8th Meeting of the EBMT Paediatric Diseases
WP 3rd Meeting of the EBMT Paediatric Nurses including Inborn errors WP educational day

7th – 9th June 2012
8th Meeting of the EBMT Paediatric Diseases WP
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7th – 9th June 2012
Prague

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## Scientific Programme

### June 7th, Thursday

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<td>Chairs: Christina Peters, Thomas Klingebiel</td>
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<td></td>
<td>Transplantation for ALL along with innovative approaches to prevent relapse</td>
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<td>Michael Pulsipher</td>
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<td></td>
<td>OP1: Dolgopolov I. – High-dose chemotherapy in relapsed and refractory Hodgkin’s lymphoma</td>
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<td>OP2: Fedorova A. – Allogeneic HSCT in the first-line therapy of childhood anaplastic large</td>
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<td>cell lymphoma: a choice for minimal residual disease positive patients?</td>
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<td>13:45–15:00</td>
<td><strong>Session II (Malignant Diseases 2)</strong></td>
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<td>Chairs: Rupert Handgretingier</td>
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<td>Haploidentical transplantation as a platform for the treatment of refractory leukemia and solid tumors</td>
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<td>Rupert Handgretingier</td>
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<td>OP3: Rettinger E. – Immunotherapy with sequential infusions of cytokine induced killer cells for pediatric patients after haploidentical stem cell transplantation</td>
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<td>OP4: Toporski J. – Haploidentical HSCT in children with refractory acute leukemia – the Lund experience</td>
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<td><strong>Session III (GvHD)</strong></td>
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<td>Criteria for chronic GvHD grading, chronic GvHD therapy in children</td>
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<td>David A. Jacobsohn</td>
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<td>15:30–16:00</td>
<td>Tea/Coffee Break</td>
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<td>16:00–17:30</td>
<td><strong>Session IV (Marrow Failure Syndromes)</strong></td>
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<td>Chairs: Jackie Cornish, Maria Domenica Cappellini</td>
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<td>Thalassemia, why transplant</td>
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<td></td>
<td>Maria Domenica Cappellini</td>
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<td>OP5: Matthes-Martin S. – HSCT for sickle cell disease with reduced intensity conditioning regimen</td>
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<td>OP6: Dalle JH. – Outcome of busulfan and fludarabine-based reduced intensity conditioning regimen for related and unrelated HSCT in Fanconi anemia patients</td>
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<td>OP7: Waespe N. – Haploidentical HSCT after fludarabine/alemtuzumab conditioning as a life-saving emergency treatment in critically ill patients with severe aplastic anemia</td>
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<tr>
<td>17:45–18:00</td>
<td><strong>“Being a transplanted child”</strong></td>
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### June 8th, Friday – IEWP Educational Day

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<tr>
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<tr>
<td>08:00–18:00</td>
<td>Registration</td>
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<td>Accompanying Exhibition</td>
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<td>08:30–18:00</td>
<td>Poster Session</td>
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<td>08:30–09:30</td>
<td><strong>Session V (Donor Selection)</strong></td>
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<td>Chairs: Isaac Yaniv, Carlheinz Muller</td>
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<tr>
<td></td>
<td>Search and selection of unrelated donors – established systems and recent developments</td>
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<td>Carlheinz Muller</td>
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OP 8: Sedlacek P. – Search for matched unrelated donor in children of Caucasian origin – national study
OP 9: Sufliarska S. – 16 years of allogeneic HSCT in children in Slovakia

■ 09:30–10:30 Session VI (Primary Immunodeficiency 1)
  Chairs: Robbert Bredius, Andrew Gennery
  Update of transplanting SCID
  Andrew Gennery
  OP10: Mielcarek M. – Outcome of HSCT in children with primary immunodeficiencies and metabolic disorders – single centre study
  OP11: Krol L. – Allogeneic HSCT in pediatric patients with hyper-IgM syndrome: single centre experience

■ 10:30–11:00 Tea/Coffee Break

■ 11:00–12:00 Session VII (Monitoring, Pharmacokinetics)
  Chairs: Jacek Wachowiak, Arjan Lankester
  Monitoring of ATG levels, clinical relevance
  Robbert Bredius
  OP12: Główka F. – Pharmacokinetic study of high-dose treosulfan i.v. in paediatric patients before HSCT
  OP13: Sobiak J. – Etoposide plasma concentration on day “0” and outcome of allogeneic HSCT in children with acute lymphoblastic leukemia
  OP14: Breuer S. – Definition of a risk-adapted algorithm for diagnosis of impeding graft rejection based on early chimerism in the T- and NK-cell lineages in pediatric patients undergoing allogeneic HSCT
  OP15: Lankester A. – Exklusive persistance of recipient T lymphocytes after effective myeloablative stem cell transplantation in children with acute lymphoblastic leukemia: possible contribution to anti-viral immunity

■ 12:00–12:30 Satellite Symposium MSD
  “Unmet Medical Needs and Current Standards of AF Treatment for IFI in Children with Hematologic Malignancies”
  Speaker: Thomas Lehnhbecher

■ 12:30 – 13:30 Lunch

■ 13:30–14:00 Session VIII (Fungal Infections)
  Chair: Petr Sedlacek, Paul Veyes
  OP17: Ball LM. – Emerging azole resistance of Aspergillus species. A proposal for a new management strategy in children undergoing HSCT with suspected invasive fungal infection

■ 14:00–15:00 Session IX (Primary Immunodeficiency 2)
  Chairs: Tayfun Güngör, Bobby Gaspar
  Outcome of transplant for CGD
  Tayfun Güngör
  Gene therapy for PID
  Bobby Gaspar
  Pharmacological SCID-ADA treatment
  Fulvio Porta

■ 15:00–15:30 Tea/Coffee Break

■ 16:00–17:00 Poster Session chaired by members of scientific committee

■ 17:00–18:00 ALL-SCT International – Study Committee Meeting
  (Congress Hall 2)
  Christina Peters

Programme
June 9th, Saturday

09:00–10:30  **Session X (Viral Infections)**  
Chair: Jean Hugues Dalle, Tobias Feuchtinger  
**State of art: management of refractory viral infections post allo SCT**  
Tobias Feuchtinger  
OP18: Geyeregger R. – Short-term in vitro expansion allows affordable generation of multiple virus-specific T-cells for a broader clinical application  
OP19: Feghoul L. – Molecular decryption of an epidemic of group C adenovirus infections in children recipients of HSC  
OP20: Feghoul L. – ADV load in stool predicts invasive dissemination in pediatric recipients of HSC  
OP21: Liles A. – Effectiveness of cidofovir in the treatment of adenovirus  
OP22: Sterkers G. – Cellular and humoral immunity elicited by influenza vaccines in pediatric HSC

10:30–11:00  **Tea/Coffee Break**

11:00–12:00  **Session XI (Late Effects) – joint with PNWP**  
Chair: Anita Lawitschka (PD), Eugenia Trigoso (PN)  
**The latest in late effects after pediatric HCT**  
Michael Pulsipher  
OP23: Guerola BT. – Renal function follow-up in a cohort of children after HSCT  
OP24: Lynne M. – Immediate and long-term somatic effects and health related quality of life of bone marrow donation during early childhood  
OP25: Bresters D. – Pubertal development and ovarian function after HSCT in childhood  
OP/N1: Begli S. (PN) – Aspects of donating HSC: the sibling donor perspective  
OP/N2: Baslo R. (PN) – Intense patient- and family-centered nursing care: the hallmark of pediatric transplant nursing

12:00–12:15  **Closing of the Meeting**
Chemotherapeutic and Hematopoietic Cell Transplant (HCT) approaches to childhood ALL and AML are well-established treatment modalities resulting in long-term disease-free survival. Because the intensive therapy associated with some HCT regimens and Graft versus Host Disease (GVHD) increases the risk of certain late effects, HCT is reserved for intermediate or high-risk populations where a survival benefit can be demonstrated. I will review principles of comparative studies of HCT and chemotherapy and then discuss innovative approaches to addressing the main challenge in improving outcomes after HCT for ALL—prevention of relapse.

**Chemotherapy versus Transplant for Pediatric ALL**

**Defining Risk Groups, Comparing Chemotherapy and HCT Approaches**

Over the past decade precise measures of response (minimal residual disease (MRD)) detected by molecular or flow cytometric methods combined with refined detection of genetic lesions and gene expression patterns have allowed more precise delineation of patients at highest risk for relapse with chemotherapy. While many consider HCT an appropriate alternative for high-risk patients, how do we compare HCT with chemotherapy approaches over time as outcomes using both modalities improve and more accurate risk factors are developed?

The first consideration is ensuring the integrity of the risk factor. Validation of a proposed risk factor must be performed in multiple distinct cohorts to confirm general applicability. As refinement or modification of risk factors occurs, analysis should reflect the most current state of knowledge risk factors for a given condition. This issue is vital in comparing HCT with chemotherapy outcomes because mixing a lower risk group with a high-risk cohort will generally favor chemotherapy. A notable example is found in published comparisons of approaches to treat high-risk infant leukemia. The Children’s Oncology Group (COG) recently published nearly identical survival between infants with MLL-rearranged leukemia receiving HCT, 48.8% or chemotherapy, 48.7%. The problem with this study is that all MLL+ infants were analyzed together, despite published information from the same cohort that infants with high WBC (> 200 K/μL), age <6 months, and CD10- phenotype did much worse with chemotherapy. The cohort as a whole didn’t appear to benefit from HCT, but what about the high-risk group? The Interfant – 99 study analyzed infant risk groups separately and demonstrated that in infants with high-risk MLL by their definition (<6m + WBC > 300 K/μL or poor predisone response at day +8) 5-year overall survival of patients achieving remission improved from 19% with chemotherapy to 66% with HCT (p = 0.001). Because the COG study combined risk groups they missed an opportunity to define outcomes in their highest risk cohort and their conclusions about the value of HCT in infants may have been misleading.

Other important considerations when attempting to assess chemotherapy versus HCT for a given cohort: appropriate patients, time-points and approaches must be compared. Patients who do not achieve remission generally do not benefit from any approach, and an excess of these patients in a chemotherapy or HCT arm will bias the results. Patients not achieving remission should either be excluded from the analysis or analyzed separately. Correction of the chemotherapy arm for median time to transplant is essential to remove early relapse bias. Most importantly, chemotherapy and HCT approaches being studied need to be as uniform and era-appropriate as possible. Mismatched unrelated donor and haploidentical approaches currently have inferior outcomes compared to matched unrelated and sibling donor approaches and must be analyzed separately.

**Indications for HCT in ALL: Background**

Because HCT has been reserved for very high-risk ALL patients with known poor outcomes using chemotherapy, attempts at randomized studies of HCT vs. chemotherapy have been thwarted by non-compliance. In clinical scenarios where indications have remained relatively static (early BM relapse), significant data are available that demonstrate efficacy. For newer indications, especially if the patient population is small, cooperative groups often allocate patients to HCT based upon their most current definitions of very high risk for failure with chemotherapy.

**Current and Emerging Indications for HCT in Pediatric ALL: CR1**

With constant discovery of new risk factors and introduction of targeted therapies into treatment schemas, the designation of which patients would benefit from HCT in CR1 is challenging. Primary induction failure (PIF) has been universally fatal unless patients achieve CR with subsequent courses of chemotherapy. While a percentage of patients achieving CR may survive with chemotherapy alone (younger patients with lower risk B-cell disease), outcome with HCT in the modern era of PIF patients in CR1 after appropriately matched HCT has exceeded 60%. Similar to PIF, patients with persistent levels of minimal residual disease (MRD) measured by flow cytometric or RT-PCR techniques have been shown to do poorly, especially if the MRD persists after both induction and consolidation.

Most Ph+ ALL patients in the pre-TKI era had a survival advantage with HCT. COG study AALL0031, which combined an intensive chemotherapy approach with daily imatinib has
is unclear. Patients with early or late relapsing (>18 m or >36 m from diagnosis) B-lineage isolated CNS disease have 4 year EFS approaching 80% with chemotherapy; HCT for these patients may be unnecessary. Many adverse risk factors have been identified for patients with IEM: short remission duration (<18 m from diagnosis), T-lineage disease, a history of previous cranial irradiation, and the presence of submicroscopic disease in the BM. Different groups have proposed HCT for subsets of these patients, but studies fully comparing outcome with these higher risk cohorts undergoing HCT vs. chemotherapy have not been performed. The largest study comparing chemotherapy approaches with HCT for IEM patients showed similar survival outcomes, however, relevance of the study to current practice is unsure because the study reflected treatment in the 1990s, and HCT outcomes have improved over the past decade.

Indications for HCT in ALL: CR3

Any patient suffering a second or subsequent relapse, whether IEM or involving BM is considered a candidate for HCT. Reported survival is generally poor, ranging from 20–30%. A significant contributor to treatment failure in this group is transplant related mortality, which can exceed 40% when unrelated donors are used. Excessive TRM in this population is likely related to the high burden of prior therapy and attendant pre-transplant comorbidity these patients bear. Novel HCT approaches that decrease TRM while maintaining low levels of relapse are a critical need for this population.

Approaches to Prevention of Relapse

Transplant-related mortality (TRM) after allo-genic HCT has decreased markedly in the past decade, leaving relapse as our greatest challenge to success after HCT for ALL.

Modifications Pre-HCT that Could Prevent Relapse

The strongest association with relapse pre-HCT has been detection of measurable MRD. This has lead many investigators to attempt to decrease relapse by giving extra courses of chemotherapy to decrease MRD. Although it is known that patients with the lowest risk of relapse are identified by achieving MRD negative status after reinduction, it is not clear that patients with high MRD after reinduction who eventually obtain MRD negative status have higher survival. Currently, most HCT physicians recommend no more than 2–4 attempts at reinduction, as protracted attempts at intense chemotherapy may lead to significant complications that may make patients ineligible for HCT.

Many investigators have proposed bridging strategies that include novel immunological agents. The hypothesis that using immunological agents to bring chemotherapy resistant patients into an MRD negative status will improve cure has yet to be proven, but seems logical. Agents such as blinatumomab, a bridging molecule that binds to CD3 and CD19, thus putting T-cells in direct contact with B-cells and thus enhancing cancer targeting of B-cell ALL, offer promise as a way to make resistant MRD+ patients MRD- and thus “bridge” them to HCT. Other CD19 immunotoxins, such as moxetumomab, bind CD19, leading to internalization of the molecule and subsequent cell death via an attached pseudomonas toxin. Both of these agents are being evaluated in relapsed, refractory B-ALL patients and have good single agent activity and future studies may show the efficacy of a bridging approach.
is a large cohort of children who fail in spite of such monitoring and manipulation. Specific interventions aimed at increasing GVL, such as IL-2 therapy have not shown benefit. The COG conducted a large trial randomizing the addition of sirolimus, an agent with immunosuppressive properties as well as potent anti-leukemic activity in ALL, to GVHD prophylactic regimens. This trial showed a decrease in acute GVHD, but slightly increased toxicity and relapse resulted in the agent showing no survival advantage. This is an important lesson—any intervention that potentially interferes with the GVL effect may not increase survival.

Several other agents are being considered for use post-HCT with trials either underway or in development. Blinatumob, moxetumomab and other immunotoxins may work in this setting. Chimeric antigen receptors (CARs) are very promising and can be used in either an autologous or allogeneic setting. This approach puts a surface receptor on T-cells that targets CD-19 cells, bringing the T-cell and cancer cell into direct contact. The receptor has internal elements that activate T-cells, causing expansion. In some cases, long-term expansion and activity of these cells has been noted, indicating that ongoing tumor surveillance may be possible.

**Summary**

Children with high-risk ALL may benefit from HCT, but ongoing studies are needed to define when HCT is of benefit. The highest risk for failure in children undergoing HCT is relapse, and several approaches pre-HCT (minimizing disease burden), during the HCT procedure (use of ideal approaches and stem cell sources), and post-HCT (immune manipulation and targeted strategies) need to be developed in the coming years to improve cure of these very high risk children.

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**EP 2 – Haploidentical transplantation as a platform for the treatment of refractory leukaemia and solid tumors**

Rupert Handgretinger  
Children’s University Hospital, University of Tuebingen, Germany

Allogeneic stem cell transplantation plays a crucial role in the treatment of high risk hematological malignancies. Besides the preparative regimen, the anti-malignancy effect of allogeneic transplantation is increasingly harnessed to prevent post-transplant relapses and to improve the outcome. Over the last decade, we have mainly focused on the anti-malignancy potential of haploidentical transplantation for various malignancies. In the beginnings, we have used purified CD34+ positively selected peripheral stem cells from parental donors and many patients with leukemia have been treated with this approach. We have demonstrated in these patients that recipients of CD34+ stem cells from NK alloreactive donors had a lower risk of relapse compared to patients whom received grafts from non-alloreactive donors. We could also show that acute lymphoblastic leukemia is a NK-susceptible disease in children. In order to retain the bulk of donor NK cells in the graft, we have switched from CD34+ positive selection to the negative depletion of CD3+ T-cells of mobilized grafts in haploidentical transplantation. With these grafts, large numbers of CD356+ NK cells are co-transplanted and more than 100 children with various malignancies have been treated with this approach. More recently, we have introduced 8B T-cell depletion, which retains beside NK cells yδ T lymphocytes in the graft. Using this approach, a rapid reconstitution of the innate immune system (NK-cells and γδ T-lymphocytes) as well as of the adaptive immune system could be seen in the first patients.

