

collected ($R2=0.86$). In particular, those subjects who reached the target of $\geq 2 \times 10^6/\text{Kg}$ CD34+ cells by one single LA, showed a minimum ratio of 180.

Conclusions: In our retrospective experience we did not observe any difference in the collection time and blood volume for GM and no-GM to reach the target. The ratio > 180 suggests that the target of CD34+ cells can be reached by one single LA, with shorter procedures, minimizing the subject's risks. We propose therefore a prospective study in order to adjust the total blood volume reducing the LA time in GM who showed a ratio ≥ 180 . These data will be confirmed using specific kits with additional bags that allow serial sampling during LA.

P604

PBPC collections: procedures, efficiency and risks

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Objectives: We tried to evaluate the efficiency, safety and risks of three techniques which were used for autologous PBPC collections: (a) Large-volume Leukapheresis (LVL), (b) Standard Collections, and (c) a new modified technique which was called as „Mix“ collections.

Methods: We evaluated the results of 136 PBPC collections which were performed in 98 patients who suffered from multiple myeloma, DLBCL, MCL and Ewing sarcoma. (a) In 93 LVL (more than 3 TBV /total blood volumes/ of the patients were processed; anticoagulation: ACD – A and Heparin), in (b) 16 Standard procedures (less than 3 TBV were processed; anticoagulation ACD – A), and in (c) 27 „Mix“ collections (less than 3 TBV of patients were processed; anticoagulation: ACD-A+ Heparin). Collections: Cobe Spectra, Caridian.

Results: In patients (a) with a good effect of mobilization (pre-collection CD 34+ cells in blood higher than 20 /ul) we prepared almost the same median dose of CD 34+ cells from the Standard and „Mix“ collections, 3,8 and $4 \times 10^6/\text{kg}$. In LVL the median yield of CD 34+ cells was $8 \times 10^6/\text{kg}$. In patients (b) who were mobilized weekly (precollection CD 34+ cells in blood lower than 20/ul), LVL enabled to prepare $1,5 \times 10^6$ of CD 34+ / kg from one collection, while the median yield of CD 34+ cells from Standard and „Mix“ collections was 0,9 and $1,2 \times 10^6/\text{kg}$. Three mild adverse reactions in patients were observed – hypotension (1) and hypocalcemia (2) which were corrected in the course of procedure.

Conclusion: We observed the similar efficiency in Standard and Mix collections in well mobilized and weakly mobilized patients. LVL enabled to get higher yield of CD 34+ cells than the Standard and MIX collections in well mobilized patients as well as in weakly mobilized patients. We can recommend LVL in all patients who can tolerate it due to a greater chance of collecting high yields of progenitor cells. In the weakly mobilized patients LVL offers a greater chance of collecting at least a minimum amount of CD 34+ cells needed for transplantation. „Mix“ collections may be used as an alternative technique in circumstances in which standard or LVL can not be recommended, e.g. in patients who do not tolerate citrate or the high extent of procedure (cardiac arrhythmia, unstable vital signs etc.).

P605

Haploidentical stem cell transplantation in children with advanced malignancies

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Haploidentical SCT is the therapeutic modality for patients lacking HLA-identical donor. RIC ensures good tolerability and safety, early stable full donor chimerism with potential GvT effect. Since 2001 we performed 44 haplo-transplantations from relatives in 38 pts with poor-prognostic malignancies: 9-AML,

4-ALL, 4-CML, 5-JMML, 1-MDS, 3-NHL, 7-NBIV, 4-EWS and 1-melanoma. RIC regimen included Fludarabine 180 mg/m², and ATG 40 mg/kg in combination with Busulfan 8 mg/kg (n=31) or Treosulfan 30000 mg/m² (n=7). The PBSC with a median number of $6.3 (2-12.5) \times 10^6$ CD34+ cells/kg were infused after incubation in vitro with vincristine and methylprednisolone. T-depletion was not performed. GvHD prophylaxis consisted of short methotrexate and cyclosporine A or tacrolimus.

