16 (87%) of patients. 2 patients (12%) achieved reduction in M protein of at least 75%, and 6 patients (37%) achieved complete remission (CR). Median duration of response was 22 months (range 8-72). Toxicity grade 2-3 were marked in 20% cases on 35 courses VMmP (neutropenia –1, gastrointestinal side effects – 4, steroid diabetes – 2). 3/16 patients (19%) experienced infection complications (pneumonia –2, fungal mucositis grade 3 - 1). Comparison with historical data of our patients treated with MP indicated that VMmP regimen induced significantly higher rates of overall response (87% versus 60%) and CRs (37% vs 13%). Patients, receiving VMmP in comparison to MP had significantly higher rates of overall response (87% versus 60%), complete remissions (37% vs 13%). Unfortunately VMmP therapy has not improved survival rate of MM patients (median survival 35 mounts). However, VMmP regimen showed important advantages over standard MP as primary treatment for MM. For VMmP treated patients higher rates of overall response and CRs with a shorter duration of therapy were achieved. Therapy was well tolerated and improved the quality of life.
PREDICTORS OF MULTIPLE MYELOMA PROGRESSION IN PATIENTS WITH MYELOMA KIDNEY.

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Renal involvement in patients with multiple myeloma has been described as a sign of poor prognosis. Few studies address the value of clinical and laboratory parameters as predictors of multiple myeloma progression in patients with myeloma kidney comparing patients without myeloma kidney. The results of these studies are controversial.

The frequency and prognostic role of the different parameters were studied in 79 patients with multiple myeloma (58 patients with myeloma kidney and 21 patients without myeloma kidney) treated by alkylating agents (melphalan or cyclophosphamide with prednisone).

The following factors were associated with multiple myeloma progression in patients with myeloma kidney: “aggressive” form of multiple myeloma (p<0.001), performance status (ECOG) >=2 (p=0.002), pathologic serum immunoglobulins >15% (p=0.045), serum creatinine >150 mkmol/l (p=0.029), serum urea >10 mmol/l (p=0.013), urea acid >400 mkmol/l (p=0.048), creatinine clearance <70 ml/min (p=0.025), hyperkalemia (p=0.013), hemoglobin <115 g/l (p=0.011), erythrocytes <3.0*10^12/l (p=0.010), platelets <170*10^9/l (p=0.040), lymphocytes <30% (p=0.008) at full blood count results, at bone marrow analysis - decreased (<1%) promyelocytes count (p=0.026), lymphocytes <4.3% (p=0.008), immature plasma cells >30% (p=0.005).

In patients without myeloma kidney these factors were not predictors of multiple myeloma progression.

Predictors of progression in multiple myeloma treated by alkylating agents are different in two groups of patients: with myeloma kidney and without myeloma kidney.
12. BONE MARROW STATUS IN MULTIPLE MYELOMA.

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In cytological investigation of bone marrow an ability of leukocytes cells, mainly of granulocytic or monocytic line, to form cellular associations with erythrocytes resembling rosettes was detected. In the previous studies it was demonstrated that hydrolytic enzymes possess a potential to penetrate into adjacent erythrocytes with their subsequent lyses. It is suggested that these cellular associations should be termed erythroclastic marrow clusters (EMC). The aim of the paper is to explore cellular content of the marrow in patients with multiple myeloma (MM) in conjunction with its cluster forming capacity.

42 patients with multiple myeloma were examined before the initiation of cytostatic therapy (average age 60,5±1,44). The disease stage was determined by universally accepted criteria of B. Durie and S. Salmon. Along with routine clinical and laboratory examination an EMC count in the marrow aspirates per 500 myelocaryocytes was performed. The disease course dynamics was monitored for 2 years after the treatment start.

There was no difference in plasmatic cells number in patients with various MM stages that confirms irregular nature of their distribution. However, in patients with chronic renal failure significantly higher plasmatic cells content (51,6%) was found in comparison to patients with normal plasma creatinine level (12,6%; p=0,004). Probably in MM with Bens-Johns protein secretion diffuse infiltration of the marrow by plasmatic cells occurs more often.

Close direct relationship between EMC number and circulating immune complexes number was established that allows proposing participation of immune mechanisms in their formation. In MM patients who had less EMC content a resistance to chemotherapy and earlier lethality were often revealed. It might be suggested that the intensity of EMC formation defines the content of circulating immune complexes in MM patient’s serum and marrow granulocytes functional status.
Oligoclonal Myeloma is a rare immunochemical tape of Multiple Myeloma (MM). Among 659 patients (type G), two had paraproteins IgG3 and IgG4. Both patients had bone pain, anemia, elevated ESR, plasma cell bone marrow infiltration (accordingly, 19 and 22.5%), stage 3 of disease. Total level of serum protein was moderately elevated (96g/l and 93.2g/l, accordingly), serum protein electrophoresis have shown the M-gradient (14.8 g/l and 10.2 g/l). Normal IgG level was moderately decreased, whereas IgA and IgM were significantly decreased. IgG3 and IgG4 were related with monospecific antibody by immuno-electrophoresis. The Bense-Jones proteinuria type K was related.

The peculiarities of clinical patterns were tumor nodules (plasmacytomas on cytological analysis), often infection complications. The predominant clinical syndrome was renal insufficiency. The complications due to antibody deficiency (despite low paraprotein level) were significant. The survival time was short (respectively, 20 and 24 mo).
Pathogenesis and treatment of Acute Leukemia.

1. FLT3 LIGAND AND ITS RECEPTOR IN REGULATION OF HDM2 IN ACUTE MYELOGENOUS LEUKEMIA.

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Acute Myelogenous Leukemia (AML) is characterised by an arrest in maturation of myeloid cells and an increase in the number of immature myeloid cells in the marrow. AML is a heterogenous disease and is divided into nine different subtypes. Numerous genetic defects are known in AML, but the most common is mutations in the fetal liver tyrosine kinase receptor 3 (Flt3).

Hdm2 corresponds to Mdm2 in mouse and is an oncogene with E3 ubiquitin ligase activity. It binds to and inactivates p53 by binding and ubiquination, but may also ubiquinate ß-adrenergic receptor and its adaptor protein [1]. Its main functions is to regulate the levels of p53 in the cell by three different ways: It blocks the transcriptional activity of p53, exports p53 from the nucleus to the cytoplasm and/or promotes the degradation of p53. There is increased expression of Hdm2 mRNA in about 70 % of AML cases [2].