Beside the allo-reactive status, the functional status of the NK cells is important and patients with a high persistent NK activity post-transplant have a lower risk of relapse compared to patients with a persistent low NK activity. Therefore, strategies which aim to augment the NK activity post-transplant should lead to a lower risk of relapse in pediatric leukemia or other malignancies. In order to activate the NK cells after haploidentical transplantation, we are clinically investigating several strategies. One is the additional and repetitive post-transplant adoptive transfer of donor-derived NK-cells which have been activated ex-vivo by overnight incubation with Interleukin 15 (IL-15). The infusion of the IL-15 activated NK cells is followed by treatment with low dose Interleukin 2 to induce in vivo proliferation of the IL-15 activated NK cells and to augment their cytotoxicity. Since NK cells can exert antibody-dependent cellular cytotoxicity (ADCC), we also investigate whether the additional use of monoclonal antibodies directed against tumor targets would increase the graft versus tumor effect post-transplant. Moreover, newer reagents such as T-cell engaging bispecific antibodies have shown to be effective in the post-transplant treatment of chemorefractory leukemia. In addition, the transfer of donor-derived virus-specific T-cells directed against adenovirus, cytomegalovirus and EBV is a safe and effective approach to control viral reactivations and diseases resistant to standard antiviral therapies. A similar approach is currently under investigation for the adoptive transfer of tumor-specific T-cells. Further prospective clinical studies will hopefully show which strategies or combinations are associated with the most effective graft versus tumor effects.

Rupert Handgretinger  
Children’s University Hospital, University of Tuebingen, Germany
rupert.handgretinger@med.uni-tuebingen.de
Chronic GVHD remains one of the major barriers to successful allogeneic hematopoietic stem cell transplant (HSCT). Children have many years to grow and develop after HSCT; therefore, finding ways to decrease this complication as well as complications of its therapy is of paramount importance.

During this session, Dr. Jacobsohn will review the recommendations for staging and grading chronic GVHD, as set by the NIH Consensus. Prospective studies carried out by the Chronic GVHD Consortium have already begun to narrow down which grading criteria by the NIH Consensus should be used, and these will be presented. For example, we have identified that NIH 0–3 staging for skin actually is predictive of long-term outcome, therefore, it may be a useful system to use for response assessment of skin cGVHD.

Dr. Jacobsohn will also review risk factors for survival and non-relapse mortality in a large cohort of pediatric patients with cGVHD. These data may be useful to help risk-stratify and guide therapy at initial diagnosis of cGVHD.

Finally, some of the novel therapies for cGVHD, such as photopheresis and pentostatin, will be presented.

DA. Jacobsohn  
Division Chief of BMT, Children's National Medical Center, Washington, DC, USA

Abstract not available.

C. Muller  
Abstract not available.

A. Gennery  
Reader in Paediatric Immunology and Haematopoietic Stem Cell Transplantation, Institute of Cellular Medicine, Newcastle University/Honorary Consultant in Paediatric Immunology, Great North Children's Hospital, Newcastle upon Tyne, UK

"What are the chances of success, doctor?" – one of the most common questions to which parents want an answer when counselled about haematopoietic stem cell transplantation (HSCT) for severe combined immunodeficiency (SCID). The answer is not so straightforward, and depends on whether survival only is considered, or long-term issues of immunoreconstitution, immune function, and quality of life are also considered. Simple survival data from the European SCETIDE registry shows ten year survival approaching 90% if an HLA geno-identical donor is used, compared to 60% for an HLA mis-matched donor. Immunophenotypic differences are also important in determining successful outcome. Survival is better for patients with T-B+NK- SCID, than for those with T-B-NK+ phenotype (10 year survival 70% vs 51%, p < 0.0001) (1). Within immunological phenotypes, there are genotypic differences in survival, even when similar donors are used. For example, long term outcome for patients with RAG and Artemis SCID are different, likely due to underlying genetic defect, but specifically due to the type of conditioning regimen employed – myelo-ablative regimens lead to significant long term complications in those with Artemis deficiency (Neven, personal communication).

Whilst an HLA-identical genotypic donor is ideal, not all patients have such donors available. To decrease length of time to transplantation, T cell depleted donors have been used to achieve T cell reconstitution. European centers have
moved from CAMPATH depletion to stem cell selection using an organic iron bead attached to an anti-CD34 antibody to isolate purified CD34+ HSCs from the other cells by passing the HSC source through a magnetic column. Immune reconstitution appears less complete using this method, with more complete myeloid donor chimerism following CAMPATH depletion (2), likely to lead to better long-term immune function (3). Recent results using CD3/CD19 depletion through organic iron bed technology have demonstrated faster time to T cell engraftment and to viral clearance than with CD34+ selection in SCID patients, although patient numbers are small (Slatter, personal communication). Umbilical cord blood stem cells are an alternative to haplo-identical T cell depleted HSC. A recent study compared outcome of transplantation using either cord blood or T cell depleted haplo-identical parents as the stem cell source (4). Cord blood recipients had more complete chimerism, faster lymphocyte count recovery and more rapid B cell reconstitution, but OS was similar between groups. Conditioning plays a major part in outcome. Myeloid chimerism is achieved in ADA SCID without conditioning (5), but necessary in T-B-NK+ SCID to achieve thymopoiesis (6). New conditioning regimens using treosulfan have lead to good outcomes for SCID transplantation, with 85% overall survival (7) (personal communication) and good chimerism. Long term effects, including preservation of fertility are unknown. The use of serotherapy in cord blood transplantation affects outcome, including speed of viral clearance and speed of immune reconstitution. Whilst no serotherapy leads to more rapid reconstitution, there is more GVHD (8), and low dose serotherapy may be a suitable alternative with more rapid immune reconstitution than high dose, but less GVHD than no serotherapy (Lane, personal communication). Cell lineage chimerism may be important, depending on the SCID genotype. Donor B cells are required for full B cell immunoreconstitution in common gamma chain or JAK3 SCID (9), but not that due to IL7Ra SCID. Finally, age at transplant is an important predictor of survival. Newborn SCID patients have a significantly better survival than probands (10). Newborn screening for SCID will likely improve survival, but raises significant questions about appropriate conditioning regimens and timing of transplantation.

References


Andy Gennery
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EP 7 – Occurrence of human antibodies to thymoglobulin before and after hematopoietic stem cell transplantation

CM. Jol-van der Zijde*, RGM. Bredius*, AMJ. Hoogendijk, MR. Egeler, FJ. Smiers, AC. Lankester, MJD. van Tol
Department of Pediatrics, Leiden University Medical Center (LUMC), Leiden, the Netherlands (*equally contributed; RGM Bredius is presenting author)

Introduction

Serotherapy (e.g., anti-thymocyte globulin, ATG) given as part of the conditioning prior to HSCT is administered not only to reduce the risks of graft rejection/relapse but also to prevent Graft-versus-Host Disease (GvHD). However, serotherapy has a major impact on the immune reconstitution as it will lead to complete immune depletion (lymphocyte depletion) of the transplanted patient and will also partly or completely deplete the T-cells from the infused graft.

Furthermore, first line treatment regimens for severe aplastic anemia (SAA) contains ATG. Clearance of total ATG (rabbit or horse IgG) and active ATG (IgG capable of antigen-specific binding to cells) is more rapid in kidney transplant patients developing anti-rabbit or anti-horse antibodies. The clinical impact of sensitization on treatment outcome in these settings might be different from the HSCT setting. In this setting, only one study has reported a few cases in which anti-ATG antibodies appeared shortly after transplantation, without signifying a potential clinical effect.

Previous exposure to ATG predisposes to the development of antibodies against ATG, and to serum sickness. In SAA patients, serum sickness is reported to be associated with poorer outcome. Enhanced clearance may also occur in HSCT recipients, particularly after sensitization against rabbit ATG (in SAA patients in case of a second course of ATG or at re-transplantation).
Patients and methods

Concentrations of ATG and anti-ATG antibodies were measured in 72 pediatric (1st) HSCT recipients treated with ATG as part of the conditioning, and in 18 SAA transplanted patients who received an upfront immunosuppressive therapy with at least one course of ATG (median 8.5 months, range 6–48 months before HSCT), followed by HSCT conditioning containing ATG.

Total ATG and anti-ATG antibodies were measured with an ELISA. Active ATG, capable of binding to HUT cells, was measured with a quantitative flow cytometry assay (1).

Results

In some patients total ATG levels decreased much faster than expected; i.e., more than a 2 log decrease in a time span of four days (Figure 1).

Anti-ATG antibodies post-HSCT appeared only in children receiving a full graft (20 out of 59); in none of 13 patients with an ‘ex vivo’ CD3/CD19 depleted or CD34 enriched graft an anti-ATG antibody response occurred (p = 0.014). Four patients developed IgG anti-ATG antibodies early (before day 22) post-HSCT (table 1). They engrafted fast (n = 4; mean day + 19; range 16–21) and had steep drops in ATG levels and showed rapid T-cell recovery, which was associated with a significantly increased risk of acute GvHD (see Figure 2). In six patients IgG anti-ATG responses occurred later (range 28–46 days) after HSCT engraftment was significantly later (mean day + 27; range 17–33; p = 0.05) and without an increased risk of GvHD. A total of 10 children only mounted an IgM (and IgA) anti-ATG response, which was without major impact on ATG levels. Although the incidence of acute GvHD was significantly higher in patients who produce IgG anti-ATG early post-HSCT, the overall survival of these patients at 1 year after HSCT was comparable with patients without anti-ATG (Figure 2). A total of only 5 patients developed chronic GvHD: 3 of 20 patients (15 %) in the anti-ATG group versus 2 of 52 patients (4 %) in the patient group without anti-ATG; this was not a significant difference (p = 0.095).

In 75 % of the SAA patients (six out of eight) with prior ATG treatment, IgGand/or IgA anti-ATG antibodies were detectable in serum obtained before the start of HSCT conditioning, whereas IgM anti-ATG antibodies were absent. Anti-ATG antibodies were undetectable in 10 patients without upfront ATG treatment prior to HSCT.

Three out of the 6 sensitized SAA patients with IgG anti-ATG antibodies prior to conditioning showed such a severe adverse reaction, i.e., severe inflammatory response syndrome (SIRS), upon the first dose of ATG that a switch to alemtuzumab was necessary. This need for a switch to alemtuzumab is significantly more frequent than without upfront ATG treatment prior to HSCT.

One patient (UPN 691, Figure 3) produced a strong anti-ATG response consisting of IgM, IgA and recipient allotype IgG antibodies from day + 3 after HSCT onwards, resulting in a complete ATG clearance on day + 6. This antibody response, which was without major impact on ATG levels, although the incidence of acute GvHD was significantly higher in patients who produce IgG anti-ATG early post-HSCT, the overall survival of these patients at 1 year after HSCT was comparable with patients without anti-ATG; this was not a significant difference (p = 0.095).

Table 1. Characteristics of patients with an InG anti-ATG response to ATG conditioning

<table>
<thead>
<tr>
<th>UPN</th>
<th>HSCT nr</th>
<th>Diagnosis</th>
<th>Graft</th>
<th>In vitro T-cell depletion graft</th>
<th>First day anti-ATG</th>
<th>Max IgG</th>
<th>Max IgM</th>
<th>Max IgA</th>
<th>Allotype IgG anti-ATG</th>
<th>Day of engraftment</th>
<th>Day of aGvHD 2–4</th>
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UPN, unique patient number; aGvHD, acute GvHD; HLH, hemophagocytic lymphohistiocytosis; JMML, juvenile myelo-monocytic leukemia. -1- Max IgG: maximum concentration of IgG anti-ATG; Max IgM: maximum concentration of IgM anti-ATG; Max IgA: maximum concentration of IgA anti-ATG; +++: > 150 AU/mL; ++ 50–150 AU/mL; +: 10–50 AU/mL; and -: < 10 AU/mL.

Allotype of IgG anti-ATG: -: no informative difference between recipient and donor type; D/r: switching from donor plus recipient to donor only.

Figure 1. Total and active ATG levels and anti-ATG antibodies in association with immune recovery in a child after HSCT. Total ATG (first row), active ATG (second row) and anti-ATG antibodies (third row), in a patient with an early appearance of IgG anti-ATG post-HSCT. Lymphocytes, T-, B-, and NK-cell counts are depicted in the lower panel. *Day of onset of GvHD. Grey lines represent the normal range of total ATG and active ATG (dashed line P50, solid lines P5 and P95).
Figure 2. Incidence of acute GvHD and overall survival. Estimated cumulative incidence of acute GvHD grade II-IV (Figure A) in the children with anti-ATG (n = 20) and in those without anti-ATG after being transplanted with a non T-cell depleted graft (n = 39). Group A patients with early IgG anti-ATG (solid line), group B patients with late IgG anti-ATG (dashed line) and patients without anti-ATG (dotted line) were compared in figure B and C. Figure B shows the cumulative incidence of acute GvHD grade II-IV in children receiving a full graft, and Figure C shows the overall survival of the patient groups.

Figure 3. Total rATG, anti-rATG Abs and lymphocyte recovery in UPN 691. Total ATG concentration (a) and anti-ATG Ab levels (b) in patient UPN 691 with a very early appearance of IgG anti-ATG after HSCT are shown. Lymphocytes (Ly), T-, B- and natural killer (NK)-cell counts are depicted in panel (c). R: day of onset of rejection; gray lines in panel (a) represent the normal range of total ATG (dashed line P50; solid lines P5 and P95); chimerism based on PCR amplification of variable number of tandem repeats in lymphocytes.

Figure 4. Flowchart of ATG treatment in SAA patients. In pre-treated patients anti-ATG should be measured prior to a second ATG course. *Screening of patient for antibodies against ATG; serum (500 μL) can be sent to the LUMC; anti-ATG antibody titer will be reported within two weeks. **Follow up of patients to check for anti-ATG and active and total ATG levels; weekly sampled (500 μL serum; top and 1–8 weeks) and measured in bulk after finishing the complete course post-SCT (C.M.Jol@LUMC.nl).

Discussion and conclusions

Conditioning regimens containing ATG are advised in various guidelines from international transplantation societies. Blood levels of total and/or active ATG are, however, not measured on a regular basis in allogeneic HSCT recipients. We detected anti-ATG antibodies from day 16 after HSCT onwards, in 20 of 72 children (28%) receiving ATG prior to HSCT as part of the conditioning regimen. In patients with an informative allotypic IgG marker, we demonstrated that this response is of donor origin. An IgG anti-ATG response was always associated with rapid clearance of active and total ATG. When the response occurred within the first three weeks after HSCT, it was accompanied by an increased risk of severe acute GvHD.

To date only limited data are available on the pharmacokinetics (PK) of ATG in adult and pediatric HSCT recipients. Consequently, dosing schedules of ATG have been developed empirically in the absence of detailed PK studies. The efficacy of patient- and HSCT-related variables on the ATG peak levels or on the time to reach sub-therapeutic levels of active ATG in relation to outcome parameters are largely unknown. Prolonged elevated levels of active ATG may lead to delayed immune recovery of donor-derived T-cells and increased susceptibility to infections, whereas too low ATG levels will increase the risk of graft rejection or GvHD, as illustrated in the present study in patients with rapid clearance of ATG due to early production of IgG anti-ATG antibodies.

In this study, anti-ATG antibody responses of donor origin were observed early after HSCT in the absence of any measurable peripheral blood leukocyte recovery. Restoration of immunity after HSCT is generally a slow process. The contribution of newly generated B- and T-cells of donor origin during the first weeks after transplantation may be negligible. Therefore, the initial response against the xenoprotein ATG has likely been derived from the mature donor lymphocytes co-infused with the graft. This hypothesis is supported by our observation that anti-ATG antibody responses in this cohort are exclusively present in recipients of an under- depleted bone marrow or peripheral blood stem
cell graft and absent in recipients of a depleted graft. Indeed, in the transplant setting using a full graft, antigen-specific immunity can be adoptively transferred from donor to recipient. The results of our study indicate that development of IgG anti-ATG antibodies has a major impact on HSCT related toxicity, i.e. acute GvHD, and should be taken into consideration in future transplantation protocols. The present study further indicates that patients, who received ATG prior to HSCT as part of the first line immunosuppressive treatment of their original disease such as SAA, are at a high risk of being sensitized to ATG. Based on the findings, it is strongly recommended to screen for anti-ATG antibodies in ATG pretreated patients and, if positive, to choose an alternative anti-T-cell therapy, i.e., alemtuzumab, for their transplantation conditioning (figure 4). However, with a longer serum half-life, alemtuzumab may seriously delay T-cell reconstitution and, subsequently extend the immunocompromised state. Using this approach, severe adverse reactions upon ATG administration and major clinical complications early after HSCT may be prevented in ATG sensitized patients.

In conclusion, measurement of total and active ATG levels and insight in the development of anti-ATG antibodies may have major implications for future transplantation policies. Detailed analyses in a larger patient cohort are needed to fully appreciate the relationship between ATG levels and immune reconstitution, GvHD and other clinical outcome parameters such as graft rejection, infections, chimerism, relapse rate, day-100 mortality and event free survival.

Publications


2. Jol-van der Zijde CM, Bredius RG, Jansen-Hoogendijk AM, Smiers FJ, Lankester AC, van Tol MJ. Antibodies to anti-thymocyte globulin in aplastic anemia patients have a negative impact on hematopoietic SCT. Bone Marrow Transplant. 2012.