Three pts with leukemia progression at the time of SCT didn't recover and died. Four pts with JMML/MDS rejected and relapsed in first 2 mos after SCT. Thirty three pts (82,5%) recovered with full donor chimerism. Incidence of acute GVHD was gr. I-II 22 pts (64%), gr III- 6 pts (18%), gr. IV- 0%. Acute GVHD was successfully treated with steroids and ATG. Incidence of chronic GVHD was 52% (mainly extensive). Five pts were retransplanted (4-JMML/MDS, 1-CML). Two of them engrafted and one of them is alive and disease free at 3 years. Nine pts are alive and well with a median f-up of 41 (8.1-88.5) mos, 20 pts died from relapse, 2 pts died from acute GVHD, 7 pts from chronic GVHD and infections. Relapse rate at 1 yr was 75% and 33% for solid tumor and leukemia/lymphoma pts. DFS and EFS were 54% and 22% with a median f-up of 45 and 25 mo for hematological malignancies vs. 20% and 8% at 10 and 8 mo in pts with solid tumors, respectively. Haploidentical transplantation following reduced-intensive conditioning is feasible and safe if an appropriate GvHD prophylaxis is given. This strategy is applicable to very high risk and potentially incurable patients if no conventional donor is available. Heavier conditioning regimen does require for kids suffering from JMML/MDS.

P606

Efficacy, complication rates and cost-effectiveness of chemotherapy + G-CSF and single agent C-CSF as mobilizing regimens for autologous PBSC: analysis of 125 patients with haematological malignancies

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Mobilized PBSC have largely replaced conventional, unprimed BM as source during the autologous transplant setting, because of a faster hematopoietic reconstitution, less transfusion requirements, less infective complications and earlier hospital discharge. Choosing the optimal mobilizing regimen is the goal for achieving the sufficient amount of PBSC for autologous transplant. G-CSF is the standard agent commonly administered undergoing PBPC mobilization and collection in a dose of 10 micro/gr in a 4 days regimen. The additional chemotherapy to G-SCF is still associated with higher rate of hemorrhagic cystitis, prolonged neutropenia, infective complications, secondary malignancies and other toxicities. Therefore in this study we evaluated the efficacy, complication rates and cost-effectiveness of chemotherapy+G-CSF versus single agent C-CSF as mobilizing regimens. We analyzed 125 patients with haematological malignancies (49 AML in first remission, 26 HD, 30 MM, 16 NHL, 4ALL) who underwent mobilization of PBSC in our centre and the attempt to reach $2 \times 10(6)/\text{kgCD34}$ cells. In 6% of patients adequate cell dose was not reachable and overall failure rate of mobilization of 17,5%. Furthermore 15.6% failed to harvest the optimal $4 \times 10(6)/\text{kgCD34}$ + cells with > 1 aphaeresis attempt. The analysis of factors contributing in this effect in the univariate analysis were: > 2 lines of previous chemotherapy and neutropenic events ($P=0,002$ and $P=0,005$), those also remained significant in the multivariate analysis (RR:4,4 and 6,2). No differences have been noticed between the diagnostic groups of patients. The mortality rate was 2% (intracranial bleeding and sepsis). The statistical analysis preformed for analyzed patients transplanted with single G-CSF as mobilizing regimen, compared with the chemotherapy + G-CSF group showed $P<0,0001$ for

febrile days, microbiological positive isolates, days of hospital stay, transfusion requirements. The median cost of PBSC collection in the Chemotherapy + G-CSF group was E 8550 (E220-10110) compared with the G-CSF group alone E3110 (E2200-4120) that showed $P < 0.0001$. Taking these results in consideration for the potential candidates for ASCT, transplant centers should consider the use of less myelosuppressive agents or dose reduction strategies for the mobilization and autologous stem cell procurement.

P607

Peripheral progenitor cell collection is safe in paediatric donors: a single-centre experience from India

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The use of peripheral blood stem cells (PBSC) for stem cell transplantation has greatly increased in adults. This trend is now reflected in paediatrics, where healthy children are donating PBSC or donor lymphocytes via apheresis for their siblings. There have been concerns over the safety of PBSC collection in paediatric donors. We have reviewed 52 PBSC (40 allogeneic and 12 autologous) collections in paediatric donors (aged 18 months to 18 years) at our institution over a period of 13 years from 1996 to 2009. The donors had received a median of 5 days of growth factor ($10 \mu\text{g}/\text{kg}$ body weight). All donors had a femoral apheresis catheter placed on the morning of the procedure under intravenous sedation. Anxiolytic agents like midazolam or lorazepam were used during the process if required. The extracorporeal line was primed with irradiated leucoreduced packed red cells when donors weighed less than 40 kg (32 procedures were primed). The anticoagulants used were a combination of heparin in bolus and continuous infusion of acid citrate dextrose at a reduced rate to avoid citrate toxicity. All donors were given calcium intravenously in between and at the end of collection.