Flt3 is expressed by immature hematopoietic cells and is important for the normal development of stem cells and the immune system. Flt3 has a Length Mutation (Flt3-LM) in exon 14 in 30 % of AML cases. This mutation is proposed to result in a constitutive active receptor. We have previously shown that the Flt3-LM is altered during AML relapse, probably making this mutation of little value for PCR-based relapse monitoring [3]. The ligand for Flt3 (FL) is expressed by e.g. marrow stromal cells and synergises with other growth factors to stimulate proliferation of stem cells, progenitor cells, dendritic cells and natural killer cells. We have tested the effect of FL and other related growth factors on primary AML blasts, in terms of proliferation and cytokine secretion [4]. AML blasts with Flt3-LM gave in general a weaker proliferative response when stimulated with growth factors, but this effect was modest. No significant effect was seen on spontaneous apoptosis. To determine witch pathways involved in the weak proliferative response, we examined components of the p53 pathway.

We have examined Hdm2 in relation to the Flt3 in primary AML blasts by immunoblotting. The Flt3 protein was detected by immunoblot in more than 60% of the AML patient cells. Apparently, the protein level of Flt3 correlated negatively with the protein level of Hdm2.
Treatment of AML patient cells with FL resulted in downregulation of Flt3 and up regulation of Hdm2. AML patient cells with mutated Flt3 showed weaker down regulation of Flt3 and up regulation of Hdm2. These results may suggest that Hdm2 and Flt3 are mutually regulated, and that Flt3-ligand indirectly alter the p53 system in AML. Experiments are in progress to examine the effect of inhibitors of Flt3 on Hdm2 and p53 in AML cells.

References:
2. GENE EXPRESSION ANALYSIS IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA, CHILDREN AND ADULTS.

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Advance in ALL therapy is one of the successful stories of cancer treatment during 20th century. Nowadays the survival of children with ALL is around 81% while adult treatment is less successful and survival seldom exceeds 30%.

The aim of our study was to find if there is difference in the expression profile that can indicate a biological difference in ALL patients: in children versus adults, in subgroups of children and in subgroups of adults.

Freshly frozen samples of bone marrow or peripheral blood with blast cell counts exceeding 75% were used to prepare and label RNA for hybridization. Thirty eight archive samples from 16 adults and 22 children have been included in the study. All patients have a follow up time of more than 1 year and they have been extensively investigated for presence of important cytogenetic abnormalities using FISH and SKY analysis when possible. The Affymetrix genechip platform Hu95ver2a containing around 12600 genes, some of which are in duplicates, was used to study the expression profile of ALL.

Unsupervised cluster analysis revealed that patients group according to cytogenetic abnormalities irrespective of age. We have found so far no difference in the gene expression profile between children up to 18 y.o and adults. There were distinct groups formed by T-cell ALL, ALL with MLL gene translocation, TEL/AML1 gene fusion, hyperdiploidy, monosomy 20, BCR-ABL gene fusion, and a few samples that didn’t fulfill cytogenetic criteria and didn’t form separate clusters.

Each cytogenetically defined group has a unique expression profile able to predict the cytogenetic abnormality. Seven to 10 genes were enough to predict cytogenetic abnormality. These are the subject for more detailed investigation since those genes may indicate new mechanisms involved in leukemia development.
3. OUTCOME IN AML PATIENTS TREATED IN CONSECUTIVE RUSSIAN MULTICENTER TRIALS.

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From September 1992 till May 2003 Russian multicenter trials comprised 790 AML and 111 APL patients treated in 32 hematological departments. The first AML-92 study has shown that etoposide (VP) in a five-days course started at day 10 after 7+3, did not increase CR rate (+VP=65,6%;-VP=58,6%) and induction death rate (+VP=22,3%;-VP=20,7%), but sufficiently improved 10-years probability of continuous CR (+VP=50%;-VP=29%, p=0,05), mostly due to patients from favorable risk group (L<30*10⁹/µ – 60% vs L>30*10⁹/µ – 20%, p=0,001; AML M1-2 – 60% vs AML M4-5 – 42%, p=0,01). It was also revealed that aggressive maintenance with «7+3» was not necessary after «7+3»+VP induction/consolidation, but was important after induction/consolidation without etoposide. The second trial AML-95 has proved that daunorubicin dose escalation in «7+3» course from 45 mg/m² to 60 mg/m² did not improve neither CR rate (45 mg/m² – 64,6% and 60 mg/m² – 64,6%) nor 6-years DFS (45 mg/m² – 28% and 60 mg/m² – 29%). It was also demonstrated that the efficacy of one year or three years maintenance is equal (DFS with 1 y – 28%, with 3 y – 15%, n.s.). The third trial AML-99 has shown a low outcome in patients after two consolidation courses with intermediate doses of ARA-C (1 g/m² bid, 1-5 days) with daunorubicin (45 mg/m² 1-3 days) in comparison with standard one year maintenance with “7+3”: relapse rate at one year 26,6% vs 4,5%. The fourth ongoing trial AML-01.01 compares four vs two 7+3+VP courses with one year maintenance vs treatment cessation after two «7+3»+VP induction courses and two consolidation courses with high doses ARA-C ( 3g/m² bid 1-3 days) with daunorubicin (45 mg/m² 3-5 days) . 165 patients were randomized. Two years DFS is 56%, 52% and 59%, OS - 38%, 58% and 65%, respectively.

APL trials has demonstrated that «7+3»+ATRA induction, «7+3» consolidation and «7+3» one year maintenance provided 65% of 5-years DFS. It was revealed that one year maintenance is not sufficient for APL as 77% of all relapses are late, occurring at > 18 months of CR, so maintenance duration in ongoing APL trial was prolonged to 2 years. ATRA incorporation into maintenance protocol increased 3-years OS from 68% to 82%. APL patients with initial WBC > 10*10⁹/l constitute an unfavorable prognostic group and are supposed to be treated more intensively (autologous or allogeneic BMT). 2-years DFS in these patients is 35% comparing to 78% in patients with WBC < 10*10⁹/l.
4. SURVIVAL IN NORWEGIAN PATIENTS <60 YEARS WITH PRIMARY AML, TREATED WITH A COMMON NATIONAL PROGRAM, WHICH INCLUDES REPEATED COURSES OF HIGH DOSE ARA-C AS CONSOLIDATION THERAPY, AS WELL AS ALLOGENEIC STEM CELL TRANSPLANTATION.

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The Norwegian Registry for Acute Leukemias and Lymphoblastic Lymphomas.