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**EP 8 – Reduced toxicity conditioning and allogeneic hematopoietic stem cell transplantation in chronic granulomatous disease: Favorable outcome after high-dose fludarabine, serotherapy and submyeloablative busulfan**

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Allogeneic hematopoietic stem cell transplantation (HSCT) is able to cure chronic granulomatous disease (CGD) but for patients with significant pre-transplant disease complications graft versus host disease (GvHD), non-engraftment and toxicity of the conditioning regimen contribute to relevant treatment-associated morbidity and mortality. Efficacious reduced intensity conditioning regimens providing sustained myeloid engraftment for such high risk patients are still lacking. Thirty-seven CGD patients (n = 29 male; n = 7 female; n = 24 X-linked; n = 13 autosomal-recessive) from 15 centers underwent HSCT after conditioning with 180 mg/sqm fludarabine (d-8 to-3), rabbit ATG (Fresenius: 40 mg/kg; d -4 to -1 or Genzyme: 7.5 mg/kg, d -5 to -3) or alemtuzumab (0.5–0.6 mg/kg; d -8 to -6), and submyeloablative doses of busulfan (d-5 to -3). At transplant, 28/37 (76 %) patients suffered from overt infectious or inflammatory disease manifestations, the others suffered from chronic pulmonary disease or had previous severe invasive infections. HSCT was performed at a median age of 12 years (3–39 ys) from matched sibling (n = 13), matched family (n = 3), matched unrelated (n = 13) or mismatched unrelated donors (n = 8). Bone marrow (n = 31) was the main stem cell source whereas PBSC (n = 6) was used less frequently. Pharmacokinetic (PK) monitoring for busulfan was performed in 29 patients aiming at a targeted submyeloablative cumulative area under the curve (AUC) of 45–65 mg/L × h (10 962–15 834 micromol × min) (myeloablative range 80–120 mg/L × h). In 9 patients where PK monitoring was not feasible, 30–50 % reduced busulfan doses (6.4–12.8 mg/kg iv, median 9.5 mg/kg) were administered. The total administered cumulative busulfan doses ranged between 6.4 and 26.4 mg/kg (median 9.5 mg/kg) corresponding to an estimated cumulative AUC of 13 and 64 mg/L × h (median 51 mg/L × h). Conditioning was well tolerated and no sinusoidal obstruction syndrome was observed. The rate of acute GVHD grade III–IV and chronic limited GvHD was 8 % (3/37) and 11 % (4/35), respectively. Neutrophil and platelet engraftment was observed after a median of +1 9 and +21 days, respectively. Two patients with low cumulative AUC for busulfan experienced autologous reconstitution. Both patients underwent successful retransplantation. After a median follow-up of 11 months (range 2–103), the overall and event free survival rates are 95 % (CI 94–100) (35/37) and 89 % (CI 85–95) (33/37), respectively. All surviving patients exhibit a stable ≥ 90 % myeloid donor chimerism and all pre-existing infectious and inflammatory lesions have cleared off. This protocol provides little short term toxicity and excellent myeloid engraftment resulting in high cure rates in a population of CGD patients at high risk of therapeutic failure. Targeting busulfan administration is of major importance to ensure myeloid engraftment, prevent autologous reconstitution and to reduce long-term toxicity.

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**EP 9 – Gene therapy for PID**

**B. Gaspar**

Abstract not available.

**EP 10 – Pharmacological SCID – ADA treatment**

**F. Porta**

Abstract not available.

**EP 11 – State of art: management of refractory viral infections post ALLO SCT**

**T. Feuchtinger**

Abstract not available.
EP 12 – The Latest in Late Effects after Pediatric Hematopoietic Cell Transplantation (HCT)

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Chair, Pediatric Blood and Marrow Transplantation Consortium Director, Pediatric Blood and Marrow Transplantation University of Utah-Huntsman Cancer Institute/Primary Children’s Medical Center Salt Lake City, Utah, USA

The Lifetime Impact of Post-Transplant Late Effects

Expansion of the number of indications for transplantation and improvements in the availability of appropriate alternative donor stem cell sources to patients with rare HLA types through the use of cord blood and haploidentical approaches has resulted in increased numbers of HCT performed in children annually. In conjunction with this, a reduction in the mortality secondary to relapse, infections, Graft vs. Host Disease (GVHD), and other acute transplant related complications, is leading to improved survival rates and thus an ever-increasing population of HCT survivors.

As we continue to follow HCT patients long-term, however, we are finding that in both the autologous as well as the allogeneic transplant setting, HCT survivors experience mortality rates higher than the general population. One of the largest and most comprehensive studies of HCT survivors to date, the Bone Marrow Transplant Survivor Study (BMT-SS), examined patients treated with HCT who were alive at least 2 years post-transplant and found that allogeneic HCT survivors were at a 99 fold-increased risk of premature death. Even 15 years after transplant these patients continued to have mortality rates twice that of the general population (standardized mortality ratio = 2.2). While relapse of the primary disease and chronic Graft vs. Host Disease (cGVHD) were the leading causes of death, late mortality was attributed to treatment-related causes in 25% of deaths including second malignancies, late infections, cardiac, and pulmonary causes.

While the issue of premature mortality is of obvious concern, the overall and cumulative impact of late effects in HCT survivors is also alarming. Large, comprehensive studies have shown in detail the burden of late effects in childhood cancer survivors. The cumulative incidence of late effects after childhood cancer is as high as 75%. Over 40% of survivors have severe, disabling and/or life-threatening late effects or died due to an adverse effect of cancer treatment.

What about late effects after HCT in children? Only a handful of single-center studies describe the cumulative incidence and severity of late effects in survivors of childhood HCT. Most of these studies focused on survivors with a particular disease, age and/or conditioning regimen. Pediatric HCT survivors have been reported to have a higher cumulative incidence of late effects compared to the studies of cancer survivors who did not receive HCT as part of their treatment, with 93% of survivors having at least one late effect with a median follow-up of only 7 years. In contrast, only 25% of pediatric HCT survivors had severe or disabling/life-threatening late effects, but the follow up was 1–2 decades less than the childhood cancer studies.

The experience gained through studies in pediatric cancer survivors, along with experience gained at centers studying late effects in adult HCT recipients, positions us to address these concerns directly with pediatric-focused studies. Study of late effects after pediatric hematopoietic cell transplantation (HCT) offers unique opportunities and challenges. The opportunities include an ability to discern the effects of treatment modalities on normal childhood growth and development. In addition, these individuals have decades of life ahead of them, with potentially new issues arising as they age. The challenges, however, are daunting. The numbers of transplants for any given condition are few, with even the largest centers performing only a handful of transplants for a given condition each year. This issue is magnified by the fact that children going through each developmental stage (infant, toddler, child pre-adolescent, and young adult) have different sensitivities to therapies, resulting in different complications (i.e. infants and toddlers are susceptible to neurocognitive damage with radiation and adolescents are at high risk of joint/bone issues with steroid therapy). Furthermore, ability to self-report symptoms changes throughout the continuum of transplant survivorship, both across different patients and even within a patient. Thus to be most effective, the study of pediatric late effects after HCT in children should ideally occur in a multi-institutional setting to maximize accrual. To date, such efforts have been limited.

In April of 2011, top experts from around the world gathered in Washington DC to review the current state of knowledge regarding the pathophysiology and epidemiology of late effects after pediatric HCT. The goal was to define deficits in the field and organize studies to address critical needs over the next five to ten years. A series of seven manuscripts were published in Biology of Blood and Marrow Transplantation in 2011 and 2012 that detail the conclusions of this conference and offer insight into the following key areas for children post-HCT: 1) genetic risks of experiencing late effects, 2) methodological challenges in late effects study designs, 3) specific organ effects, 4) metabolic disorders, 5) endocrine issues, 6) immune dysfunction and reconstitution, 7) quality of life (QoL), functional, and neurocognitive outcomes, and 8) recommendations for screening. Included in this brief summary are selected recommendations for screening and treatment of targeted organ, metabolic, and endocrine-related complications after pediatric HCT.

Iron overload, gastrointestinal, and hepatobiliary issues

Secondary iron overload is a nearly universal complication of HCT causing liver cardiac, pancreatic, pituitary and thyroid-related morbidity. Risk factors include high numbers of red cell transfusions and increased GI absorption of iron due to inflammatory conditions, including GVHD. Studies using serum ferritin as a marker suggest that iron levels fall slowly over time after transplant, reaching normal levels years later. In heavily iron-overloaded patients, iron reduction therapy may improve transplantation outcomes and cardiac function. Because of this, it is important to screen for iron overload after HCT using serum ferritin.

Although liver biopsy has often been recommended to quantify iron overload, recent standardization of the T2* MRI method of quantifying tissue iron will likely replace this for managing patients. The mainstay of treatment of iron overload is phlebotomy in patients with recovered normal erythropoiesis.

The majority of GI late effects are related to protracted acute GVHD and chronic GVHD. As
GVHD is controlled and tolerance is developed, most symptoms resolve. Major hepatobiliary concerns include the consequences of viral hepatitis acquired before or during the transplant, biliary stone disease, and focal liver lesions.

Renal disease
Hypertension and renal function screening should occur at all long term follow up visits. Albuminuria and proteinuria predict poor late outcomes, and it may be worthwhile to screen for albuminuria and proteinuria and consider referral to a nephrologist for patients with this and other signs of ongoing renal disease. It is also important to aggressively treat hypertension in patients post-HCT, especially when they have been treated with prolonged courses of calcineurin inhibitors. Whether post-HCT patients with albuminuria and hypertension benefit from treatment with ACE inhibitors or angiotensin receptor blockers requires further study.

Pulmonary disease
Early recognition and treatment of chronic pulmonary conditions after HCT may be important to successful outcomes: by the time patients become symptomatic, the disease is generally advanced. Comprehensive evaluation is recommended when PFTs are decreased by more than 15% or when signs or symptoms of pulmonary dysfunction are detected.

Standard treatment for obstructive lung disease combines enhanced immunosuppression with supportive care including antimicrobial prophylaxis, bronchodilator therapy and supplemental oxygen when indicated. Unfortunately, the response in patients with restrictive lung disease to multiple agents including corticosteroids, cyclosporine, tacrolimus or azathioprine is limited. New agents and better interventions are needed.

Cardiac disease/metabolic syndrome
Although cardiac dysfunction has been studied extensively in non-HCT settings, less is known regarding the incidence and predictors of CHF following HCT in childhood. Potentially cardiotoxic exposures unique to HCT include conditioning with high-dose chemotherapy (especially cyclophosphamide) and total body irradiation (TBI). Pre-HCT exposures and cardiac function have been shown to have significant impact on post-HCT cardiac function, so levels of pre-HCT anthracycline and chest irradiation should be known as post-HCT patients are evaluated for long-term issues.

Screening and follow up for cardiac/metabolic syndrome issues should include blood pressure assessment, measurement of lipid profile and fasting glucose every 5 years or yearly if abnormal, and treatment of lipid abnormalities based on current cardiovascular guidelines.

Thyroid dysfunction
Current recommendations include thyroid function studies (TSH and FT4) annually post-HCT. If TSH is elevated with a normal FT4, thyroid hormone replacement could be initiated, but since subclinical hypothyroidism may resolve spontaneously, studies could also simply be repeated in approximately 2–6 months. In patients receiving BU-based conditioning, surveillance needs to continue for at least 10 years post-HCT. However, in patients that received TBI, there is no clear plateau in the incidence out to 30 years, suggesting that screening may need to continue life-long.

Growth impairment
Current recommendations include yearly measurement of growth with an accurate stadiometer in children. Patients experiencing slow growth should have their bone age obtained annually until their epiphyses close (generally around age 17 for girls and age 19 for boys). For those children who do not appear to be tracking along an appropriate growth curve, referral to pediatric endocrinology consultation is appropriate.

The administration of GH appears to improve final height compared to similarly-transplanted controls that chose not to receive GH. The treatment effect is strongest in those less than 10 years of age at time of HCT, whereas an effect of GH on older children has been difficult to ascertain.

Bone health: low bone mineral density
Post HCT patients should be encouraged to maintain an adequate intake of calcium and vitamin D, and should be counseled about adverse effects of cigarette smoking as well as alcohol and caffeine consumption. Weight-bearing exercise, early mobilization and return to normal activities should also be encouraged.

It is currently unclear when screening for low bone mineral density (BMD) should be initiated and what is the appropriate frequency of screening after HCT. More study is needed, but we recommend DXA scan pre-HCT, 1 year after HCT, then yearly if BMD Z-score is <-1. Patients exposed to high doses of methotrexate and corticosteroids, and patients with a significant bone loss (BMD Z-score <-2), history of fractures, or endocrine deficiencies should be referred to a pediatric endocrinologist.

Bisphosphonates have been shown to be effective in preventing bone loss after HCT in adult patients. More studies are needed to determine if the use of bisphosphonates can be recommended as a routine measure in children who received HCT who either have low BMD or are at increased risk for continued bone loss (eg treatment with corticosteroids for GVHD).

Reproductive risks
For males, reproductive function should be evaluated by assessing sexual function and by performing a semen analysis. In women, it is important to monitor menstrual function, though hormonal contraception will mask any signs of ovarian failure. Even women who maintain cyclic contraception after therapy are at risk for early menopause, infertility, and long-term health problems related to early ovarian failure. Several hormones and ultrasound measures have been utilized to evaluate a woman’s fertility potential and response to fertility treatments. It appears that Anti-Mullerian Hormone (AMH) and Antral Follicle Counts (AFC) are the most sensitive measures of ovarian reserve. While these tests may help to determine ovarian reserve in transplant recipients, it is not yet clear if they will ultimately help to predict the likelihood of pregnancy or time to menopause in this population.

Early loss of ovarian function is associated with menopausal symptoms and long-term health risks including cardiovascular disease and osteoporosis. Estrogens are the most effective therapy for the treatment of menopausal symptoms and genitourinary atrophy, and these symptoms dramatically improve in transplant patients started on hormone therapy (HT). There are no clear guidelines regarding the optimal method of hormone replacement therapy in this population since little data exist comparing the long term safety and efficacy of various different forms of HT in cancer survivors. Prepubertal transplant patients should be monitored closely for development of secondary sexual characteristics after 10–11 years of age. Patients with evidence of gonadal failure should be cared for by a pediatric endocrinologist who can administer a physiologic regimen of hormone replacement.

Bone health: low bone mineral density
Post HCT patients should be encouraged to maintain an adequate intake of calcium and vitamin D, and should be counseled about adverse effects of cigarette smoking as well as alcohol and caffeine consumption. Weight-bearing exercise, early mobilization and return to normal activities should also be encouraged.

It is currently unclear when screening for low bone mineral density (BMD) should be initiated and what is the appropriate frequency of screening after HCT. More study is needed, but we recommend DXA scan pre-HCT, 1 year after HCT, then yearly if BMD Z-score is <-1. Patients exposed to high doses of methotrexate and corticosteroids, and patients with a significant bone loss (BMD Z-score <-2), history of fractures, or endocrine deficiencies should be referred to a pediatric endocrinologist.

Bisphosphonates have been shown to be effective in preventing bone loss after HCT in adult patients. More studies are needed to determine if the use of bisphosphonates can be recommended as a routine measure in children who received HCT who either have low BMD or are at increased risk for continued bone loss (eg treatment with corticosteroids for GVHD).

Reproductive risks
For males, reproductive function should be evaluated by assessing sexual function and by performing a semen analysis. In women, it is important to monitor menstrual function, though hormonal contraception will mask any signs of ovarian failure. Even women who maintain cyclic contraception after therapy are at risk for early menopause, infertility, and long-term health problems related to early ovarian failure. Several hormones and ultrasound measures have been utilized to evaluate a woman’s fertility potential and response to fertility treatments. It appears that Anti-Mullerian Hormone (AMH) and Antral Follicle Counts (AFC) are the most sensitive measures of ovarian reserve. While these tests may help to determine ovarian reserve in transplant recipients, it is not yet clear if they will ultimately help to predict the likelihood of pregnancy or time to menopause in this population.

Early loss of ovarian function is associated with menopausal symptoms and long-term health risks including cardiovascular disease and osteoporosis. Estrogens are the most effective therapy for the treatment of menopausal symptoms and genitourinary atrophy, and these symptoms dramatically improve in transplant patients started on hormone therapy (HT). There are no clear guidelines regarding the optimal method of hormone replacement therapy in this population since little data exist comparing the long term safety and efficacy of various different forms of HT in cancer survivors. Prepubertal transplant patients should be monitored closely for development of secondary sexual characteristics after 10–11 years of age. Patients with evidence of gonadal failure should be cared for by a pediatric endocrinologist who can administer a physiologic regimen of hormone replacement.
EN 1 – Educational Folder: experimental project in the Hematopoietic Stem Cell Transplant Unit

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Introduction

The therapeutic education born in the 70s by Jean Philippe Assal, in 1998 The ‘WHO European Region states that ‘therapeutic education is an ongoing process, integrated and patient-centered care’ The International Joint Commission accreditation states that ‘Health education includes both knowledge necessary during the process of care and knowledge necessary once the patient is discharged’ Inside the HSCT Unit there is much educational material aimed at the child/family, and is ongoing in our Institute the construction of computerized clinical recorders. A previous trial of an educational folder was abandoned in March 2010.

Objectives

Provide all children and their families all the elements to know and share the therapeutic procedure of bone marrow transplant, side effects and risk factors, the skills required for the safety management of the disease at home; Administration of Educational folder to all children undergoing allogeneic HSCT; Integration with written. Develop a simple tool to compile, but at the same time with the complexity that meets the relationship we have with them at all times but not always all our educational activities are written. Develop a simple tool to compile, but at the same time with the complexity that meets the relationship we have with them at all times but not always all our educational activities are written. Develop a simple tool to compile, but at the same time with the complexity that meets the relationship we have with them at all times but not always all our educational activities are written. 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Develop a simple tool to compile, but at the same time with the complexity that meets the relationship we have with them at all times but not always all our educational activities are written. Develop a simple tool to compile, but at the same time with the complexity that means a family that carries out the therapeutic procedure of a HSCT is not easy.

Conclusion

The forms of the educational process are the initial result of colleagues’ involvement.

This project will help to further improve the existing to ensure the child and his family the best care and return home safely even with social skills to manage their disease. The designed form are assumed as a starting point to work to build a flexible and effective tool for the patient education.

This project should lead to greater involvement of all team. The cultural change may lead during the trial, some structural change, adapting the organization to new educational perspective.