53 PBSC collections were done with smallest donor weighing 11 kg and a median of 2.10 times of donor blood volume was processed per procedure. The mean mononuclear cell yield for per kg of recipient weight was 5.4×10^8 in allogeneic donors and 3.3×10^8 in autologous donors. 8 donors underwent two days of collection and 1 donor for 3 days. Minor side effects like generalized myalgia due to growth factor administration, pain at the femoral catheter site, anxiety, restlessness, perioral tingling due to hypocalcemia and mild hypotension were seen in a majority of the donors. 2 donors had fever after insertion of the femoral catheter requiring intravenous antibiotics. 1 child had atrial fibrillation requiring adenosine. This event occurred in one of our earliest collections and modification of the anticoagulant protocol during harvest has virtually eliminated this potential problem in subsequent procedures.

These results show that PBSC collection in paediatric donors in the allogeneic and autologous setting is possible with minimum adverse events. The yield of MNC by using G-CSF stimulation and the volume processed appear to be adequate and safe for good clinical outcome.

P608

The duration of the ex vivo transportation of haematopoietic stem cells does not seem to impair the clinical outcome following transplantation – a single-centre study

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In hematopoietic stem cell transplantation (HSCT), using unrelated donors, stem cells are often harvest at remote hospitals, and may be handled ex vivo for several days before they are infused into the recipient. Thus, the aim of this study was to investigate whether the duration of the ex vivo transportation

of hematopoietic stem cells impairs the clinical outcome following transplantation. We retrospectively analyzed 90 consecutive patients who received unrelated donor hematopoietic stem cells at our centre between 2003 and 2008. Both children and adults were included (median age 45, range 0.5–67), whereas retransplantations were excluded. The indications for allogeneic HSCT were: AML (32%), ALL (16%), myelodysplastic syndrome (16%), CML (10%), lymphoma (12%), solid tumors (7%), metabolic disorders (3%), aplastic anemia (2%), and multiple myeloma (2%). In all recipients, the total ex vivo time of the stem cell transplant (median 28 h, range 5–53 h) was calculated and subdivided into the transportation time from the donor hospital to our centre (median 20 h, range 3–38 h), as well as the time from arrival at our hospital until the start of the stem cell infusion (median 10 h, 0.5–26 h). In univariate analysis, none of the ex vivo times were significant predictors of graft failure, relapse, non-relapse mortality or overall survival. In conclusion, the clinical outcome following HSCT seems to be independent of the time the stem cell transplant is kept ex vivo for transportation purposes.

P609

Bone-to-bone boost in poor graft function after haploidentical haematopoietic stem cell transplant: safety and feasibility.

Two case reports

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Background: Poor graft function (PGF) occurs in 5–27% of patients (pts) after allogeneic hematopoietic stem cell transplant (HSCT) and is associated with high morbidity and mortality. Graft function may be poor as result of slow or incomplete recovery of blood counts (primary PGF) or decreasing blood counts after successful engraftment (secondary PGF). Several factors may determine PGF: disease status, conditioning regimen, HSC source, HLA compatibility, T cell content, immunosuppression, GvHD, viral infections, drugs. G-CSF and Rhu-EPO are readily available and effective but with no effects on platelet. Second transplantation from the same donor, with or without conditioning therapy, can boost the haematopoietic recovery in these pts. Unfortunately, both a second peripheral CD34+ mononuclear cells (MNC) mobilization and a marrow harvest in the operating room may be contraindicated early after the first donation as not safe for donors. Intrabone SCT can overcome the risk of graft failure even with a low number of CD34+ MNC and we have performed in two adult pts with PGF a bone-to-bone boost (BBB) with a small marrow harvest from respective donors, who were unfit for a second conventional donation.

Aim: To evaluate the feasibility of the BBB technique in 2 pts with PGF.

Methods and results: pts were 2 males (57, 53 y) with a diagnosis of AML and CMML, respectively. Prolonged pancytopenia and hypoplastic marrow were documented in both cases, with diagnosis of primary PGF and secondary PGF, respectively, donor chimerism ranging from 80–100% (STR and HLA), without evidence of leukemia. The 2 donors were related, haploidentical. For the BBB procedure small quantities of bone marrow (<200 ml) were collected from the posterior iliac crest of the donors, at the bedside, during deep sedation and analgesia. After 30 minutes, necessary for graft treatment according to Institution procedure, the unmanipulated marrow harvested was infused into the posterior iliac crest of the pts at day 30 and 72 days after SCT, respectively. MNC infused doses were 0.9 and $0.4 \times 10^8/\text{Kg}$, respectively. No side effects were recorded both for patients and donors.

Conclusion: In this 2 cases the BBB technique proved feasible and safe for both donors and patients. This practice can give the chance of HSC boost to patients with PGF without the need of a G-CSF mobilization for donors and with a minimal invasive