The Norwegian Registry for Acute Leukemias and Lymphoblastic Lymphomas (short: Leukemia Registry) is a subsidiary of the Norwegian Society of Hematology and has been operative from 1.1.2000. It’s aim is to register all norwegian patients > 15 years with AML, ALL and lymphoblastic lymphomas in order to create a database for epidemiological and clinical research. One of the registry’s first clinical projects is a study of the survival of younger patients (<60 years) with primary AML. The current national chemotherapy program for these patients was implemented in 1995, and is identical to the CALG-B program as published by Mayer et al. (N Engl J Med 1994). It comprises induction treatment with daunorubicin 45 mg/m² day 1-3 and Ara-C 200 mg/m² day 1-7, and 3-4 courses with consolidation with Ara C 3 g/m² x 2 day 1, 3 and 5. Furthermore, the national treatment program also includes allogeneic stem cell transplantations according to the following guidelines:

Low risk patients are not transplanted in first remission. Standard risk patients are candidates for allo BMT in first remission if they have a family donor, and high risk patients are candidates for allo BMT in first remission if they have a family donor or a matched unrelated donor (MUD). All patients, regardless of risk group, are candidates for allo BMT in 2 or later remission, with either family donor or with MUD. The different risk groups are defined on the basis of karyotyping and number of induction courses which were used in order to reach CR. In the national guidelines, upper age limit for transplantation with family donor is 60 years and for transplantation with MUD 55 years.

The assessment of treatment results in patients diagnosed in the period 1995-2000 (116 patients), was made on the basis of the hospital records. For the patients diagnosed after 1.1. 2000 (91 patients), the relevant data were taken from the Leukemia Registry.

Results: In all 217 younger patients received treatment for AML and 174 (80.2%) reached CR. Maximum follow-up is 96 months, median follow-up 15 months. 3 years leukaemia free survival was found to be 48.8 % +/- 4.3% and 3 years total survival 41.9 +/- 4.2%.

28 patients received BMT with family donor and 6 patients were transplanted with MUD in 1.CR. 3 patients received BMT with family donor in early relapse.

Furthermore, 6 patients were transplanted with family donor and 7 with MUD in 2 remission. 4 patients were transplanted with family donor and 3 with MUD in 3. or later remission. In all 57/217 patients received allo BMT.

The results of the current treatment program are superior to those obtained with the previous national program, which was used in the period 1990-1995 and consisted of less intensive consolidation chemotherapy, but had an identical transplantation program.
5. TREATMENT RESULTS OF ACUTE LEUKEMIAS IN PATIENTS YOUNGER 60 YEARS BASED ON DATA OF HOSPITAL REGISTERS OF ST-PETERSBURG, LENINGRAD REGION AND KARELIA REPUBLIC.

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We have carried out the analysis of morbidity and survival structure in different forms of acute leukemia in St-Petersburg, Leningrad region and Karelia republic during 2000-2002 years based on data of hospital registers. Patients with acute leukemias (AL) and lymphoblastic lymphomas (LL) treated in SPSMU under the name of academician Pavlov, Region hospital of St-Petersburg, hospital N 31 of St-Petersburg and Republic Hospital of Karelia were included in study. All patients were diagnosed during the period from 01 January 2000 to 31 December 2002. Totally, 358 patients were cured in above hospitals, among them 275 patients with acute myeloid leukemia (AML), 56 - with acute lymphoblastic leukemia (ALL), 15 – with lymphoblastic lymphoma (LL). The average age of patients was 56,5 +/- 17,5 years. Hundred patients had only symptomatic and palliative treatment due to old age or severe condition. Survival analysis included only patients younger than 60 years, who were planned to undergo standard chemotherapy. Complete remissions were reached in 53% (N=60) patients with AML and in 77% patients with ALL and LL. Further relapse was registered in 35% (24/60) patients with AML and in 45% (14/31) patients with ALL and LL. Overall survival was equal for AML and ALL and has made 23%, in patients with LL – 65%. Disease free survival in AML patients was 42%. In analysis of survival depended on type of leukemia the best results were found out in patients with promyelocytic leukemia (overall survival = 72%, all patients, who reached complete remission are alive without disease signs). Significantly worse survival was in M1-M2 variants and especially M4-M5 variant of AML (20% and 11% respectively). Relapse free survival was 41% (M1-M2) and 25% (M4-M5). The main cause of death was connected to progression or relapses of disease. Early death (first 30 days from diagnosis) in assessed group was 19%, and in whole population of patients – 32%. The majority of patients were undergone similar treatment in all hematological centers: “7+3” in AML, GALL 3/95 in ALL and LL, ATRA-based treatment in patients with promyelocytic leukemia. However, treatment results are different in various clinics, probably because of organization distinctions, firstly concerning supportive therapy. High level of relapses in all clinics could confirm the lack of consolidation therapy, such as high dose therapy with or without bone marrow transplantation.
6. **LONG-TERM RESULTS OF ACUTE LYMPHOBLASTIC LEUKEMIA TREATMENT IN ADULT PATIENTS.**

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**Aim:** evaluation of the long-term outcome in acute lymphoblastic leukemia (ALL) in adults.

**Patients and Methods.** From January 1992 to December 1999, ALL was diagnosed in 53 patients (27 males, 26 females, median age 35 years, range 15-68). Immunophenotyping available in 42 patients identified the following variants: prepreB - 4, Common - 20, preB - 6, B - 2, T - 10. Normal karyotype was found in 7 patients, hyperdiploid - in 5. Ph+ chromosome was detected in 5 cases, t(4;11) - in 4, t(1;19) - in 2; t(8;14), der 9, del TAL1 - each in 1 case. Central nervous system involvement at the time of diagnosis was observed in 4 patients (7.5%). Two patients died before the treatment started, one refused chemo-therapy. B-ALL patients (2 cases) received therapy according to the B-NHL-86 protocol, the others with different ALL variants (48 cases) were treated with the modified GMALL-04/89 protocol [Problems of Haematology and Blood Transfusion, 1996, N3, p.34].

**Results.** B-NHL-86 protocol: complete remission (CR) lasting for 120 months has been achieved in one B-ALL patient; the second one (68 years) died during the induction therapy. Modified GMALL-04/89 protocol: CR was achieved in 35 patients (72.92%), including 33 patients under 50 years (84.63% out of 39), 2 patients over 50 (22.22% out of 9). Refractory patients in general were 4 (8.33%), among “under 50” group - 2 (5.12%), among “over 50” - 2 (22.22%). Early death happened in 9/48 (18.75%), 4/39 (10.25%) and 5/9 (55.6%) patients respectively. Out of 35 patients in CR 2 (5.7%) died during chemotherapy course. Relapse was registered in 23 patients (65.71%): during the 1st year - in 12 (52.17%), the 2nd - 4 (17.39%), the 3rd - 2, the 4th -1, the 5th - 2, the 6th - 2. After 6 years’ remission no relapse occurred.