Member of the Paediatric Committee EBMT NG

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Males with oligospermia as a result of previous cancer therapy may be able to donate for intrauterine insemination or IVF with intracytoplasmic sperm injection (ICSI). Menstruating women found to have decreased ovarian reserve after cancer therapies may be candidates for undergoing fertility therapies with ovulation induction, intrauterine inseminations, or in-vitro fertilization. However, existing data suggest that cancer survivors have a diminished response to ovarian stimulation and lower IVF success rates compared to couples without a history of cancer.

After a woman has experienced reproductive dysfunction as a result of cancer treatment, the option with the highest chance of a delivering a live born infant is donor egg.

Conclusions

While we know a reasonable amount regarding risks of endocrine and reproductive dysfunction, along with incidence of second malignancies, much more needs to be learned about specific organ dysfunction, bone health, inherited risk, and many other areas. Larger studies multi-center are required to expand our knowledge in this area and define ways of preventing adverse outcomes and improving the quality of life of HCT survivors.

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EN 2 – Family-Centered-Care in the Paediatric Stem Cell Transplant Setting (SCT)

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It is recognized that a definition of Family-Centered-Care (FCC) does not exist. There is no agreement also in the “core elements” of FCC. Authors define FCC as a model, a framework, a philosophy.

A literature review has been performed and results will be shown to the audience. There is a lack of articles regarding the SCT paediatric patient.

In 2010, G Mikkelsen and K Frederiksen declared that:

“FCC is the PROFESSIONAL SUPPORT of the child and the family through a process of involvement and participation, underpinned by empowerment and negotiation.

FCC is characterized by a relationship between healthcare professionals and the family, in which both parts engage in sharing the responsibility for the child’s health care.”

This presentation will pose topics for discussion around as FCC a model of care delivery to children and families in the Stem cell transplant (SCT) setting.

It is important to involve family and children and negotiate the whole process of care at the level they want and desire.

FCC developed over three decades following awakening awareness that excluding parents during a child’s hospital admission was detrimental to the child’s mental health. Literature suggests that it is time for a revision of practices and policies that espouse family-centred care as the optimum model of care in paediatrics. Questions will be discussed: is family-centred care relevant now? What does it mean to implement family-centred care? Is negotiation and decision making implemented effectively?

The main objectives of this presentation are to:

- define parent involvement and negotiation
- highlight if FCC is a relevant factor and if negotiation is implemented effectively in the SCT

Future research should be conducted in SCT setting it should be interest to explore nurses, parents and child’s perspectives.

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EN 3 – The informational needs of mothers about physical care at home for children who underwent stem cell transplants

M. Yilmaz
Izmir Katip Celebi University, Pediatric Stem Cell Transplantation, Izmir, Turkey

Objective

After children who underwent Hematopoietic Stem Cell Transplantation (HSCT) were discharged from the hospital, all of their care responsibilities were generally undertaken by mothers.

Design

This descriptive and longitudinal study was conducted to assess the informational needs of mothers related to the physical care at home of children who underwent (HSCT).

Sample

The sample included the mothers of 74 children who had undergone a transplant within the past three months according to the eligibility criteria.

Measurements

Data collection tools were: (1) Child Information Form, and (2) Informational Needs Form.

Results

The total mean number of informational needs of mothers was very high at 11.3 ± 3.1 (3–15). Both the “moderate” and “high need” rates of mothers were the highest in the areas of “medications and side effects, anticipated complications and their symptoms, skin care, pain, fatigue, nausea, vomiting, diarrhea and sleep management”.

Conclusions

The results revealed that the information needs of mothers for physical care at home of children undergoing HSCT was very high. The results can assist in planning nursing strategies and collaborations for HSCT nurses to improve the discharge process, for prevention and early detection of complications and for decreasing readmission rates by public/home health care nurses.

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EN 4 – Fever and infection management

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Background
Despite considerable progress in the management of the complications of HSCT, infections remain an important cause of post-transplant morbidity and mortality.

Definitions
Febrile neutropenia is a special situation where bacterial infections must be the main target of the empirical anti infective therapy. Patients conditioned with TBI are more likely to suffer from mucositis which is one of the main factors for streptococcal bacteraemia. 30 % of febrile neutropenic episodes are microbiologically documented with 20 % Gram + cocci and 10 % Gram – bacteria. Broad spectrum antibiotics should be administered promptly after blood cultures and sampling of any clinical site of infections whenever possible.

Fungal infections
During the last decade, with better control of opportunistic infections such as CMV infection, invasive fungal disease has emerged as an important cause of death among HSCT patients.

The most efficient protection from fungal air-born infections during the neutropenic phase of allogeneic transplant is the use of air filtration with positive pressure. This kind of isolation has been shown to decrease the early risk of Aspergillus.

Viral infections
Herpes simplex virus (HSV) reactivation can occur during pre-engraftment phase. After engraftment, the herpesviruses, particularly cytomegalovirus (CMV), are major pathogens.

Adenovirus infections
Adenovirus infections can be a cause of severe disseminated infections in allogeneic HSCT recipients. There are currently no established strategies for prophylaxis or treatment of the adenovirus disease.

Special points: septic shock
Sepsis: “The clinical suspicion of infection and evidence of a systemic response” (Kline, Nursing Care of Children and Adolescents with Cancer, 2002). Systemic response is usually defined by a combination of two or more of the following: hypothermia (C), hyperthermia (> 38 °C), tachycardia, tachypnea, leukocytosis, or neutropenia.

Septic Shock: Septic shock is a more progressive stage of sepsis, defined by persistent hypotension that does not respond to fluid resuscitation.

EN 5 – Patient safety in a paediatric transplant unit

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Children’s hospital K10 Division of Haematology-Oncology and Stem Cell Transplantation is combined with several units. There is the paediatric transplant unit (10 beds, JACIE accredited since 2008), haematology/oncology unit offering conventional treatment (9 beds), day-hospital, outpatient unit, home care, anaesthesia unit (BM biopsies, lumbar punctures) and hsc collection unit. Physicians and nursing staff is working in all these units after specific training.

Procedure for handling patient safety incident reports has been in place since 2006. There has been a web-based patient safety incidents reporting system (HaiPro) since 2009. Reports are gathered every 3 months and discussed in division’s management group (medical and nursing lead present). In these meetings is also decided actions to be taken and information to the staff. This is a no blame practice.

HaiPro web-based reporting system allows one to choose from 5 subgroups when making the report from medication error (preparation, prescription, documentation, distribution, administration and unexpected reaction for the medication). All subgroups are more detailed and allow one to be more precise.

Reported patient safety incidents in the unit 2010–2011: total number of incidents 202, happened to the patient 72.3 %, near misses 27.7 %. Incidents were related to: medication 60.9 %, communication 15.8 %, laboratory, imagining 15.3 %, other 8 %. Contributing factors: working environment, work load (21.3 %), communication, flow of information (18.8 %), knowledge, skills, training (16.3 %), not reported (27.7 %)

In the presentation the contributing factors are discussed and also the possibilities to influence to them.

EBMT NG Paediatric committee member

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**EN 6 – JACIE accreditation in a paediatric transplant unit**

M. Stenvall  
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Helsinki University Central Hospital, Helsinki, Finland

Division of Paediatric Haematology-Oncology and Stem Cell Transplantation (JACIE accredited since 2008) is combined with several units: paediatric transplant unit (10 beds), haematology/oncology unit offering conventional treatment (9 beds), day-hospital, outpatient unit, home care, anaesthesia unit (BM biopsies, lumbar punctures) and HSC collection unit. About 30 stem cell transplantations are performed every year. Of these, more than 20 are allogeneic transplantations (BM, PBSC, cord blood). Also BM and aphaeresis collections are carried out in the unit. Physicians and nursing staff is working in all these units after specific training.

Accreditation process started in 2005. A full time JACIE project manager (nurse) was named for 2006–2007. Several physicians and nurses were actively involved in the process. Application for accreditation was submitted 12/2006, document submission was 7/2007; on-site inspection took place 8–9. 10. 2007. Unit was awarded JACIE accreditation for clinical and collection programme 22. 4. 2008, as a first paediatric unit in the Nordic countries. The unit has been inspected in October 2011 for re-accreditation and is now waiting for final approval.

During the initial process there were a lot of paper work to do or existing forms had to be revised: Quality Management Plan, 4 manuals, 82 standard operating procedures, 42 orders, 36 worksheets in total. There were also 49 audit project group meetings, 18 quality group meetings and 3 internal audits prior to accreditation inspection.

Maintaining quality is a continuing process and includes monthly quality group meetings, audits quarterly (topics vary), revising SOPs regularly according to feedback and change in regulations. Nursing care audits are still to be initiated. Quality manager (physician) and quality responsible person (nurse) have time allocated for maintaining the quality process.

In conclusion this was a very positive process with a lot of ups and downs. All processes had to be spelled out and made more transparent. It’s vital to plan things properly, discuss openly, share information and involve all personnel in accreditation process.

JACIE office personnel has offered great help during the whole process, also directions on the internet are very informative and useful.

EBMT NG Paediatric committee member

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**EN 7 – EBMT NG update**

E. Wallhut

Abstract not available.
INTERACTIVE EDUCATIONAL MATERIAL FOR PAEDIATRIC BONE MARROW TRANSPLANTATION NURSES

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Disclaimer:
This project has been funded with support from the European Commission. This publication [communication] reflects the views only of the author, and the Commission cannot be held responsible for any use which may be made of the information contained therein.
ABSTRACTS

OP 1 – High-dose chemotherapy in relapsed and refractory Hodgkin’s lymphoma
IS. Dolgopolov, NN. Subbotina, VK. Boyarshinov, RL. Pimenov, ES. Belyeva, OV. Morozova, AV. Popa, GL. Mentkevich
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Moscow, Russia

From 2003 to 2009 19 pts (female 9, male 10) with early relapse (n = 13) or resistant (n = 6) Hodgkin’s lymphoma (HL) were enrolled in this study. The median age was 13.2 (4–17) yrs. All pts had previously received at least 2 CT lines and 20–35 Gy. Primary LH staging was: st IIa – 6 pts, st IIb–7 pts, st IIA – 2 and IIB–4 pts. The duration of first remission was 11 mo. (4–20) mos. Status at the HDCT was CR in 6, PR in 7 and SD or PD in 6 pts. Stem cells were harvested after 1st course of salvage CT. In pts who failed PBSC harvest bone marrow primed with G-CSF was collected. In 3 pts splenectomy was performed and in all residual tumors was found. Conditioning regimens included Ara-C – 1,000 mg/m 2/d, on d -6 through -3, and melphalan – 70 mg/m 2/d, on d -3, -2. PBSCs were transplanted in 8 pts, PBSC + BM in 9 pts and BM in 2 pts.

Hematological toxicity grade 4 was observed in all pts. Non-hematological toxicity included mucositis grade >2 (n = 16), gastrointestinal grade >2 (n = 10) and severe bleeding (n = 1). One pt with resistant HL died on d+22 from pulmonary infection. WBC and PLT levels >1,0 × 10 9/l, >20 × 10 11/l were reached by d +13 (9–26), d +180 (15–78), respectively. OS and DFS were 81 % and 73 % with a median follow-up of 70 and 76 months, respectively. OS and DFS in persistent HL vs. relapse HL groups were 66 % vs. 89 % (p = 0.3) and 45 % vs. 91 % (p = 0.027), respectively.

Aggressive therapy including HDCT with stem cell rescue seems to be a promising approach for the pediatric pts with HL who relapsed early after 1st line CT.

OP 2 – Allogenic stem cell transplantation in the first-line therapy of childhood anaplastic large cell lymphoma: a choice for minimal residual disease positive patients?
A. Fedorova, A. Kustanovich, N. Minakovskyaya, O. Aleinikova
Belarusian Center for pediatric oncology, hematology and immunology, Minsk, Belarus

Background: In spite of good treatment results for children with anaplastic large cell lymphoma (ALCL), incidence of relapses remains high (25–35 %). Relapse mostly occurs early and can be curative with allogeneic stem-cell transplantation (alloSCT) as a part of the relapse strategy. There is some data on the negative role of minimal disseminated and minimal residual disease (MRD) in this type of lymphoma. Circulating tumor cells (CTC) can be detected in bone marrow (BM) or peripheral blood (PB) by PCR in about 60 % of pts at diagnosis and confers a relapse risk of 50 %, while slow CTC clearance makes relapse occurrence almost imminent. AlloSCT represents a valid treatment option for pts with relapsed ALCL, partially due to graft-versus-lymphoma effect.

Methods and case reports: NPM/ALK-positive ALCL patients were entered into the study. Quantitative (RQ-RT-PCR) and qualitative (nested PCR with a sensitivity of 10–5) and quantitative (RQ-RT-PCR) either in BM or PB at diagnosis, after cytoreductive prephase (day 6) and before each chemotherapy courses, and the days 30, 60, 100 and further after alloSCT (Table). Case #1: a boy of 8, presented with stage IVM (BM involvement by trephine) of lymphohistiocytic subtype ALCL. Case #2: a girl of 7, with stage III, common type ALCL. Patients (pts) started treatment according to ALCL 99, high risk group (MTX 1 g/24 h, VBL), both pts had high response to dexamethasone prephase. Because of positive MRD before 3rd course, they received more intensive CC course. After the 3rd course a boy achieved complete clinical remission, while a girl had residual tumor (less than 30 %) in primary site (inguinal lymph nodes). They underwent a myeloablative conditioning regimen (a boy after the 4th course CC, a girl after the 3rd) consisting of TBI (2 × 2 Gy days -7, -6, -5), thiotepa (10 mg/kg day -4) and etoposide (40 mg/kg day -3). Donors were HLA–identical matched siblings. In Case #1, steroid resistant acute graft-versus-host disease grade II (skin II + hepar III) was observed, treated with CsA, MP 2 mg/kg, daclizumab, MCS twice. In Case #2 there were no any serious complications, CSA alone was used for prophylaxis of GVHD.

Table 1. CTC detected by PCR for NPM/ALK in BM or PB.

A

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Results: There was correlation $r = 0.58$ (p < 0.05) between nested PCR and RQ-RT-PCR. Pts remain disease free after alloSCT 20 and 6 months respectively, both MRD negative.

Conclusion: We suggest that alloSCT can be performed before clinical disease progression/ relapse in NPM/ALK positive ALC pts with very high risk of disease failure.

OP 3 – Immunotherapy with sequential infusions of cytokine induced killer cells for pediatric patients after haploidentical stem cell transplantation
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Cytokine induced killer (CIK) cells have been shown to be cytolytic against solid and hematological malignancies while possessing an anti-infectious potential. This phase I study was performed to evaluate the safety and feasibility of a sequential, dose escalating administration of in vitro expanded CIK cells in pediatric patients with hematological or solid malignancies after haploidentical stem cell transplantation (SCT).
A total of five pediatric patients were enrolled after regulatory approval. One patient with acute myeloid leukemia (AML) in 4th remission, one AML patient with 3rd relapse, one patient with alveolar rhabdomyosarcoma (RMS) in 3rd remission, one RMS patient with 3rd relapse, and one patient with 2nd relapse of a disseminated nasopharyngeal carcinoma. A total of 21 CIK cell infusions (mean 4.2, range 1–9) from haploidentical stem cell donors were administered according to dose escalation with a median number of $20.3 \times 10^6$ CD3+CD56- CIK cells/kg of body weight range (0.1–95.4 $\times 10^6$ CD3+CD56- CIK cells/kg) and a minimum interval of three weeks between infusions. AML patients in part received low-dose chemotherapy before CIK cell infusion. Median follow up was 4.5 months (range 2–8 months). CIK cell infusions were well tolerated and no signs of acute graft versus host disease (aGVHD) were observed, while best response to CIK cell infusion was complete remission in two patients, very good partial remission in one patient and stable disease in two patients. All patients showed immunological improvement in responses to CIK cell infusions. In addition, persistent infections of norovirus and rotavirus responded to CIK cell infusions.

In conclusion, this study demonstrated feasibility, absence of GvHD and efficacy of CIK cell infusions against malignant diseases and viral infections in pediatric patients after haploidentical SCT.

OP 4 – Haploidentical stem cell transplantation in children with refractory acute leukemia – the Lund experience
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Background: Patients with therapy resistant relapsing acute leukemia (RAL) have extremely poor prognosis with conventional approach. The aim of the study was to evaluate efficacy of intensified induction as well as to determine the safety and efficacy of haploidentical stem cell transplantation in the treatment of the otherwise incurable disease.

Patients and methods: Between September 2005 and December 2011 fourteen children with RAL were referred to BMT Unit at the Department of Pediatric Hematology and Oncology in Lund. There were seven boys and seven girls with the median age of 8 years (range from 0.5 to 13.6). Nine children had ALL, five had AML. Nine patients had the first refractory relapse; five had multiple refractory relapse inclusive three who relapsed after allogeneic SCT.

All children received intensified induction chemotherapy consisting in nine cases with Ciofarabine, Etoposide and Cyclophosphamide (CioEC).

Results: Nine patients responded to the induction achieving subsequent remission (n = 5) or having hypoplastic bone marrow with the number of blasts below 5% in morphology (n = 4). These nine patients were transplanted with haploidentical parent after T-cell depletion of PBSC graft.

Five children did not respond to the induction. All of them received induction with CioEC. Three children died of progressive leukemia; one is still receiving chemotherapy and one patient died because of toxicities not achieving remission. All transplanted children engrafted. Six children are alive from 2 months to 6,2 years after haplo-SCT. Two transplanted patients relapsed and died within 2 months after transplantation. One patient with AML who relapsed after previous allogeneic BMT was transplanted in SCR and died from aGvHD which was caused by his first donor – not the haplo donor.

Conclusions: Intensive induction allowed achieving subsequent CR in five out of 14 children and substantial reduction of blast cells in four out of 14 patients. Five out of nine children who received CioEC were transplanted.