In general, remission continues in 11 patients, its duration ranges from 50 to 131 months, in 9 patients exceeding 6 years. 4-years disease-free survival is 41.67%, among “under 50” patients - 44.11%; 4-years overall survival - 30% and 37.5% respectively.

**Conclusions.** The received data demonstrate the efficiency of B-NHL-86 and modified GMALL-04/89 protocols treatment in ALL patients under 50. For patients over 50 it proved to be extremely toxic, what caused a very high level of early death among the patients of this group.
7. MTT-TEST IN PROGNOSIS OF DISEASE-FREE SURVIVAL IN ALL (3 YEARS OF OBSERVATION).

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**Background:** As an important determinant of response to chemotherapy, accurate measurement of cellular drug resistance may provide clinically relevant information.

**Aims:** to evaluate the relations between blast cells sensitivity profile to chemotherapy ex vivo (according to range scale) and the probabilities of 3-year disease-free survival.

**Methods:** The blast cells sensitivity to chemotherapy was examined in MTT-assay. The material of the study – bone marrow from 100 children with newly diagnosed ALL.

**Results:** For each drug (Vcr, Pred, Dexa, L-asp, ) LC50 was determined. The degree of cytotoxicity was estimated on a ranks scale: each step of a drug dilution (from greatest to lowest) was corresponded to ranks (from 1 up to 7). The high sensitivity to a drug - value of LC50 from 1 to 4 ranks, low sensitivity - value of LC50 from 5 to 7 ranks. The probability of 3-year disease-free survival was significantly higher in patients with the high sensitivity (1 - 4 ranks) to Vcr (p=0.02), Pred (p=0.015), Dexa (p=0.012), L-asp (p=0.01) in comparison with the patients with low sensitivity (5 - 7 ranks). The probability of 3-year disease-free survival in patients with high simultaneous sensitivity to 3 of 4 drugs (Vcr, Pred, Dexa, L-asp) was 100% versus 75% in patients with low simultaneous sensitivity to these drugs (p=0.011). The leukocytosis at initial diagnosis do not influence in disease-free survival by difference sensitivity drugs in MTT-assay.

**Conclusions:** the initial sensitivity of leukemic cells to Vincristine, Dexamethazone, Prednisolone and L-asparaginase is very significant in prognosis of 3 years disease-free survival in child ALL. High simultaneous sensitivity to at least 3 of 4 above mentioned drugs appears to be the good prognostic factor.
8. CLINICAL AND LABORATORY FEATURES OF ACUTE HYBRID LEUKEMIA.

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The co-expression of myeloid antigens on leukemia cells in adult acute lymphoblastic leukemia is observed not frequently, and the data on clinical and laboratory features of these hematological malignancies are not numerous.

The purpose of our work was to characterize genetic, morphological and clinical features of acute hybrid leukemia (AHL) and acute lymphoblastic leukemia (ALL) with co-expression of myeloid antigens (CD13 or CD33).

We observed 11 AHL patients and 4 ALL patients with co-expression of CD13 or CD33 antigens at the age from 17 to 52 years which were treated in the Hematological Centre of Regional Clinical Hospital №1 (Ekaterinburg) in the period from 1997 till 2003. The percentage of blasts in bone marrow at the moment of diagnosis of acute leukemia was 78,0±20,7%. Immunophenotyping, cytogenetical and/or molecular genetical analysis were performed in all patients. Aberrant caryotypes were revealed in 7 patients (46,7%), including pseudodiploidy (4 cases, 26,7%), hyperdiploidy (2 cases, 13,3%) and hyperdiploidy in combination with structural mutations (one case, 6,7%).

The treatment of patients was carried out according to protocols of the Hematological Scientific Centre of the RAMS (Moscow). Survival probability was estimated by Kaplan-Meier.

In 66,7% of cases a primary hematological remission was achieved, in 20% of patients the early and late relapses were observed. A 46-months event-free survivability was 53,3%. The extremely adverse prognosis was observed in two patients with t(9;22)(p34;q11) (terms of follow-up were 1,5 and 4 months, accordingly). Rather favorable prognosis was determined in the group of patients with diploidy (n=8, 46-months event-free survivability was 62,5%).

We conclude that for the detection of prognostically favorable mutations in patients with AHL (ALL with co-expression of myeloid antigens) it is necessary to use molecular genetic analysis while cytogenetical method is insufficiently effective. It can help to define groups in acute leukemia with various variants of genetic abnormalities for development of therapeutic programs, including bone marrow or peripheral blood CD34(+) - stem cells transplantation.
9. PROGNOSTIC SIGNIFICANCE OF TRILINAGE MYELODYSPLASIA AND ITS ASSOCIATION WITH KARYOTYPE IN DE NOVO ACUTE NONLYMPHOBLASTIC LEUKEMIA (ANLL).

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Purpose: WHO classification 2001 defined ANLL as a separate major category of disease with multilinedged dysplasia by the present two or trilineage myeloid cells dysplasia (TLD). It is known a number of studies indicating an unfavorable prognostic role of TLD, but the reason of this phenomenon remains unclear. The aim of the study is to determine prognosis of TLD pts and its association with karyotype in cases of de novo ANLL.

Patients and Methods: 131 newly diagnosed ANLL pts (except acute promyelocytic leukemia) entered the study in Hematology dept. from 1981 to 2002. Median age 39,4 years (range 17-70), male/female ratio 61/70. Definition of dysplasia was determined according to Goasguen et al., 1992. Cytogenetic analysis was performed in 66 pts at cytogenetic laboratory of Cancer Research Center RAMS. Favorable prognostic karyotyping features included t(8;21)(q22;q22), inv(16); unfavorable changes were considered as loss of chromosome 5 and 7, rearrangements of long arm chromosome 3, specific translocations: t(9;22), t(6;9) and complex abnormalities containing 3 or more aberrations; other chromosomal abnormalities arranged intermediate subgroup. All pts were treated according to schedule “7+3” by standard dose cytosine-arabinoside and different anthracylines.

Results: TLD was diagnosed in 14 (10,7%) pts. Complete remissions (CR) rates, overall survival (OS) and disease –free survival (DFS) were similar (p>0,05) in TLD and non TLD groups: CR – 50 and 60,7%, resistance –42,9 and 23,1%, median OS – 7 and 7,9 mo (median follow-up 3,5 and 5,5 mo), 5-years OS – 28 and 22%, median DFS –22,2 and 14,6 mo, 5-years DFS – 21 and 9%, respectively. Collation of cytogenetic analysis and dysplasia in TLD group revealed significant higher frequency of unfavorable chromosomal abnormalities in TLD group vs. non TLD group (50 and 23,2%, p<0,05).

OS and DFS were significantly better (p<0,05) in combined favorable and intermediate cytogenetic subgroups (median OS – 17,4 mo, median DFS – 22,2 mo) vs. unfavorable cytogenetic subgroup (median OS – 4,4 mo, median DFS – 4,9 mo). There was no influence of TLD on survival in combined favorable and intermediate cytogenetic subgroups. There were no differences in survival in TLD and non TLD groups in unfavorable cytogenetic subgroup too. However in TLD pts were revealed significant differences (p=0,02) of OS in combined favorable and intermediate cytogenetic subgroups (median survival not achieved) vs. unfavorable cytogenetic subgroup (median OS – 4,3 mo). Otherwise in non TLD pts was noted only tendency to higher OS and DFS in combined favorable and intermediate cytogenetic subgroups vs. unfavorable cytogenetic subgroup.

Conclusion: These results indicate higher frequency of unfavorable cytogenetic abnormalities in TLD vs. non TLD ANLL. Our preliminary data permit to conjecture not only the primary unfavorable role of appropriate karyotype, but more significance of it in comparison with TLD in de novo ANLL. This assumption needs more representative study groups.
Intensive chemotherapy in treatment of malignant diseases is often hampered by bone marrow deficiency. This problem is even more severe in case, when person or his relatives oppose transfusions of blood products. In order to evaluate the benefit of erythropoietin in anemia caused by chemotherapy we treated a child with subcutaneous injections of this medicine while performing treatment for acute lymphoblastic leukemia.

A 1-year boy was admitted to Regional Children’s hospital in Chelyabinsk with acute lymphoblastic leukemia, standard risk group. We offered his parents a cytostatic treatment, according ALL-BFM90M protocol. The problem appeared at the first day of hospitalization, the family belongs to Jehovah’s Witnesses confession. They have refused the transfusion of any blood products, but at the same time insisted on intensive chemotherapy.

We expected that decreasing volume of blood is necessary in MB-91 protocol (Moscow-Berlin). We combined chemotherapy with subcutaneous injections of erythropoietin. Erythropoietin course consisted of 5 daily injections. The doses of Rekurmon or Erythropoietin were 2000ME per injection. There were only 5 erythropoietin courses.

At the beginning of the treatment patient had Er-2*10⁹ /l, Hb-66g/l, Tr-110 *10⁹ /l, Leuk-11,2*10⁹ /l. During the whole period of treatment Hb level was 80-100 g/l and Er 2,6-3,3*10¹²/l. These blood parameters permitted not to stop chemotherapy. There was not necessity to use Erythropoietin during consolidation part of the protocol. Now the boy has maintenance therapy.

So, Erythropoietin’s courses allowed to perform high intensity therapy according to protocol without blood transfusion.
11. THE EXPRESSION OF PRB AND E2F1 PROTEINS IN LEUKEMIC CELLS.

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Background: The retinoblastoma tumor suppressor protein (pRB) is a transcriptional repressor that regulates gene expression by physical associating with transcription factors (E2F family members). pRB and E2F control the cells recruitment from G₁ to S-phase of mitotic cycle and play important role in cell proliferation and differentiation. In untransformed cells, the ability of pRB to bind to E2F is regulated by its cell-cycle-dependent phosphorylation. In hypophosphorylated form, pRB binds and inhibits E2F1. The understanding of the physiological role of the pRB–E2F pathway in human cancers is of utmost importance, because pRB and its upstream regulators are often mutated in malignant cells.

Aims: to evaluate the levels of expression of principal mitotic proteins pRB and E2F1 in childhood acute leukemic cells.

Methods: the material of the study – bone marrow cells from 57 children with newly diagnosed ALL (B-lineage-26, T-lineage-4) and AML(15) and 12 children with relapse ALL. The level of protein expression pRb and E2F1 was studied by Western blotting analysis.

Results: Protein pRb expression was not observed in 9 out of 30 cases of initial ALL in children (30%) and in 9 out of 12 cases of relapsed ALL in children (75%); the level of E2F1 expression in these cases was much higher: median 2.35 in comparison with 0.8 in cases, expressed pRb.

The expression of protein Rbp was not visualized in 2/15 of initial -AML samples. Levels of Rbp protein expression in initial ALL samples was 3.79-fold lower levels than in relapsed ALL, 1.47-fold lower than in initial AML.

The expression of E2F1 protein was not visualized in 6/30 of initial All, in 3/15 of initial AML and in 4/12 of relapsed ALL. Levels of E2F1-protein expression in initial ALL samples was 1.9-fold higher than in initial AML. There was no differences in E2F1 protein expression levels between child initial and relapsed ALL.

Conclusions: The absence of pRb expression has a poor prognostic significance. It is observed more often in relapsed ALL cases. Levels of E2F1 expression are higher in cases without pRb expression.
12. MORPHOLOGICAL PECULIARITIES OF SECONDARY MYELODYSPLASTIC SYNDROME IN PATIENTS WITH HEMOBLASTOSIS.

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Modern modes of program and high dose polychemotherapy are often accompanied with myelopoiesis depression with further formation of leukopenia and anemia that are characterized by marked resistance to therapy.

The purpose of the research was a study of hematopoesis condition in patients with primary myelodysplastic syndrome (36 patients), with acute leukemia – 42 people, with non-Hodgkin’s malignant lymphomas – 73 patients before and after chemotherapy and determination of the hematopoetic dysplasia value in cytopenia development.

Clinical and laboratory data, results of treatment, morphology of marrow and peripheral blood cells were analysed in dynamics.

Before polychemotherapy (PCT) the presence of some signs of myelodisplasia was determined, and in 60,7% of the cases the changes were found in cells of three haematopoetic lineages. In primary myelodysplastic syndrome signs of myelodysplasia was observed in cells of three lineages in 100% of patients. After chemotherapy courses the occurrence of dysplastic changes in marrow cells in patients with acute leukemia and non-Hodgkin’s malignant lymphomas increased. The following erythropoiesis disturbances were observed: aniso-poikilocytosis of erythrocytes – 64% before and 92% after the therapy; cytoplasmic bridges – 56% and 77,2%; appearance of megaloblasts – 32% and in 51% of patients correspondingly. In the granulocytic lineage hypogranulation of neutrophils were found before PCT in 83,3% of patients and in 92% of patients after it, pelgeroid changes – 16,6% and 28,5% accordingly. Signs of dysmegakaryocytopoiesis were found in 25% before the treatment and in 64,2% of patients after PCT.