Two out of nine transplanted patients who had residual disease before transplant relapsed within 2 months after haplo-SCT. Transplant related morbidity included virus reactivation and pulmonary VOD in one, the youngest child. The incidence of life threatening acute GvHD was acceptable.

OP 5 – Stem cell transplantation for sickle cell disease with reduced intensity conditioning regimen
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Background and aims: Sickle cell disease (SCD) is still associated with substantial morbidity and reduced life expectancy. Disease related mortality rises from 4% in children to 14% in adolescents and young adults even in the context of care in specialized centers. Following stem cell transplantation the overall survival and the disease free survival is 90% and 95% respectively. In order to reduce transplant associated late effects, the feasibility of a highly immunosuppressive reduced intensity conditioning regimen in children with SCD and a matched sibling donor irrespective of the history of chest syndrome or stroke was evaluated.

Methods: The conditioning regimen consisted of Fludarabine, Melphalan +/- Thiopeta. ATG or Campath and CyA plus MMF were given as rejection/GvHD prophylaxis. The graft was bone marrow in 5 and cord blood in one case.

Results: Eight children and adolescents with a median age of 16.5 years and severe SCD associated morbidity were included. The median follow-up is 4 years (1–7). The conditioning regimen was well tolerated with maximum organ toxicity WHO grade II and no severe infectious complications. Neutrophil engraftment occurred after median 16 days. 5/6 patients had mixed chimerism on day...
+28. Two patients converted spontaneously to complete donor chimerism until day +100 and 2 had complete chimerism on day +365 following donor lymphocyte infusions. In the remaining children mixed chimerism is associated with 100% donor erythropoiesis. None of the patients developed acute or chronic GvHD.

**Conclusions:** Bone marrow transplantation from matched sibling donors following a reduced intensity conditioning regimen seems to be feasible and well tolerated. The confirmation of these preliminary results in a larger patient cohort may have implications on the transplant-indication for children with SCD.

**OP 6 – Outcome of Busulfan and Fludarabine-Based Reduced Intensity Conditioning Regimen for Related and Unrelated HSCT in Fanconi Anemia Patients**  
Robert Debré University Hospital, Paris

HSCT is the only curative treatment for Fanconi Anemia (FA) pts with either bone marrow failure or MDS/Leukemia. Due to characteristic chromosomal instability, the poor outcome of FA pts transplanted after conventional myeloablative conditioning regimen has been proved. Then, reduced-intensity conditioning regimen (RIC) has been considered as a model for allelogeneic HSCT particularly fludarabine-based RIC increasing the overall survival.

**Patients and method:** Between Feb’02 and Dec’10, 17 FA pts from 3 academic French centers were included: R. Debré hospital, St. Louis hospital and J. de Flandre hospital. All pts underwent HSCT because of bone marrow failure (no MDS or leukemia). All patients received the same RIC i.e. fludarabine 30 mg/m²×3d, cyclophosphamide 10 mg/kg/d×4d and IV busulfan (Bu) 0.75 mg/kg/d×3d once daily on days -5 to -2 and alemtuzumab 0.5 mg/kg once daily on days -5 to -2. No further immunosuppression or GVHD prophylaxis was administered. Conditioning was well tolerated with stable respiratory and cardiovascular function while still on ventilator support. PBSC of the patient’s mother. Conditioning comprised fludarabine 40 mg/m² sq.m. once daily on days -5 to -2 and alemtuzumab 0.5 mg/kg once daily on days -5 to -2. No further immunosuppression or GVHD prophylaxis was administered. Conditioning was well tolerated with stable respiratory and cardiovascular function while still on ventilator support.

**Results:** Median age at diagnosis and at HSCT were 4.7 years (range 1.8–9.3) and 7.4 years (range 4.4–15.2), respectively. 12 pts presented with less than 3 FA-related malformations and 5 with more than 3.2 pts received more than 20 transfusions before HSCT, whereas 8 received less than 20 and 7 did not receive any. Graft versus Host Disease (GVHD) prophylaxis consisted of CSA associated with MMF or corticosteroids. Donors were either matched related (sibling, n = 6; other, n = 2) or unrelated (10/10, n = 6; 9/10, n = 3). Stem cell sources were BM (n = 10), UCB (n = 4) and PBSC (n = 2). Median follow-up was 32 months (range 3-102). Successful engraftment was obtained in all patients with a median time for neutrophil recovery of 17 days (range 10–42) with 100% donor chimerism for all. During transplant procedure, 13 pts experienced at least one severe infectious complication. Five pts died from TRM. 36 months OS was 69% (95% CI: 50–96). Cumulative incidence of grade 2 to 4 acute GVHD was 71% (95% CI: 41–87). 5 pts presented with either limited (n = 4) or extensive (n = 1) cGVHD and 36 months cumulative incidence of cGVHD was 33% (95% CI: 11–58). To date, no pt had secondary malignancy.

**Discussion:** Our study confirms the good results before obtained by using flu-based RIC in FA pts. Indeed, satisfying engraftment and long-time survival rates were obtained without any TBI, irrespective of the stem cell source and the donor type. As demonstrated by an on-going study of EBMT registry, the risk of secondary malignancy in these pts is statistically correlated to cGVHD. Then, this rate still remains here probably too high in our study, even though only one pt presented with extensive cGVHD and no pts developed any secondary malignancy (but explained by short follow-up). Suppression of one alkylating agent may reduce both cGVHD incidence and other tissue injuries leading to secondary malignancies. Then, we claim for suppression of Bu for related donor HSCT since 2011 without any cGVHD observed to date (3 patients), with same chimerism. The aGVHD incidence was not different. In FA pts receiving transplant from unrelated donor, the relative impact of either low-dose IV-Bu or low-dose irradiation on toxicity and especially development of secondary malignancies remains to be evaluated.

**OP 7 – Haploidentical stem cell transplantation after fludarabine/alemtuzumab conditioning as a life-saving emergency treatment in critically ill patients with severe aplastic anemia**  
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Kinderspital, Division of Immunology and BMT, Zürich, Switzerland

**Introduction:** Treatment of acquired severe aplastic anemia (SAA) comprises immunosuppressive therapy (IST) and allelogeneic hematopoietic stem cell transplantation (HSCT). HSCT is attempted in children as a first line treatment in presence of an HLA-matched sibling donor or as a second line treatment after unsuccessful IST with an HLA-compatible unrelated volunteer donor. In the absence of a suitable donor, haploidentical transplantation from the parents or a sibling has been attempted before with mixed results. As fungal infections in SAA have a high mortality and often progress before a compatible donor can be found, early transplantation can be life-saving.

**Case report:** We present the case of a 9.5-year-old girl with idiopathic very severe aplastic anemia refractory to one cycle of IST with cyclosporine A, anti-thymocyte globulin, prednisone, and filgrastim. As a complication of prolonged neutropenia, she acquired invasive bilateral pulmonary aspergillosis leading to respiratory failure requiring ventilator support. Due to her massively impaired general condition in complete myeloid aplasia rapid transplantation was mandatory and haploidentical allelogeneic HSCT was performed with peripheral blood stem cells (PBSC) of the patient’s mother. Conditioning comprised fludarabine 40 mg/sq.m. once daily on days -5 to -2 and alem- tuzumab 0.5 mg/kg once daily on days -5 to -2. No further immunosuppression or GVHD prophylaxis was administered. Conditioning was well tolerated with stable respiratory and cardiovascular function while still on ventilator support. PBSC of the mother were obtained after stimulation with filgrastim and a total count of 7.8×10⁶ CD34+ cells/kg were transfused. A low CD3⁺ count of 1.7×10⁶ cells/kg was achieved by CD34+ positive selection. Rapid neutrophil engraftment with absolute neutrophil count ≥ 0.5×10⁹/l was seen on day +10. Formal platelet engraftment with platelet count ≥ 20×10⁹/l was seen on day +35. Respiratory functions improved and the girl was extubated on day +14. Fever disappeared and the size of aspergillosis lesions regressed. No significant GVHD or viral reactivations were noted. As she was diagnosed to have an intracardiac thrombus and, in addition, four aspergillus lesions were noted. As she was diagnosed to have an intracardiac thrombus and, in addition, four aspergillus lesions persisted in close proximity to major bronchi the girl was kept on systemic anticoagulation and antifungal therapy. After prolonged rehabilitation she was discharged home on day +125. Sadly, the girl succumbed on day +201 due to a sudden pulmonary bleeding and secondary thrombocytopenia.

**Conclusion:** Even in severely ill SAA patients with infectious complications needing intensive care support, haploidentical HSCT is feasible with fludarabine/alemtuzumab-based conditioning. Rapid neutrophil engraftment can be
achieved with transfer of a high count of CD34+-positive selected cells. It is unclear if persistent fungal lesions or secondary thrombocytopenia after alentuzumab treatment caused the lethal bleeding complication in our patient.

OP 8 – Search for matched unrelated donor in children of Caucasian origin – national study
Department of Pediatric Hematology and Oncology, 2nd Medical School and University Hospital Motol, Prague, Department of Pediatric Hematology and Oncology, University Hospital Motol, Laboratory of DNA Diagnostics, Institute of Hematology and Blood Transfusion, Czech Stem Cells Registry, Institute for Clinical and Experimental Medicine, Prague, CZ

Allogeneic stem cell transplantation (SCT) using unrelated donor to treat malignant or non-malignant disorders became a standard procedure in patients who lack matched family donor. Only around 15% of Caucasian children with malignancy and less with non-malignant disease have matched sibling available. This study describes the chance for children in Czech Republic to identify within the time available unrelated donor for SCT.

In between I/2000–XII/2010 we have initiated preliminary or formal donor search for 279 children (174 males, 62%) of Caucasian origin (median age 8.3 years, range 0.1–18.8) with malignant (n = 188; 67%, median age 10.2 years) or non-malignant disease (n = 91; median age 4.0 years) because no matched (9–10/10) donor was available within the close family members. Children with other ethnic origin were excluded from this analysis. HLA genotyping of loci HLA-A, B, C, DRB1 and DQB1 on allele level was performed using SBT (sequencing based typing) and PCR-SSP (polymerase chain reaction with sequence specific primers).

Searches were initiated in median of 3 days after HLA genotyping was finalized (range 0–2.106 days) and were performed as needed for both national and/or international donors. In average samples of 2 donors/patient were obtained for final confirmatory typing. Finally 197 for SCT indicated children (70%) underwent allogeneic transplant in median of 97 days from search initiation) using matched unrelated donor (MUD, 9–10/10, n = 143,73%), mismatched unrelated donor (MMUD, 7–8/10, n = 31, 16%), unrelated cord blood (UCB, n = 20) or haploidentical family donor (n = 3). In 4/20 patients 9–10 matched cord blood was used. Transplants using mismatched alternative donors were performed in median 132 days since start of search. However none patient died due to the donor unavailability. 38/279 patients died without transplant for toxicity (n = 6) or disease progression (n = 32) in median 100 days (1–1298) after search initiation. In 10/38 identical donor was already available, but SCT was already cancelled. Harvested unrelated donors were identified in Czech (24%), German (48%) and other European registries, 13% were harvested outside the Europe. Since I/2000 till XII/2011 329 first allogeneic SCT were performed at our center using MFD (24%), MUD (52%), MMUD (14%), UCB (8%) or Tcell depleted graft of haploidentical family donor (2%).

We finally conclude that MUD could be identified in about 75% of children of Caucasian origin in need for allogeneic SCT in reasonable time. However, in everyday life, for significant number of patients (25%), especially in those with diverse racial and ethnic backgrounds, we may not be able to rapidly identify a suitably matched donor. For those alternative donors should be identified in time not to delay transplant according to center preference using less matched unrelated donors, cord blood or haploidentical family donors.

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OP 9 – 16 years of allogeneic stem cell transplantation in children in Slovakia
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Introduction: Allogeneic hematopoietic stem cell transplantation (alloHSCT) is considered the best therapy for a number of pediatric patients with certain life-threatening malignant and nonmalignant diseases. The traditional preference for stem cell source – bone marrow – didn’t change much in children in the last decades, despite increasing proportion of peripheral blood used as stem cell source in the adult setting.

Methods: We retrospectively analyzed 143 alloHSCT from 87 human leukocyte antigen (HLA) matched related donors (MRD), 53 HLA-compatible unrelated donors (MUD) and 3 haploidential donors in 135 patients, performed between December 1995 and December 2011 in the only pediatric BMT Unit in Slovakia. Patients aged 1 to 18 years underwent alloHSCT for malignant (90 patients) and non-malignant (46 patients) diseases. The proportion of peripheral blood as stem cell source was 63% whereas bone marrow was 33%.

Results: The patients received a median of 4.2 ± 10.5×10⁸ cells/kg of body weight. 135 patients (95%) engrafted after a median of 14 days, 4 patients (3%) died prior to engraftment and 3 patients experienced autologous recovery. Acute graft-versus-host disease (GVHD) grade II to IV developed in 54 (40%) out of 136 patients who engrafted after alloHSCT and chronic GVHD developed in 46 (36%) out of 127 patients evaluable for chronic GVHD. 80% of patients with chronic GVHD had peripheral blood as stem cell source. The 5-year probability of overall survival is 49.5% and 86.6% in the cohort of malignant and non-malignant diseases respectively. Overall transplant related mortality (TRM) in this heterogeneous group of patients was 17%.

Conclusion: Based upon our experience, peripheral blood as stem cell source can be safely used in alloHSCT from related and unrelated donors for malignant and non-malignant diseases. It did not increase TRM in the analyzed group of patients, though the majority received peripheral blood, but the patients tend to have more severe chronic GVHD, especially in the related setting.

OP 10 – Outcome of hematopoietic stem cell transplantation in children with primary immunodeiciencies and metabolic disorders – single centre study
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Background: Increasing number of donors, high-resolution HLA typing, reduced-intensity conditioning regimens, better infection preven-
tion, detection and treatment, improved supportive care, and Graft-versus-Host Disease (GvHD) prophylaxis and treatment have resulted recently in an increased overall survival (OS) rate among children with primary immunodeficiency (ID) and metabolic diseases (MD) who underwent hematopoietic stem cell transplantation (HSCT).

Aim: The aim of the single centre report was to evaluate the clinical outcome of patients with ID and MD after allogeneic HSCT.


Results: There was no difference in OS between the retransplanted group (9 pts, 14.8%) and the group with single procedure (77.8 ± 14% vs. 57.2 ± 7%; p = 0.231). OS was not affected by the type of transplant (10 matched sibling donor HSCT with 80.0 ± 13% OS; 24 haploidentical HSCT with 62.5 ± 10% OS, 26 matched unrelated donor HSCT with 50.3 ± 11% OS; p = 0.477). There was no influence of the stem cells source on OS (bone marrow HSCT with 77.8 ± 14% OS, 47 peripheral blood HSCT with 57.1 ± 7% OS, 4 cord blood HSCT with 50.0 ± 25% OS; p = 0.488). There was no effect of the number of transplanted CD34+cells/kg on OS (≥ median with 56.2 ± 11% OS, 2005 (n = 41) 66.1 ± 8%; p = 0.095. Stage III–IV of acute GvHD occurred in 8 pts (14.3 % in ID and 8.3 % in MD; p = 0.584), whereas the extensive chronic form was found in 3 cases (61.9% in ID and 9.1% in MD; p = 0.379). Infections were the main cause of death in 17 children (66.7% in ID vs. 80.0% in MD), GVHD in 3 cases (16.7% in ID vs. 0.0% in MD) incl. 1 person who died of aGVHD grade IV (skin II, gut IV, liver III) and 2 of extensive chronic GVHD (lung, liver), other complications caused death in 3 pts (16.7% in ID vs. 20.0% in MD) incl. pulmonary embolism, graft rejection with the progression of MD and 1 cardiac arrest due to unknown cause; p = 0.619.

Conclusion: Careful pre-HSCT assessment (incl. infection status and CNS progression of MD) ensure a risk/benefit analysis prior HSCT and the best outcome in patients with ID and MD. The best curative option for leukodystrophy is HSCT performed in pts with Loes score < 8.

OP 11 – Allogeneic stem cell transplantation in pediatric patients with hyper-IgM syndrome (HIGM): single centre experience

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Immunodeficiency with hyper-IgM (HIGM) is a rare genetic disorder characterized by recurrent infections in association with markedly decreased serum IgG, IgA, and IgM levels but normal or elevated serum IgM or IgG levels. HIGM is caused by impairment of immunoglobulin isotype switching. Apart from humoral immunity also the T-cell function is affected. The cornerstone of the therapy are preventive measures, antifungal prophylaxis and immunoglobulin substitution. The hematopoietic stem cell transplantation (HSCT) is the only curative therapy currently available. Between 2007 and 2012 three children with HIGM were transplanted in the Czech Republic. Patient 1 underwent HSCT from HLA-identical sister in 2007 at 12 months of age with myeloablative conditioning (Busulphan and Cyclophosphamide). Leukocyte engraftment occurred on day +17 and bone marrow aspiration smear showed trilin ear hematopoiesis on day +28 with 35% of autologous hematopoiesis. Treatment was complicated by acute graft versus host disease (GVHD) grade II and reactivation of cytomegalovirus (CMV). Immunosuppression and IVIG substitutions were stopped 2.5 months after HSCT. Approximately 2 years after HSCT patient received first donor lymphocyte infusion (DLI) from original donor for increasing autologous hematopoiesis with some effect. During next 2 years he received few more DLIs but only with a transient effect. Autologous hematopoiesis is now more than 80%. Patient is at home on antibacterial and antifungal prophylaxis without major infection complications and without the need of IVIG substitutions. Patient 2 was transplanted in 2009 at the age of 4.5 years using matched unrelated donor (MUD) with myeloablative conditioning (Thiotepa, Fludarabine, Treosulfan). Leukocyte engraftment occurred on day +21 and bone marrow aspirate smear showed trilin ear hematopoiesis on day +28 with a complete chimerism. Autologous hematopoiesis appeared on day +35. Immunosuppression was discontinued 2 months after HSCT. Now, more than 2 years after HSCT the patient is at home without major infectious complications, although IVG substitution continues and he has more than 85% of autologous hematopoiesis. Patient 3 was transplanted in 2011 at age 1.5 years using MUD with myeloablative conditioning (Busulphan, Fludarabine). Leukocyte engraftment occurred on day +16. Treatment was complicated by acute GVHD grade II and due to progression of exanthema on steroids 2 doses of ATG have been administered. Bone marrow aspirate smear showed trilin ear hematopoiesis on day +28. Now, more than 100 days after HSCT the patient is at home without major infectious complications and he has 0.1% of autologous hematopoiesis. HSCT in HIGM patients is the only curative method although it is very difficult because of high risk of graft rejection. The prognosis of patients is uncertain. There are no easy and clear criteria for 2nd HSCT.