The obtained data reveal high occurrence of marrow myelodysplasia in non-Hodgkin’s malignant lymphomas and acute leukemia, which were more significant after PCT and persisted in the period of remission. This phenomenon is due to tumor and chemotherapy influence on haematopoesis. The peculiarity of the secondary medicamentally induced myelodysplasia is absence of erythroid cells hyperplasia, monocytosis, less expressed megaloblastoid features, although the latter occur more often after chemotherapy. There was direct correlation of dysplasia intensity of hematopoietic cells and the duration and level of cytopenia. Leukopenia and refractory anemia which persist for a long time require dynamic control and, if necessary – therapeutic correction.
13. ADHESION AND HOMING OF BONE MARROW HAEMATOPOETIC PROGENITORS OF MDS PATIENTS TO NORMAL BONE MARROW STROMAL CELLS.


The myelodysplastic syndromes (MDS) are clonal stem cell disorders associated with a variety of abnormalities of mature and maturing cells including surface antigen abnormalities. The different adhesion molecules take part in homing of haemopoietic progenitor cells to bone marrow and mobilization of haemopoietic cells in peripheral blood. Insights in the abnormalities seen in adhesive patterns in MDS will help to develop the new strategies for the treatment of this disease. The purpose of the present research was to study the adhesion interactions and homing of haemopoietic cells and particular haemopoietic progenitor cells to stromal microenvironment in patients with MDS and to compare that from healthy donors. The adhesion of bone marrow mononuclear cells and haemopoietic progenitor cells (CFU-GM and BFU-E) to stromal layer from long-term bone marrow cultures and fibroblast monolayer was evaluated in vitro through 2 hours and during 3 days of cultivation. Adhesion of MDS progenitors to normal stromal layer (13,2±5,9%) was less as compared to the binding to donor progenitors (27,7±3,7%). The percent of attachment haemopoietic progenitor cells from MDS bone marrow to stromal layer and fibroblasts during 3 days of cultivation was lower than that in donors. Adhesion of haemopoietic progenitor cells to bone marrow stroma can be modulated via specific adhesion molecules. To determine which adhesion molecules may be involved in these cell-cell contacts we examined the expression of adhesion receptors on fibroblasts obtained from bone marrow of MDS patients and healthy donors. It was shown that there was no difference in \( \beta \)-1 family integrins expression (VLA-1, VLA-2, VLA-5) on MDS and normal fibroblasts. However MDS fibroblasts expressed significantly less VICAM-1 integrin as compared with normal fibroblasts. Our findings demonstrate that reduced adhesive properties of MDS haemopoietic progenitor cells and VCAM-1 expression on the cell surface of MDS fibroblasts may be one of the important reasons of disturbance of intercellular interactions in MDS.
14. CYTOKINE NETWORK OF IGE-PRODUCTION IN HEMATOLOGICAL MALIGNANCIES.

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Different factors regulate the growth and differentiation of the malignant cells and the same factors change IgE-production in patients with hematology malignancies. Well known that, the level of main cytokines in vivo and in vitro is regulated by the relation between Th1 and Th2. The production of IgE is strongly depended on that relationship. In hematological malignancies as NHL, HD, ALL, PV and AML blood-levels of IgE are significantly higher (2 – 10 times) than in healthy donors, CLL and CML. Blood-levels and secretion in vitro of main cytokines, which regulate IgE-synthesis (IL-4, γ-IFN and TNF-α) would be to change significantly too and would depended by variant of hematology malignancies and therapy effects. Also, it had been to appear, that levels of cytokines depend by activity and relationship of the receptors for histamine H1-, H2- and H3-receptors) on the immunocompetent cells. And the level of EPO in vitro and vivo influenced to the secretion of cytokines and IgE-productions. We found inverse dependency between IgE level and EPO in vivo and in vitro. In NHL and ALL patients with neuroleukemia levels of TNF-α, γ-IFN and IL-4 in spinal liquor increase comparatively without neuroleukemia. Moreover IL-4 was not only positive factor for IgE-production, but that will be to modulate in vitro activity of the leukemic blasts and bone marrow cells (BMC) in patients with hematology malignancies. But the results (stimulation/inhibition of cells and modulation of IgE-production) are differences between groups of hematology diseases and normal immunocompetent cells. Erythrokaryocytes of bone marrow in PV and AML-M6 show high cytokine-levels production as Th- and strongly modulate activity of peripheral blood mononuclear cells (PBMC) and BMC-proliferation and Ig-synthesis. The special erythroide suppressor factor had been discovered. It suppressed strongly BMC proliferation, significantly stimulated IgE-production by normal and patients’ with lymphomas and PV lymphocytes and strongly suppressed a synthesis of Ig other classes. In conclusion that scientific problem hasn’t restricted only of the mechanisms of regulation IgE-synthesis in hematology malignancies, but results would be used for prognosis and cytokine’s therapy of the same disturbance and manipulation of bone marrow cells around transplantation.
15. POLYMORPHISMS G2677T AND C3435T OF MDR1 GENE AND RESISTANCE TO CHEMOTHERAPY OF PATIENTS WITH LYMPHOPROLIFERATIVE DISEASES

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P-glycoprotein, the gene product of MDR1, belongs to the ATP-binding cassette superfamily of membrane transporters involved in active transport of wide spectrum of substances. Many antineoplastic agents including antracyclines, Vinca alcoloids and taxanes are P-glycoprotein substrates. Functional genetic polymorphisms in the MDR1 gene influence the distribution and bioavailability of substrates of P-glycoprotein and that may lead to alteration of sensitivity of tumour cells to chemotherapy.

We investigated MDR1 polymorphisms in exon 21 and exon 26 and analyzed distribution of MDR1 polymorphic alleles in patients with lymphoproliferative diseases (non-Hodgkin’s lymphoma, chronic lymphocytic leukaemia). Polymerase chain-reaction - restriction fragment length polymorphism assay was applied to assess G2677T and C3435T MDR1 polymorphisms in 53 patients after chemotherapy. Odds ratio was applied to estimate association of MDR1 genotypes with resistance to chemotherapy.

The risk of resistance to chemotherapy for patients with T2677T genotype was a 4.5-fold higher than for G2677G genotype carriers and a 6.8-fold higher in comparison with heterozygotes and sub-jects homozygous for G-allele. The carriers of T3435T genotype had a 6.3-fold increased risk of resistance to anticancer agents compared to C3435C genotype carriers and had 6.8-fold increased risk in comparison to the combined category (C3435C and C3435T). The T2677T T3435T haplotype conferred a statistically significant 10-fold increase in risk of resistance to chemotherapy relative G2677G C3435C haplotype and in 17-fold increase in risk in comparison the sum of all other combinations genotypes. All differences described above were statistically significant.

Conclusions: Our results suggest that polymorphisms of MDR1 gene may be associated with drug resistance of haematological malignancies. T2677T and T3435T genotypes conferred a statistically significant increase in risk of patient to be resistant to chemotherapy.

Supported by RFBR grant N 02-04-48328.
Chronic lymphocytic leukemia (CLL) is characterized by differences in types of progression, responses to chemotherapy (CT) and survival. Despite the presence of several prognostic markers, sometimes it is difficult to predict the course of the disease in patients with early stages.

The aim of the study was to evaluate and estimate the prognostic significance of p-53mut, bcl-2 and CD38 expression levels during the course of the disease and to compare it with effects of CT.

105 patients were evaluated, median age was 58 years (age range: 34 - 78).

Results. Biological markers of malignant cells of peripheral blood and bone marrow were investigated immunocytochemically by avidin-biotin method with monoclonal antibodies ("DAKO"). Our data showed the link between the expression levels of these markers and the stage of the disease. The highest levels of these parameters were associated with stages III and IV of CLL. CD38 expression was not detected in cases of non-progressive courses in comparison with progressive courses. There was a strong link between expression levels of these markers and responses to therapy. Poor response to therapy was associated with highest primary levels of the markers. Incidence of remissions in the group of patients with high CD38 (>30%), bcl-2 (>50%) expression levels was 24%, in the group with low expression - 76%. During courses of CT, expression of p-53 and bcl-2 markers decreased. Extent of this decrease correlated with the CT regimen. The most significant decrease was revealed in patients treated with fludarabine, cyclophosphan and mitoxantron (FCM) in comparison with FC and leukeran, and CHOP. Patients with primary high bcl-2 and p-53 expression levels after the courses of FCM had higher incidence of complete remissions (56%), than those after standard CT programs. Our findings show that p-53mut, bcl-2 and CD38 markers may be used as an additional prognostic factors to predict the course of the disease and response to therapy.
17. DETECTION AND COMPARISON OF HYPER EXPRESSION LEVELS OF MDR1 AND MRP GENES IN PATIENTS WITH NON-HODGKIN LYMPHOMA.

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The phenomenon of multi-drug resistance is one of the major reasons of standard chemotherapy failure in patients with hematological malignancies. In more than 90% cases multi-drug resistance occurs due to overexpression of the P-glycoprotein, encoded by the MDR1 gene. P-glycoprotein functions as transmembrane ATP-dependent pump, decreasing the intracellular concentrations of different toxic substances. The second important type of multi-drug resistance is associated with overexpression of multi-drug resistance associated protein (MRP) which acts similar to P-glycoprotein.

Therefore the aim of our study was to detect the level of expression of the MDR1 and MRP genes and to compare the frequency of appearance in patients with non-Hodgkin’s lymphoma before and after chemotherapy.

The samples of venous blood were collected to the sterile tubes, erythrocytes were separated and the total cellular RNA was isolated from each sample. Level of expression of the MDR1 and MRP genes was measured by reverse transcription polymerase chain reaction (RT-PCR) method using beta-actine and L11 ribosomal protein (RPL 11) genes as internal standards. 72 Patients with different immunomorphological types of Non-Hodgkin’s lymphomas were taken in the study.

The high expression levels of MDR1 or MRP genes were detected in 60% of all cases. In 85% of the cases the high level of MDR1 gene in respect to MRP gene expression was detected, in 15% cases the high level of the MRP gene and in 9% cases the high expression levels of both genes. The results showed significant correlation between high expression levels of the MDR1 and/or MRP genes and the standard chemotherapy failure.
Immunomorphologic diagnostics of tumors is one of the most significant methods in modern oncology and oncohematology. Municipal center of immunomorphological diagnostics of tumors was established in 2001 in Novosibirsk. 1052 immunomorphologic analyses were made for patients with tumors from hospitals of Novosibirsk and other cities of Siberian region (Tomsk, Omsk, Barnaul, Novokuznetzk, Kemerovo et al.). Main problems during performing analyses were concerned with interpreting data, but not with technical complicity. The purpose of a study was to perform an analysis and to evaluate the key points in making an immunomorphologic diagnosis. We used a panel of 60 monoclonal antibodies during exploring the tumor immunophenotype. Clinical data, morphological features of a tumor, cytogenetical and immunophenotypical characteristics were mentioned when making a certain diagnosis. The structure of examined patients consisted of patients with solid tumors (30%), patients with reactive states of hemopoesis (15%), and patients with hemoblastosis. While analysing the data we got to know that the basis of the methodology of making an immunomorphological diagnosis is as follows: 1) considering the heterogenity of lymphoma immunophenotype that demands using the additional markers (T-cell: CD1α, 2,3,4,5,7,8, 45RO, TCR-α/β, TCR-δ/γ; B-cell: CD20, 23, 79α, κ-chain Ig, λ-chain Ig et al.); 2) considering the incorrespondence of immunophenotype and clinical aggressiveness of lymphomas (24,5% incidents), demanding studying proliferation and apoptosis in tumor sample (Ki-67, Cyclin D1, bcl-2, p-53, CD95, APO-TACS et al.); 3) necessity of differential diagnostics between lymphomas, reactive states of hemopoesis and solid tumor metastases, which needs using the oncomarkers (c-myc Oncoprotein, ALK), anti gene MDR product – antibodies (p-Glycoprotein) and 25-30 tissue-specific monoclonal antibodies; 4) requirements of skills of the immunomorphologist, experienced in morphological dyagnistics, immunological studies and clinical practice; 5) necessity of combining immunocytochemical and immunohistochemical studies of a tumors; 6) individual marker panel to a concrete clinical situation. Observing these conditions is the only way to improve tumor diagnostics and therapy effectiveness significantly.
19. CLINICAL PROGNOSTIC FACTORS AND MULTI-DRUG RESISTANCE (MDR) PHENOMENA IN CHRONIC LYMPHOCYTIC LEUKEMIA AND LOW GRADE NON-HODGKIN’S LYMPHOMAS.

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The aim of investigation was the estimation of frequency and prognostic role of the clinical factors and MDR genes expression in the patients with chronic lymphocytic leukemia and low grade Non-Hodgkin’s lymphomas. Forty one patients with low grade Non-Hodgkin’s lymphomas and 63 patients with chronic lymphocytic leukemia were included in the study. Expression of MDR genes (MDR-1 gene, MDR-related protein - MRP, topoisomerase IIα, glutathione S-transferase - GSTπ) was investigated by reverse transcription polymerase chain reaction in the bone marrow samples in untreated (23 cases) and previously treated (23 cases) patients.

The study showed that the following factors were associated with disease progression in patients with low grade Non-Hodgkin’s lymphomas: high and intermediate value of MPI (p=0.001), in the bone marrow analysis - lymphocyte count >50% (p=0.002), myelocyte count <13.9% (p=0.020), erythroid cells count <=15% (p=0.002), peripheral blood leucocytes count >9.0*10⁹/l (p=0.021), lymphocytes >40% (p=0.001) and neutrophils <50% (p<0.001).