Partly supported by the Institutional support of University Hospital Motol

OP 12 – Pharmacokinetic study of high-dose treosulfan i.v. in paediatric patients before haematopoietic stem cell transplantation

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Treosulfan is an alkylating agent registered as Ovastat, first for treatment of advanced platinum-resistant ovarian carcinoma. Nowadays, treosulfan is increasingly applied in high doses 12–14 g/m2 as a promising myeloablative agent with low organ toxicity in children (1).

Presented here results of the pharmacokinetic study of treosulfan in children constitutes a continuation of our first research project which results were published in BMT 2008 (2). The new group of paediatric patients consisted of 16 children aged 5 months – 16 years (median 7.5 years) and came from two 2 transplantations centres.

Treosulfan (Ovastat, Medac, Hamburg) was administered intravenously to paediatric patients for three consecutive days, as 1–2 h infusion. Daily
doses of 12 g/m² and elevated to 14 mg/m² were administered to 10 and 6 patients, respectively.

Blood samples were drawn through indwell-
ing venous access 8 times in the intervals of 0–12 hours and immediately acidified to avoid artifi-
cial ex vivo degradation of treosulfan. Urine sam-
pies were collected up to 12 hours. Treosulfan concentrations in children plasma and urine were determined by a validated HPLC method with refractometric detection.

The plasma concentrations were used to cal-
culate pharmacokinetic parameters based on two 
compartment disposition modelling with first or-
der elimination using the WinNonlin pharmaco-
kINETIC program. The AUC increase with dose and 
mean value was from 11 90.2 mg/h/L for 12 mg/
m², to 2675.6 mg/h/L for 14 mg/m². Cmax also increased significantly with dose and amounted to 506.1 mg/L for lower dose and 1085.6 mg/L for 14 mg/m² dose. Volume of distribution (Vss) and total clearance (CL) were 14 L and 8.3 L/h for 12 g/m² and lower values of 9.5 L and 6.6 L/h were obtained for greater doses. It has no influence on terminal half-life of treosulfan that was near the same for both doses and amounted to 1.4 h. The similar T0.5 (1.43 h and; 1.68 h) was retrieved from urine data, but the mean percent of parent drug eliminated with urine was about 30% of the total dose eliminated during the first 12 hours.

Conclusion: The increase of Cmax as well as 
AUC was not proportionate to dose as well as the 
decrease of total clearance and Vss with dose dem-
strates significant variability of the parameters 
in children. However literature data suggests linear 
pharmacokinetics of treosulfan next studies are 
required to explain the unproportional changes of 
the important pharmacokinetic parameters.

References
1. Wachowiak J, Sykora KW, Cornish J, Chybicka A, Kowalczyk 
Jr, Gorczyńska E, Choma M, Grund G, Peters C. Treosulfan-
based preparative regimen for allo-HSCT in childhood he-
matological malignancies: a retrospective study on behalf 
of the EBMT Pediatric Diseases Working Party. Bone Marrow 

2. Główek FK, Kowalczyk-Juńca M, Grund G, Wroblew T, Wa-
chowiak J. Pharmacokinetics of high dose intravenous treo-
sulfan in children undergoing treosulfan-based preparative 
regimen for allogeneic haematopoietic stem cell transplan-


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Etoposide (VP-16) plays an important role 
in conditioning prior to allogeneic HSCT (allo-
HSCT) in children with ALL, but VP-16 demonstr-
states variability in pharmacokinetic (PK) pa-
rameters. Therefore it was investigated whether 
VP-16 plasma concentration on day of HSCT 
demonstrates any impact on outcome of allo-
HSCT in childhood ALL.

The study concerned 28 children (aged 5–18 
years) with ALL, including 17 in CR1 and 11 in 
CR2 transplanted from MSD (n = 19) or MUD 
(n = 9). Conditioning regimen consisted of FTBI 
(12.0 Gy with dose reduction to 9.4 Gy in lungs), 
followed by single 4-hour infusion of VP-16 high-
dose (60 mg/kg) given 3 days (n = 5) or 4 days 
(n = 23) prior to allo-HSCT. In addition patients 
transplanted from MUD received ATG. For GvHD 
prevention cyclosporin A (CsA) alone was given 
after MSD-HSCT, whilst CsA and methotrexate 
after MUD-HSCT. Blood samples were collected 
before VP-16 infusion, at the end of infusion and 
subsequently at 2, 4, 8, 24, 48, 60, 72, 96, and 120 h 
after the end of infusion. The VP-16 plasma con-
centration was determined using HPLC method 
with UV detection and a three compartment 
model was used for PK parameters calculations. 
Values of calculated PK parameters were variable. 
Maximum concentration observed at the end 
of infusion was 131.64 μg/ml to 346.95 μg/ml. In 
24 children (82%) VP 16 was present on standard 
time-point of transplantation, i.e. 72 h 
after the end of infusion (0.10–1.52 μg/mL). 
11 children (39%) had detectable concentration 
of VP-16 even 96h after the end of its infusion 
(0.12–0.85 μg/mL).

Non-relapse mortality did not occur. Time 
of engraftment and rate of acute and chronic 
GvHD did not differ significantly in children 
demonstrating no VP-16 and in those with de-
tectable VP-16 in plasma when transplanted.

All relapse occurred exclusively in children 
demonstrating detectable VP-16 concentration 
in plasma (0.10–0.61 μg/mL), including 2 in 1st 
complete remission (CR1; 4 and 18 months after 
MSD-HSCT) and 2 in CR2 (3 and 16 months after 
MUD and MSD-HSCT, respectively).

In children with HR-ALL in CR1 the 5-year 
probability of leukemia free survival (pLFS) in 
8 patients demonstrating no VP-16 was 100%, 
whilst 78% in those 9 with still detectable VP-16, 
but the difference was statistically not signifi-
cant (p = 0.183). In children with ALL in CR2 the 
11-year pLFS in patients without (n = 5) and with 
still detectable VP-16 (n = 6) when transplanted 
was 100% and 62%, respectively (p = 0.142).

Conclusions:
1) As many as 82% of children with ALL con-
ditioned for allo-HSCT with FTBI+VP-16 
(60 mg/kg) demonstrated detectable plasma 
concentration of the drug 72 h and almost 
40% of them 96h after the end of VP-16 
administration.

2) VP-16 plasma concentration observed on 
day of HSCT shown no significant impact 
on engraftment and GvHD occurrence, but 
adverse impact on pLFS, probably due to 
injury to allogeneic GvL effector cells pre-
sent in transplant material.

3) VP-16 PK guided allo-HSCT may improve 
pLFS in children with ALL.

OP 14 – Definition of a risk-
adapted algorithm for diagnosis of impending graft rejection based on early chimerism in the 
T- and NK-cell lineages in pediatric patients undergoing allogeneic stem cell transplantation 
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Early diagnosis of impending graft rejection 
after allogeneic hematopoietic stem cell trans-
plantation (SCT) is crucial for timely onset of effec-
tive therapeutic measures to prevent graft loss.

Chimerism testing of peripheral blood leuko-
cytes by fluorescence in situ hybridization 
(FISH) and polymerase chain reaction (PCR) is a 
standard procedure in post-transplant moni-
toring of engraftment and rejection risk and also 
of relapse risk in leukemia. For evaluating the risk 
of graft loss, chimerism is mostly tested in total 
leukocytes. However, because the significance 
of different leukocyte subsets for rejection does 
not necessarily correlate with their absolute 
and relative levels, testing of sorted leukocyte 
fractions could be more informative.

We have therefore investigated the predic-
tive potential of early leukocyte subset-specific 
chimerism for graft loss in children undergoing 
SCT. Within 10 years, a total of 192 consecutive
pediatric patients transplanted for the treatment of malignant and non-malignant diseases after reduced-intensity or myeloablative conditioning were analyzed. Upon first appearance of leukocyte counts amenable to cell sorting of CD15, CD56, CD3, CD4 and CD8 positive cells, surveillance of lineage-specific chimerism was initiated at a median of 20 days after SCT. In 23 patients graft rejection was observed between 24 and 492 days post-transplant (median 63 days). The first chimerism analysis of T and NK cells identified three different risk groups irrespective of the conditioning regimen: recipient chimerism (RC) levels in T cells below 50% indicated a very low risk of graft loss (1.4%), whereas high levels of RC (>90%) both in T and NK cells heralded rejection in the majority of patients (90%) despite therapeutic interventions like donor lymphocyte infusions (DLI). RC > 50% in T cells and ≤ 90% in NK cells defined an intermediate-risk group in which timely immunotherapy frequently prevented rejection. These results indicate that early analysis of T- and NK-cell chimerism may be instrumental in the risk assessment and therapeutic management of imminent graft rejection.

Based on these findings, we propose an algorithm for risk adapted chimerism monitoring and treatment of impending graft rejection according to the combined analysis of early T- and NK-cell chimerism levels.

**Patients and methods:** To obtain further insight in chimerism profiles after myeloablative SCT in children with ALL in first and second remission, we performed a retrospective analysis in a cohort of n = 71 consecutive and evaluable ALL patients transplanted with (mis) matched family and unrelated donors between 2000–2010. Chimerism was routinely performed every 2–4 weeks within the first 3 months and once monthly thereafter until 1 year post-SCT in both peripheral blood mononuclear cell (PBMC) and granulocyte fractions.

**Results:** Relapse occurred in n = 31/71 patients which was preceded by mixed chimerism in both PBMC and granulocyte fractions, particularly in the early relapse cases (<6–12 months after SCT). Persistent mixed chimerism (>5% recipient signal beyond day +60) was also documented in n = 2/40 patients with continuous complete remission, and lasted 8 and >12 months, respectively. In these patients, mixed chimerism was exclusively present in the PBMC fraction. Notably, repetitive analysis of bone marrow mononuclear cells revealed complete donor chimerism and molecular remission. Detailed analysis of peripheral blood leukocyte fractions demonstrated that mixed chimerism was exclusively found within CD3+/CD4+ and CD3+/CD8+ T-lymphocyte subsets. The episode of mixed T lymphocyte chimerism coincided with reactivation and clearance of adenovirus and cytomegalovirus, respectively. Adenovirus-specific T lymphocytes of recipient origin could be isolated during this period.

**Conclusion:** Recipient memory T lymphocytes have the capacity to exclusively resist successful myeloablative and anti-leukemic SCT conditioning. These T lymphocytes may contribute to anti-viral immunity post-SCT. In these specific cases mixed chimerism is not correlated with ALL relapse, and thus potentially harmful immunotherapeutic interventions may be avoided.

**OP 15 – Exclusive persistence of recipient T lymphocytes after effective myeloablative stem cell transplantation in children with acute lymphoblastic leukemia: possible contribution to anti-viral immunity**

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**Introduction:** In children transplanted with high-risk and relapsed acute lymphoblastic leukemia (ALL), monitoring of peripheral blood and bone marrow chimerism after stem cell transplantation (SCT) has been demonstrated to be a valuable parameter to predict leukemia recurrence. In patients with mixed chimerism, cessation of immune suppression and infusion of donor lymphocytes may result in elimination of residual recipient hematopoiesis, achievement of full donor chimerism and prevention of leukemia relapse.

**Conclusion:** To obtain further insight in chimerism profiles after myeloablative SCT in children with ALL in first and second remission, we performed a retrospective analysis in a cohort of n = 71 consecutive and evaluable ALL patients transplanted with (mis) matched family and unrelated donors between 2000–2010. Chimerism was routinely performed every 2–4 weeks within the first 3 months and once monthly thereafter until 1 year post-SCT in both peripheral blood mononuclear cell (PBMC) and granulocyte fractions.

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**Conclusion:** Recipient memory T lymphocytes have the capacity to exclusively resist successful myeloablative and anti-leukemic SCT conditioning. These T lymphocytes may contribute to anti-viral immunity post-SCT. In these specific cases mixed chimerism is not correlated with ALL relapse, and thus potentially harmful immunotherapeutic interventions may be avoided.


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**Objectives:** Invasive fungal disease (IFD) and especially invasive mucormycosis (IM) became at our hospital one of the most difficult complications in the haemato-oncological patients during last years. Hence we wanted to analyse our own experience with IM.

**Methods:** We performed the analysis of all cases of IM treated in Department of Paediatric Haematology and Oncology from I/2005 to XII/2011. We evaluated the risk factors, clinical and laboratory signs and therapy in these cases by the use of basic statistical methods.

**Results:** We observed 58 cases of IFD, 13 cases of IM were detected (7F/6M, aged 0.75–17 yrs.) in our cohort (22.4% of IFD; 11 proven/2 possible IM). Ten of the patients were neutropenic at time of IM diagnosis. Diagnoses of those patients were mostly haematological malignancies. 9 patients were treated for acute leukaemia (7 ALL/2 AML), 2 for CNS tumour, 1 lymphoma (NHL) and 1 with advanced myelodysplastic syndrome. All but one patient (surgery for CNS tumour only) received chemotherapy or immunosuppressive drugs. Three were allogeneic HSCT recipients treated for GvHD before the diagnosis of IM. The mean time from the first clinical symptoms to the diagnosis was 16 days (range 3–35 days). Infection was detected most frequently in lungs (6 pts), paranasal sinuses (2 pts), skin (2 pts), among the rest of the patients liver, gut and CNS were affected. Dissemination occurred in 4 cases. Morphological diagnosis of IM was obtained in 11 cases; in 10 cases the cultures grew mucormycetes (B Rhizopus spp., 1 Absidia sp. and 1 Mucor sp). The PCR detection was performed in 7 IM and in 6 pts result lead to early start of appropriate therapy. Amphotericin B application (ABL) was started in 10 patients, 6 of them were treated with the combination of amphotericin B and extensive surgery. Diagnosis post-mortem was made in 3 cases. The mortality decreased rapidly during the years – it was 4 of 5 in the period of 2005–2008 and only 2 of 8 patients in 2009–2011. Seven of 13 patients survived the IM. The combination of surgery and amphotericin B administration is the only factor associated with the survival (p = 0.005; Fisher’s exact test). We didn’t prove the reconstitution of neutropenia at the time of diagnosis as the factor associated with better outcome.

**Conclusion:** The incidence of IM at our department was 22.4% of IFD in haematological patients, which is much higher than the published data from Pagano et al with IM incidence of 2.6% of IFD. The most frequent underlying disease is acute leukaemia which is in agreement with the literature. The mortality decreased with the improvement in diagnostic techniques and early diagnosis.
OP 17 – Emerging azole resistance of Aspergillus species. A proposal for a new management strategy in children undergoing stem cell transplantation with suspected invasive fungal infection

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Background: Aspergillus sp. causes 29% of invasive fungal infections in children undergoing HSCT (1). Empirical or pre-emptive treatment for invasive aspergillosis is most Dutch pediatric departments is voriconazole, based on the results of Herbrecht (2).

Based on Antimicrobial Susceptibility testing, 6% of the A. fumigatus are azole resistance, mainly in the Netherlands and the UK (3). Phenotypic testing can detect azole resistance within a few days, but requires a positive culture.

It is important to realize that some azole-resistant strains appear in patients not having received any prior azole treatment, besides those isolated from patients being exposed to azoles for an extended duration.

Azole pharmacokinetics determines response to treatment and in children trough levels may be inadequate with recommended doses. In a pediatric subpopulation it has been shown that trough concentrations > 1.0mg/L are associated with a higher likelihood of success and that each voriconazole trough concentration.

Discussion: In light of emerging resistance to azoles, a new management strategy is required, namely to direct antifungal treatment based on isolate susceptibility and DTM to avoid azole under dosing.

The Dutch pediatric oncology supportive care group together with the pediatric mycology network is proposing this new management strategy, which will include prospective data collection inclusive of DTM and sensitivity testing of isolates, on all Dutch pediatric oncology patients, including those undergoing stem cell transplantation, treated for invasive mycosis. We propose liposomal amphotericin B as first line therapy in children suspected of invasive Aspergillus until isolate susceptibility is determined. Central nervous system dissemination poses a special problem as CNS penetration is poor with most antifungal agents other than azoles. The possible propensity for neurological dissemination of azole resistant Aspergillus sp, as clinically observed, will be evaluated.

We suggest that this issue also be addressed by the PDWP in conjunction with the infectious diseases WP to determine the epidemiology of azole resistant mycosis in children undergoing HSCT and registered with the EBMT.

References

To promote awareness of an emerging problem and suggest a new study within PDWP.

OP 18 – Short-term in vitro expansion allows affordable generation of multiple virus-specific T-cells for a broader clinical application

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Objectives: Adenoviral infections after paediatric allogeneic haematopoietic stem cell transplantation (HSCT) are a significant cause of morbidity and mortality. Reconstitution of antiviral immunity by transferring donor-derived adenovirus (ADV)-specific T-cells represents a promising treatment option. However, often limiting factors are time-consuming production, high costs, specificity of T-cells, need for leukapheresis and/or a narrow time span (21 days) between first detection of ADV-loads (> 10E6 copies in stool) in patients and observation of lethal disease.