The following factors were associated with disease progression in patients with chronic lymphocytic leukemia: lymphadenopathy (p=0.001), splenomegaly (p<0.001), stage C (p<0.001), in the bone marrow analysis - pathologic forms of lymphocytes >3% (p=0.012), lymphocytes >60% (p=0.015), prolymphocytes >5% (p<0.001), monocytes <10% (p=0.001), in peripheral blood count – the hemoglobin level <120 g/l (p=0.010), erythrocytes <3.3*10¹²/l (p=0.027), leucocytes >9.0*10⁹/l (p<0.001), lymphocytes >80% (p=0.006).

To determine a possible effect of the chemotherapy on MDR-1, MRP, topoisomerase IIα, GSTπ expression patients were classified into two groups (i.e. treated or untreated patients). In untreated patients the increased level of MDR-1 gene expression (p<0.001) and decreased level of topoisomerase IIα expression (p<0.001) were found, as compared to the group of untreated patients. In order to know whether these markers were associated with a clinical resistance to chemotherapy, we compared the expression of MDR-1, MRP, topoisomerase IIα, GSTπ – in two groups: with the clinical response and without clinical response (disease progression). In patients with disease progression we found the increased level of MDR1 (p<0.05), MRP (p<0.01), GST-π (p<0.05).

Clinically MDR phenomenon results from the interaction of a large number of cellular events. We suggest that the constitutive overexpression MDR-1, MRP, GSTπ is one of the mechanisms of MDR phenomenon in patients with chronic lymphocytic leukemia and low grade Non-Hodgkin’s lymphomas.
The aim of the study was to define risk factors of *Pseudomonas species* infection in patients with hematological malignancies.

**Materials and methods:** 67 patients with hematological malignancies and infection complications from hematological department of the Novosibirsk Regional Clinic were included (27 men, 40 women, the age 16-82 years old). Non-Hodgkin’s lymphoma (25.4%) and acute leukemia (41.3%) were diagnosed more frequently. The infection complications developed after chemotherapy in 87% cases. Neutropenia (decrease of white blood cells less then 1000 cells in 1 mkl) was noticed in 82% patients. Pneumonia was diagnosed in 65.5% cases, acute or chronic bronchitis – in 19.4% cases, febrile neutropenia with bacteremia – in 14.9% cases. Antibi-otic prophylaxis with Co-trimoxasol or Ciprofloxacin was used in 44.7% patients. Etiology of infection was determined in all cases. Identification of microorganisms from different clinical materials (sputum, BAL fluid, blood, pulmonary tissue) was performed by common Russian techniques. Susceptibility of microorganisms was determined by disco-diffusion method on Mueller – Hinton agar with antibiotic discs (BBL, USA). Interpretation of microbiological findings was accordingly to NCCLS criteria.

**Results:** *P. species* caused 13.6% of infections complications in oncohematological patients (n=19). *P. aeruginosa* was revealed in 11 patients. 100% of *P. species* had susceptibility to Ceftazidime, 83.4% - to Ciprofloxacin and Imipenem, 85.7% - to Cefepime, 77.8% - for Amikacin and Piperacillin, 75% - to Cefotaxime and Ceftriaxon. Susceptibility of *P. aeruginosa* to Cefotaxime and Ceftriaxon was not tested. More frequently *P. species* were revealed in patients with pneumonia (p=0.025), with previous of chronic pulmonary diseases (OR=176.5; 95%CI 15.96-4712.89; p<0.001), with neutropenia (p=0.045), with artificial lung ventilation (OR=10.62; 95%CI 1.62-87.70; p=0.001). *P. species* didn’t correlated to chemotherapy and preceding antibiotic prophylaxis in our group of patients. Death rate and severity of infection complications were with the same frequency in patients with and without risk factors of *P. species* infection.

**Conclusion:** infection lung lesion (pneumonia), presence of chronic pulmonary diseases, neutropenia, artificial lung ventilation were prognostic factors for *P. species* infection development.
Early diagnostic and prevention of infection complications are very actual in children’s oncology. We use the hypothesis that peculiarities of myelopoiesis are different in patients with and without infection complications. The goal of the research is to estimate the compensation ability of the stem haemopoietic cells in patients during chemotherapy of leukemia and lymphoma.

Two groups of the patients in remission after 33-th day BFM-95m protocol were included: I – with severe infection complications (n=37), II – with neutropenia and moderate infection episodes (n=25). There were no differences in the level of neutropenia and GSM-doses between these two groups. CD34+ peripheral stem haemopoietic cells tested in venous blood at luminescent microscope. Control group consists of 25 healthy children, CD34+ cells level at this group 0,7±0,1.

The unexpected phenomenon has been found in comparable analysis: CD34+ cells level in the I group – 12,0±1,8; II group – 5,7±0,6. Probably, this is the result of proliferation of cells with abnormal differentiation. It may be the illustration of universal phenomenon in nature: hyperproduction with hypofunction. Further investigations can confirm the importance of increasing CD34+ cells level as prognostic marker infection complications in patients on chemotherapy.
Primary amyloidosis is a monoclonal disorder with plasma cell discrasia and production of pathologic monoclonal amyloidogenic paraprotein. Recognition of nature of the disease makes immunosuppressive therapy the main one, that allow improves patient quality of life and prognosis. The patient A. is under supervising of hematologist of Republic Hospital since October 2001. The diagnosis of primary amyloidosis based on clinical (loss of weight, weakness, fatigue, cardiomegaly, refractory congestive heart failure, sensorimotor peripheral and autonomic neuropathy and renal insufficiency), morphologic (liver biopsy revealed deposition of amorphous mass with Congo red produced an apple - green birefringence under polarized light) and histochemical data. The patient received treatment with melphalan – prednizolon regimen (melphalan 10 mg per day 7 consecutive days and prednizolon 60 mg per day 7 consecutive days). He had received 10 such courses with 40 days intervals with definite positive answer: patient general condition was greatly improved, congestive heart failure was compensated, sensorimotor peripheral and autonomic neuropathy disappeared, renal function became stable. Ultrasound examination revealed considerable reduction both size of liver and degree of thickening of left ventricular wall. The median survival of patients with primary amyloidosis presenting with congestive heart failure is 6 months, so we consider the results of therapy with clinical improvement and stable condition during 21 months as evidence of effectiveness of immunosuppressive treatment. This case reflects the role of immunosuppressive therapy as the most effective way of management of patients with primary amyloidosis.