Results: We established a 12 day short-term in vitro expansion protocol to generate ADV-specific T-cells. After short-term expansion, 85.8% (mean) of all viable T-cells showed high proliferation, resulting in a 435 fold (median) increase of ADV-streptamer+ T cells. The percentage of central memory T cells, which are expected to induce long lasting immunity, was not significantly altered during the culture period. Furthermore, short-term expanded ADV-specific T cells showed high cytolytic activity and low or even absent alloreactivity. The same protocols have been successfully applied to expand CMV, EBV, and BKV-specific T-cells.

Materials and methods: PBMCs were stimulated with the PepTiler-ADV5 Hexon. On days 3 and 9, cultured cells were treated with IL-15. On day 6, cells were restimulated with PepTiler-pulsed autologous monocytes. All reagents are available as GMP-compliant products. All assays, including determination of absolute numbers and percentages of proliferating and expanded T cells, cytotoxicity assays and mixed leukocyte reactions were analysed by flow cytometry.

Conclusions: This study provides a fast, feasible and simplified clinical-grade protocol potentially useful for improved adoptive immunotherapy after HSCT.

OP 19 – Molecular decryp tion of an epidemic of group C adenovirus infections in children recipients of hematopoietic stem cells

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Introduction: Adenovirus infections are important causes of morbidity and mortality in units of hematopoietic stem cell transplants (HSCT). Several cases of infection with Adenovirus group C were observed between September and December 2010 in the pediatric unit of HSCT at the hospital Robert-Debré, including 3 C1 and 5 C2. Some of these infections have caused disseminated infections resulting in death. These infections may be due to reactivation or transmission between patients. The high sequence homology within the Adenovirus group C makes it difficult to exclude the circulation of different strains.

Objective: To analyze the variability of different strains of group C adenovirus isolated from patients and the environment to assess if inter-patient transmission occur.

Materials and methods: The genetic variability was analyzed on strains detected in the pediatric unit of HSCT between September and December 2010 from patients (C1A, C1B and C1C)
and C2 (C2A, C2B, C2C, C2D and C2E) and from the environment (C2env). Samples from patients infected with adenovirus C1 and C2 from other departments and sequences from GenBank® were included as controls. Structural genes, fiber and hypervariable region 7 (HVR7) of hexon, and non structural genes, E1, E3 and DNA polymerase, were sequenced for phylogenetic.

Results: Phylogenetic analysis of fiber, E1 and E3 showed no significant differences between the sequences of patients. Analysis of HVR7, fiber, E1, E3 and DNA polymerase concatenated sequences showed for C2 viruses distinct clusters in favor of independent events of viral reactivation and one cluster of closely related strains highly suggestive of interpatient transmission and for C1 viruses an unlikely interpatient transmission. The cluster of closely related C2 viruses occurred in two rooms sharing a common space.

Conclusions: The differentiation of strains of adenovirus group C requires analysis of several regions of the genome. Our results show that several cases may be associated with independent events. Our results also show a strong association of epidemic strains compatible with a nosocomial transmission. However, the high sequence homology of C2 variants emphasizes the need to confront the epidemic data to phylogenetic data in order to establish formal links between cases. These data show the importance of monitoring patients at risk for severe Adenovirus infection and the need to reinforce hygienic measures to control the spread of virus in the environment when positive patients are detected.

OP 20 – Adenovirus load in stool predicts invasive dissemination in pediatric recipients of hematopoietic stem cells

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Introduction: Adenovirus infections are a major cause of morbidity and mortality in pediatric transplantation of hematopoietic stem cells (HSCT). The intestinal tract is the most common cause of systemic adenovirus infections in this population. Early detection and quantification of adenoviral infections in stool for predicting the risk of dissemination represent a major challenge.

Objective: To define the threshold of adenoviral DNA viral load (VL) in stool associated with a risk of dissemination in blood.

Methods: Between September 2010 and April 2011, all patients hospitalized in HSCT unit were weekly tested for adenovirus in blood and stool. Detection and quantification of adenoviruses were performed by real-time PCR with a limit of quantification of 200 copies/ml (Argene, ViroFlu, France). The predictive value of dissemination in blood was determined by VL in pairs of plasma and stool as close as possible (median = 2 days).

Results: Among the 51 patients 28 had a digestive infection, 16 of the 28 patients presented positive detection in blood and 9 with a plasma VL >10 000 copies/ml. The predictive value was calculated from 72 pairs plasma/stool from 26 patients. VL stool was significantly higher in viremic patients (8.62 x 10^8 copies/ml) than non-viremic patients (2.74 x 10^8 copies/ml).

Conclusion: In a pediatric population of HSC transplants, the detection of adenovirus in blood is preceded by a digestive replication. Quantification in stool is predictive of risk of dissemination in blood. These results must be considered for the monitoring of adenovirus infections and pre-emptive treatment in patients at risk of disseminated infections.

OP 21 – Effectiveness of cidofovir in the treatment of Adenovirus

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Our study will investigate the effectiveness of cidofovir in the treatment of Adenovirus in the blood following allogeneic stem cell transplant in a cohort of patients for whom routine blood, stool and urine screening is performed after transplant.

Adenoviruses are a group of DNA viruses currently consisting of more than 50 different recognized serotypes which have been classified into 6 sub groups (A-F). Adenovirus infection remains a significant cause of mortality after bone marrow transplantation and may initially present itself as respiratory tract infections, gastroenteritis and haemorrhagic cystitis.

This retrospective analysis is based on patients that tested positive for Adenovirus on the Bone Marrow Transplant Unit at the Royal Manchester Children’s Hospital between January 2008 and December 2011.

Out of a possible 151 patients that were tested through Virology PCR (polymerase chain reaction) for adenovirus between January 2008 and December 2011, 22 tested positive.

Virology screening is part of the routine infection surveillance within the bone marrow Transplant Unit. At least weekly virology PCR blood samples are obtained and tested for CMV, EBV and Adenovirus infection. Stool and urine samples are also obtained weekly. If Virology PCR blood samples are found to be positive (adenovirus load greater than 500 copies per ml) before one occasion the decision to treat is taken.

Stool, urine or nasopharyngeal adenovirus infection where localised and not part of a disseminated disease is not routinely treated.

Data was then collected from the 22 patient’s notes and analysed. We considered the age, demographic information, time post transplant, the adenovirus viral load (expressed as log viral copies/ml blood) and length of treatment with cidofovir. Two negative results allowed us to stop treatment.

Invasive adenovirus must be promptly treated with antiviral therapy and reduction of immune suppression to allow recovery of a adequate cell mediated immune response to this virus. Intrahepatic cidofovir 1mg/kg three times a week is given as the first line of treatment, and if levels of Adenovirus continue to increase, cidofovir may be increased to 5mg/kg once a week or antiviral therapy changed to intravenous ribavirin along with cidofovir or substituted may be given at 8mg/kg three times a day for 24 hours, reducing to 5mg/kg three times a day afterwards.

Our data with these intervention strategies demonstrate no adenovirus related death and no significant treatment related toxicity.

OP 22 – Cellular and humoral immunity elicited by influenza vaccines in pediatric hematopoietic stem-cell transplantation

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Background: Immunity induced by influenza vaccines following hematopoietic stem-cell transplantation (HSCT) is poorly known.

Immunity induced by influenza vaccines following hematopoietic stem-cell transplantation (HSCT) is poorly known.
**Population and methods:** In this case series study, proliferative T-cell and humoral responses to influenza vaccines evaluated vaccine immunogenicity in 14 pediatric recipients (mean age: 6 years). Cases were vaccinated against the 2009 H1N1 pandemic strain (H1N1pdm2009), either alone (n = 9) or in association with the seasonal trivalent vaccine (n = 5). Median lag time from HSCT to vaccination was 5.7 months. The nature of HSC was HLA-identical related bone marrow graft in 10/14 recipients. Fourteen age-matched non-vaccinated recipients were included as controls. In addition, cytokine (IL2 and IFN-gamma) responses to influenza were evaluated by an intracellular accumulation method in part of the recipients.

**Results:** Vaccines opposed to non-vaccinated recipients evidenced higher proliferative responses to H1N1 (p < 0.0001; median stimulation index: 42 vs 1; median lag time from HSCT to investigation: 335 days in both groups) and higher titers of antibodies specific for the 2009 H1N1 pandemic strain (p < 10; median lag time from HSCT to investigation: 107 days). Specifically, 11/14 (79%) vaccines evidenced proliferative responses to H1N1, of whom 5/7 evaluable had protective (> 1/40) antibody titters. Five out of 7 recipients vaccinated against H1N1 but not against H3N2, evidenced proliferative responses to both H1N1 and H3N2 strains (median stimulation-index: 96 and 126 respectively). Finally, IL2 responses predominated over IFN-gamma responses.

**Conclusion:** Influenza vaccination elicited substantial cell-mediated immunity and to a lesser extent protective humoral immunity at early times post-HSCT in this pediatric series. Monovalent H1N1pdm2009 vaccine induced cross-reactive T-cell responses to seasonal H1N1 and H3N2 strains. Protective (IL2) rather than effector (IFN-gamma) cytokine profiles were elicited.

**OP 23 – Renal function follow-up in a cohort of children after stem-cell transplantation**

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**BACKGROUND:** Major advances in hematopoietic stem cell transplantation (HSCT) have resulted in more long-term survivors. Some of them develop acute renal failure (ARF), an appropriate follow up program in this field is mandatory.

**Objective:** To assess glomerular filtration rate (GFR) in a cohort of children survivors of hematopoietic stem cell transplantation (HSCT). This study was undertaken to analyze the follow up of our previously described cohort (1).

**Patients and methods:** Retrospective cohort study. Consecutive sampling of all patients undergoing HSCT in our Unit between January 2007 and December 2008. We analyzed GFR values (estimated by Schwartz formula’s application) pre-HSCT, early post-transplant, and one and two years after HSCT in the overall cohort. We also recorded demographic and clinical variables such as age, underlying disease and type of HSCT.

**Results:** In the study period, 41 patients underwent HSCT (21 autologous, 11MUD, 3MRD, 6 unrelated cord blood) and were included in the initial cohort. The median age was 6.34 years (0.83 to 15). All of them had malignant underlying disease, the most common was acute myeloid leukemia (40%), followed by acute lymphoblastic leukemia (34%) and Ewing sarcoma (12%). The median GFR decreased significantly from 169 ml/min/1.73 m² in the pre-TPH to 143 in the post-HSCT (follow-up on day +100) (p = 0.007). Eight patients suffered ARF in the early post-HSCT period. Ten patients died during their first year of follow up. (MRT: 1, late sepsis: 6, progression of the disease: 3) and 4 were followed in a different hospital. Twenty seven patients were evaluated for long term follow up. Median GFR at one and two years post TPH were 160 ml/min/1.73 m² and 163, respectively. Three out of 8 children with early post HSCT ARF died within a year and all but one (Chronic renal failure stage I) reached normal GFR one year after transplant. Within two years all patients in the cohort, including those with post-HSCT IRA, had a normal GFR.

**Comments:** In our cohort, all patients, including those with acute renal failure in the early post HSCT, reached normal glomerular function at two years of follow up. GFR should be closely estimated in the early post HSCT but afterwards yearly evaluation seems enough.

**OP 24 – Immediate and long term somatic effects and health related quality of life of bone marrow donation during early childhood**

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Bone marrow (BM) donation in early childhood is uncommon. Late effects of childhood donation have not been investigated. We aimed to study the immediate and long-term side effects (SE) in pediatric donors. All children who donated BM between 1968 and 2002 in the paediatric transplant unit in Leiden, before the age of 13 years were invited in this study. Group I was a retrospective analysis of medical records and computerized laboratory data (n = 197). Group II was a prospective cohort of childhood donors who completed a self reporting health and quality of life questionnaire. In total 145 were invited of whom 54% (n = 79) responded.

All donors were evaluated as medically fit to donate by a qualified pediatrician. Thirteen donors in group I (7%) had a previous medical history; seven children had persistent problems at the time of physical examination (PE) prior to donation; four of these had a severe adverse event during or immediately after harvest.

An average 18 ml BM/kg/donor weight was harvested (range 6–47 ml) with significant haemoglobin loss (mean 1.58 mmol/L; SD = 0.46; p = 0.000). Forty-five percent of the children were exposed to donor blood transfusion. Despite transfusions, Hb levels after donation compared to pre-donation, were reduced in 89% (n = 113/147), with a median loss of 2.5 g/dl (SD 0.46; range 0.3–5.2; median Hb 10.5 g/dl; p = 0.00001).

From self reports in group II donors, 91% considered themselves healthy. Fourteen donors mentioned regular upper airway infections, or problems related to asthma or allergies. Three donors reported diagnosis of an autoimmune disease (rheumatoid arthritis, hypothyroidism and Crohn’s disease). Many other donors reported non-autoimmune joint problems such as bone fractures and contusions or hospital admissions for minor surgery. Two donors suffered from epilepsy and two others were anemic; none of these donors had a medical history at time of donation. Two donors required medical intervention for
severe psychological problems, five suffered from long-lasting fatigue. One donor reported persisting back pain following a second donation. Six donors reported multiple health issues. The mean total score of the General Health Questionnaire did not differ significantly from the healthy adult Dutch population. Utilizing the Short Form 36 Healthy Survey, all donors showed significantly higher raw scores in the sub domains than the healthy Dutch population.

These results show that young children both in the immediate and long term physically tolerate BM donation. Presently no specific guidelines in relation to donation procedures in young children have been established. Our results are important in the development of such recommendations.

**OP 25 – Pubertal development and ovarian function after hematopoietic stem cell transplantation in childhood**

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**Aim of the study:** To assess the cumulative incidence of gonadal failure in female survivors of HSCT in childhood and the need for hormonal induction of puberty in girls with different stages of puberty at the moment of SCT. To assess in girls pre-pubertal at HSCT cumulative incidence of the need for hormonal induction of puberty. To assess HSCT related risk factors for gonadal failure in girls.

**Patients and methods:** In a retrospective cohort study, girls who fulfilled the following requirements were eligible: 1) HSCT during childhood (<18 years) at the pediatric department of Leiden University Medical Centre (LUMC), 2) survival at least 2 years after HSCT, 3) age more than 10 years at start of the study.

The following data were collected: age at time of study and at HSCT, diagnosis/indication for HSCT, conditioning regimen, date of last follow-up, date of menarche, Tanner stage, hormonal levels involved in the pituitary gonadal axis status (FSH, LH, estradiol), date of diagnosis of gonadal failure, hormonal induction of puberty/hormonal replacement therapy.

**Results:** Of 141 of the girls who fulfilled the inclusion criteria, 109 girls were in follow-up at our center and thus included in the study. The cumulative incidence of gonadal failure was 60.6% (66/109). Cumulative incidences were significantly different (p < 0.001) depending on puberty stage at HSCT: 48.6% (34) in pre-pubertal girls, 80.0% (24) in early pubertal girls and 100% (6) in post-pubertal girls. Cumulative incidence differed significantly (p = 0.011) according to diagnosis: 71.0% in hematopoietological disease, 45.2% in benign hematological disease and 33.3% in immunological disease. 67.2% of girls who received a conditioning regimen including TBI and 52.9% of girls who received only chemotherapy developed gonadal failure (p = 0.127). In girls conditioned with chemotherapy only, cumulative incidence was 70.6% after a regimen including Busulfan and 17.6% after a regimen without Busulfan (but including other alkylating agents in most cases; p < 0.001). Hormonal induction of puberty was needed in 31/109 girls (28%). Estrogen substitution in 63/109 (58%).

**Conclusion:** Gonadal failure and a need for hormonal induction of puberty occur in the majority of girls after HSCT. Risk factors include older age/post-pubertal stage at HSCT and chemotherapy conditioning regimens including Busulfan.

**ON 1 – Management tool for pediatric transplant patient**

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**Background:** The HSCT requires a series of self-care that is not always easy to perform, especially with regard to the pediatric patients. The average stay in our hospital is usually one month, which can often be long and tiring for children who have had a transplant. Using this tool, created with the collaboration of nurses, hospital doctors and teachers, we developed a simple routine for pre and post-transplant days to make them more comfortable.

**Methodology:** In our hospital, we have designed a box-shaped graphic that breaks down each of the activities that the patient must carry out in their daily routine before and after transplantation.

These activities include:
- Food
- Cleanliness
- Change of bed linen
- Homework, etc.

Children also assessed, using a color code (green, yellow, red) on their general condition.

**Outcomes:**
- Motivate the patient to carry out these activities.
- Identify those activities that require more help and/or support for its realization.
- Create a simple routine and practice to make them feel better during their hospital stay.
- Using a non-verbal shorthand to communicate with staff, especially in the hardest moments of the transplant.
- Allows staff to know the subjective state of the child.
- Get a plan of systematic and flexible self-care.

**Results:** With this tool, we believe we have better communication and collaboration with the pediatric patient, since some moments are embarrassing and scary, making this task difficult.

**ON 2 – The Power of Knowledge**

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„Hospital without Pain” is one of „Safra” children’s hospital’s main goals of the last decade. In our Pediatric Hemato Oncology department a special anesthesia room was designated to perform many procedures under general anesthesia regarding the disease and treatment for our children. To sustain this goal the nursing staff initiated a quality of care program containing guidelines, protocols and a pantomime motion picture with emphasis on reduction of anxiety levels in children and their parents. The purpose of this research was to reduce the level of anxiety and thereby develop an appropriate teaching protocol.

**Method:** Examine the anxiety levels of children age 14 and above, before and after their...
first general anesthesia experience. Examine the anxiety levels of parents before and after their children’s first anesthesia experience.

**Tools:** Spielberg questioners (1973; 1970) on anxiety levels were preformed for children 14 to 18 years old before and after first anesthesia, as well as for their parents. 19 children and 23 parents answered before and after questionnaire, followed by minimal oral preoperative instructions by the nurses.

**Results:** Among the group of the parents we found a decrease of 45% in general anxiety levels and decrease of 55% in situational anxieties. Among the group of the children a decrease of 61% in situational anxiety levels was found. The participants answered the question regarding the way they would prefer receiving the information: 23.3% of the parent's preferred written material, 41.9% of them prefer visual tools (film or Power Point computer presentation). Among the children group, 33% preferred visual tool and 20% written material.

**Conclusions:** Anxiety levels of parents and children's decreased when preoperative anesthesia education including written materials was provided by nurses. Today this project, providing general anesthesia in our department, has become a routine protocol prior to anesthetizing children. Our research results validate the need for appropriate pre anesthesia information. Our Pamphlets, along with a short clown pantomime film for children who do not yet read and our non Hebrew speaking multi national population, fulfill this need.

**ON 3 – Home care after bone marrow transplant. Our experience**

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The bone marrow transplant is increasingly used in the treatment of pediatric oncology patients, during the last years. BMT has become a part of the intensive therapy in some children malignancies.

The pediatric oncology unit in hospital La Fe in Valencia is a reference center for our community and a surrounded area of 200km.

The average number of BMT in our unit is 25 per year (allogeneic and autologous). The most frequent diagnoses are ALL, AML and high risk neuroblastoma.

The Home care Program in our hospital was implemented in 1997, and allowing early discharge with patients and families been supported at home through our home care team.

A nurse and a pediatric oncologist are on call 24 h 365 days (shifts are completed with other professionals of the pediatric oncology unit). Parents are encouraged to call us anytime. The most frequent nursing aspects we attend are: CVC control, observe and detect early symptoms, pain management, treatment follow up, psychological support, “return home” guidelines and coordination with other health professionals if the patient lives outside our area.

According to our experience, counting on the support provided by the home care team made families feel safer and more confident at home.

**ON 4 – Prophylaxis of gastrointestinal infections with carrot juice during an allogeneic stem cell transplantation**

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**Background:** Bacterial miss-colonization in the gastrointestinal tract can be a potential source for infection during immune suppression as part of stem cell transplantation, which often causes serious clinical and nursing problems.

Damaged mucosa, as a result of radiation or chemotherapy, as well as poor oral intake and malnutrition, facilitate the appearance of invasive enteral infections.

After reviewing the literature in the subject, the traditional treatment with antibiotics and anti-mycotics was questioned.

Problems associated with pharmacological oral decontamination such as resorption, resistance, toxicity, side effects and interactions lead us to search for an alternative.

We decided to validate the use of antiadhesive prophylaxis with “Moro Juice” in 2002.

**Methods:** A water based preparation of cooked carrots contains oligosaccharides, which prevent the adherence of microorganisms to the intestinal mucosa.

Starting about ten days before till sufficient oral nutrition or/and absolute neutrophil count (ANC) > 0.5 G/l after stem cell transplantation, patients receive 10ml/kg bodyweight of this “Moro-Juice”.

Different forms are offered, they are adapted to patient’s age and needs. An application by a nasal-gastric tube is possible.

**Results:** There was no increase neither in septicemia with enteric bacteria nor in fung-al colonization in comparison to a historical matched control group.

**Conclusion:** In the pilot study and in our nursing experience the carrot juice can be regarded as equivalent method to prevent enteral bacterial infections.

It has been shown that this natural therapy, which blocks the adherence of various enteropathogenic microorganisms, is as least as efficient as pharmacological oral decontamination and significant more cost effective.

**Future prospects:** We would like to continue to monitor the compliance of drinking the carrot juice and observe its preventive effects, and if necessary improve it.

**ON 5 – Cord blood transplant in pediatric patients**

King Faisal specialist hospital & RC experience

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Studies indicated that in Saudi Arabia there is more than 60% chance of finding a matched donor among the patient’s family members which is a relatively high percentage. However, it is estimated that approximately 40% of Saudi patients do not have suitable matched related family donor which raised the issue of finding alternative source of stem cells to transplant those patients. The alternative was either umbilical cord blood (UCB) or matched unrelated marrow donor (MUD). As of now, very few patients have full matched unrelated donors (MUD). This was an indication to start transplanting patients with un-related umbilical cord blood at King Faisal Specialist Hospital in 2003. Since then more than (180) pediatric patients diagnosed with different diseases were transplant-ed using CBUs from international and national cord blood banks. The results were encouraging and matching to the reported international results.

Unrelated umbilical cord blood transplantation is now established viable option for many pediatric patients who require SCT transplant but lack a suitable family donor. In UCB transplants, the degree of HLA matching has been closely associated with long-term survival. Therefore, most of the cord blood banks are attempting to provide a large and diverse inventory of HLA typing to extend
A 4 month old boy was admitted to the children’s ward with symptoms of vomiting, diarrhea and shortness of breath. Within days his respiratory symptoms worsened and he was transferred to PICU (Pediatric intensive care unit), where mechanical ventilation was started. He was diagnosed X linked-SCID and preparations for a SCT began. Since the infants condition was not improving it was decided to perform a haplo-transplantation using the father as donor. The father was a one locus 9/10 allele match.?? The infant was not conditioned and received cyclosporine A from day -1 and MTX on day +1, +3, +6 and +11 as GvHD prophylaxis.

Due to Pneumocystis jiroveci pneumonia the infant needed further respiratory support and was put on HFO and ECMO. To ensure positioning of the ECMO catheters and ET tube he was kept sedated and on continuous pain medication. On day + 36 he was transferred to children’s ward in CPAP, awake, but lethargic. On day + 60 the infant was discharged from hospital, smiling to family members and looking like a healthy, little boy.

Nursing challenges:
1. Change a PICU room into a SCT room with laminar hepa-filtration.
2. Keep isolation precaution in a room with a lot of technical equipment and 3 or more persons present at all times.
3. Preparing a SCT handbook for PICU nurses with little experience in caring for children undergoing SCT.
4. Nurse specialist(SCT) working as consultant for PICU nurses.
5. Using ideas from Family center Nursing and Paulo Freire’s empowerment theories to teach parents to participate in caring for their critically ill infant.
6. Preparing the parents for the change from a critical care setting with 2–3 nurses present in the room at all times to a children’s ward with less staff and less monitoring of the infant.
7. “Reading” the infant correctly in order to keep weaning the infant off pain and sedative medication.

Results:
Within cohort of patients, 4 patient no GvHD. 11 patient have oral c-GvHD associated with skin and nails involvement; 1 patient (AML sec., JMML, 7 month old), developed cGvHD and died 6 month later; 1 patient (AML, 13 years old), developed first oral and skin GvHD simultaneous. Response was good to CsA + MP, 4 month later gut GvHD remitted with Etanercept and several upper respiratory tract infections, pulmonary fibrosis. He presented with CMV Pneumonia requiring in intensive care and died 1,6 year after Tx with lung GVHD and respiratory infection.

Results: Beta Thalasemia group (3 pts) developed severe clinical manifestations: ulcers, mucosal damages, leukoplakia, xerostomia. 7 patient have minor signs of oral GvHD – mucosal atrophy and erythema; 5 patient reported no mouth pain, being able to eat. 13 patients are alive, patients have a good status with good quality of live.
OP/N 1 – Aspects of donating hematopoietic stem cells: the sibling donor perspective

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on behalf of the Leonardo da Vinci Project No. 2009–2182/001 – 001 titled “Interactive Educational Material for Pediatric Bone Marrow Transplantation Nurses”

Objectives: Psychological support given to sibling donors throughout the HSCT process is particularly important in order to help them overcome the potentially crisis condition. The first aim of this study was to investigate donors’ awareness about their siblings’ disease and to determine the extent of support provided by family members, friends and/or the professional staff. The second aim was to evaluate the process from donor’s perspective through a diary and to enable the health staff gain awareness about how donors perceive BMT process.

Methods: BMT unit psychologist conducted these interviews by using face-to-face and semi-structured techniques. Sibling-Donor information form was used during the interviews.

Results: Among all 22 donors included in this study, 73% were aware of their siblings’ diagnosis and 54.5% received the first information from their parents (F1). Donors thought that the most informative explanation was presented by health staff (F2). Of these 22 donors, 59% believed that they were sufficiently informed about the process and 68% thought that they were sufficiently supported during this process. Donors stated that they were especially supported by their parents (64%). Some donors (27%) also received support from their teachers and friends (F3). Some of them (27%) stated that they were informed about the HSCT process through internet search. The initial feelings the donors had at first time when they learned that they were going to be donors were worry, confusion, and happiness in different combinations.

Conclusion: The stem cell donating process is a stressful time for donors. An assessment of donor’s interest in getting sufficient level of information about HSCT process is important to provide optimum support. A parent’s perspective about the role of donor in family’s life during this treatment process, the role of a health staff in informing sibling donor to prepare for stem cell harvest, the importance of giving opportunity to donor ask questions, and, to determine the extent of parental support provided to donor are important topics. If topics listed above are sufficiently taken into consideration by health staff, donor’s psychological needs would be efficiently provided. In this context, diary is used as a symbol. Since the school-aged-donor simply express her/his thoughts and feelings by writing on a diary. Thus, we wanted to analyze the stressful period from donor’s perspective and to enable the health staff gain awareness.

Figure 1. Who gave the first information about BMT to the donors

Figure 2. Who gave the most informative explanation about BMT to the donors

Figure 3. Who gave support to the donors during BMT process

OP/N 2 – Intense patient – and family – centered nursing care: the hallmark of pediatric transplant nursing

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In the crucible that is pediatric transplant nursing, the memory of complicated patients often becomes etched in our collective conscience. Prolonged patient-centered care and tense times shared with parents elevate these patients to family status among the staff; our daily moods are often reflections of their current medical condition.

R, a 16 year old girl, underwent a second allogeneic transplant from an unrelated donor for treatment of myelodysplasia 3 years after a previous unrelated transplant administered for relapsed acute lymphoblastic leukemia. Complications included a disfiguring Aspergillus infection requiring multiple surgical procedures, marginal hematopoietic reconstitution, Fusariosis, CMV antigenemia, EBV-related lymphoma, HHV-6 encephalopathy, veno-occlusive disease, severe renal failure, and gastrointestinal GVHD. She was an inpatient for 6 uninterrupted, tension-filled months. We did all that we could to preserve R’s dignity and to enhance her quality of life, including sneaking her into a Justin Bieber concert while she was attached to a BPAP machine, sending her abroad for a weeklong vacation, and arranging for local celebrities to visit her bedside. During periods of encephalopathic confusion, we worked together with R’s parents to keep her centered and focused on positive aspects of her life. Explanations of her care and her status were ongoing and integral to daily routine. We helped R to cope with distorted body image and to maintain contact with her peer group. Our efforts were more than rewarded by R’s smile, by her warmth, and by watching her dance (in a wheelchair) at her gala surprise 17th birthday party. Her death two weeks later was traumatic for us all.

We will discuss how intensity of patient- and family-centered care administered by a dedicated staff of nurses distinguishes the standard of care on the pediatric transplant ward.
Outcome of allo-HSCT was retrospectively analyzed in 24 patients (median age 6.6 years, range 1–19, 12 boys, 12 girls) transplanted between January 1991 – December 2009 for Fanconi anemia (FA, n = 13) and Diamond-Blackfan anemia (DBA, n = 11) in Polish pediatric HSCT centers. FA group (n = 13): 4 (3.8%) patients were transplanted from MSD and 9 (69.2%) from MUD. 6 (46.2%) patients received bone marrow (BM), 6 (46.2%) peripheral blood stem cells (PBSC), and 1 (7.6%) cord blood. Median CD34+ cells dose was 10.4×10^6 (range 3.8–36.6×10^6/per kg of recipient b.w.). Engraftment was achieved in 10 (76.9%) patients after MUD-HSCT and MUD-HSCT was 100%.

Conclusions
1. MSD-HSCT is effective and safety procedure for children with congenital insufficiencies of bone marrow;
2. Haploidentical-HSCT in children with B-DA is still prone to a very high risk of failure and death.

PP 2 – Similar results between cord blood and bone marrow transplantation in high risk acute lymphoblastic leukemia in pediatric patients
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Introduction: High risk acute lymphoblastic leukemia (ALL) in pediatric patients has a poor prognosis treated only with chemotherapy. Allogeneic stem cells transplantation (allo-SCT) can cure a high proportion of these patients. We analyzed retrospectively the results of allo-SCT using bone marrow (BM) versus cord blood (CB) in our centre.

Patients and methods: From 2003–2011, 36 high risk ALL patients were submitted to an allo-SCT: 17 patients have received CB (group A) and 19 patients BM (group B). The median age was 5 years (range 1–19). In group A, 6 (36%) patients were transplanted from MSD and 13 (76%) from MUD, whilst two out of 2 patients transplanted from haploidentical donor died 0.8 and 2.6 months after HSCT due to infection. All 9 (81.8%) children after MSD- or MUD-HSCT are alive, with survival duration of 9.5–174.5 months (median 129 months) and 2.1–6.5 months (median 3.7 months), respectively. 5-years EFS as well as 5-years OS after MSD-HSCT and MUD-HSCT was 100%.

Results: The median time for neutrophil engraftment was longer in group A (21 days) vs group B (16 days) (p < 0.05). 100 days-TRM was similar between both groups (20% vs 20%) p = ns.

Conclusions: Umbilical cord stem cell transplantation is a good option in patients lacking an HLA-identical donor with no statistical differences in OS in comparison to bone marrow, although is associated with a higher relapse rate and higher transplant related mortality.

PP 3 – Costs and cost-effectiveness of allogeneic stem cell transplantation in children are predictable
St. Anna Children’s Hospital, Vienna, Austria

The overall costs of pediatric SCT including donor search and the first year post SCT in a cohort of 141 consecutive children transplanted in a single institution were calculated. Costs were correlated with patient and transplant characteristics and a risk score for transplant related mortality (TRM).
Cost-effectiveness was calculated as overall costs per surviving patient. Gained life years were extrapolated from the overall survival and the costs per expected gained life year were calculated.

Overall median costs were 136,382 € with a wide range from 26,987 € to 601,348 €. Costs increased significantly with age, the use of donors other than matched siblings and with advanced disease. There was a strong correlation of costs with a simple TRM risk-score: the median total costs were 89,550 € (score 0), 127,349 € (score 1), 156,578 € (score 2) and 274,915 € (score 3) (p < 0.001). Cost-effectiveness decreased with the TRM risk-score: the costs per survivor increased from 93,209 € (score 0) to a maximum of 1,216,348 € (score 3).

Costs of pediatric SCT vary substantially, however the combination of the three variables age, disease and donor is predictive for costs and cost-effectiveness. Costs per life year gained are within the broadly accepted range in life-threatening hematopoietic diseases even in the most cost intensive patient cohort.

**PP 5 – Intensive conditioning regimen may improve the results of haploidentical HSCT in children with JMML. Clinical case report**

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Aim: To present a case of DKC associated severe aplastic anemia transplanted in our center with a reduced intensity regimen (RIC).

Case report: We report a 4 year old boy diagnosed with DKC – oral leukoplakia, nail dystrophy, severe hypotrophy (weight 8 kg), and severe aplastic anemia requiring weekly red cell and platelet transfusions with secondary hemochromatosis. The RIC included Fludarabine 25 mg/m²/day, day 1-6; Cyclophosphamide 10 mg/kg/m², 2 days, Thymoglobuline 3,5 mg/m²/day, 4 days; the patient received sibling HLA compatible peripheral stem cells – 5,2×10⁶/kg; the graft versus host disease prophylaxis consisted of Cyclosporine A and short term Methotrexate (day 1, 3, 5, 11). We observed a rapid engraftment for neutrophils on day 14 and for platelets on day 16, without severe complications during aplastic phase. We also noted a complete donor chimerism on day 16.

**Background:** Dyskeratosis congenita (DKC) is a rare inherited bone marrow failure syndrome characterized by the classical clinical triad of abnormal skin pigmentation, oral leukoplakia, and nail dystrophy secondary to mutations in the components of the telomerase complex – telomerase reverse transcriptase (TERT), telomerase RNA (TERC) and dyskerin. DKC frequently evolve into aplastic anemia, requiring intense transfusional support. The only curative treatment is bone marrow transplantation, but due to the rarity of disease, the best approach for transplant procedure is not standardized.

**Aim:** Aim to present a case of DKC associating severe aplastic anemia transplanted in our center with a reduced intensity regimen (RIC).

**Case report:** We report a 4 year old boy diagnosed with DKC – oral leukoplakia, nail dystrophy, severe hypotrophy (weight 8 kg), and severe aplastic anemia requiring weekly red cell and platelet transfusions with secondary hemochromatosis. The RIC included Fludarabine 25 mg/m²/day, day 1, 6, 11 days, Cyclophosphamide 10 mg/kg/m², 2 days, Thymoglobuline 3,5 mg/m²/day, day 4, the patient received sibling HLA compatible peripheral stem cells – 5,2×10⁶/kg; the graft versus host disease prophylaxis consisted of Cyclosporine A and short term Methotrexate (day 1, 3, 5, 11). We observed a rapid engraftment for neutrophils on day 14 and for platelets on day 16, without severe complications during aplastic phase. We also noted a complete donor chimerism on day 16. The patient developed grade I skin GVHD and CMV reactivation after day 45. The Cyclosporine was progressively stopped without GVHD increase. The patient presents after 18 months continuous complete donor...
Diagnosis: At 2 years of age he was admitted to Gaslini Children's Hospital of Genoa and detection of the abnormal presence of mevalonic acid in urine and low activity of mevalonate kinase (MK) in lymphocytes led to the diagnosis. Molecular analysis revealed homozygous mutation of MK gene. Clinical condition failed to improve with first line anti-inflammatory therapy (ibuprofen, prednisone, IL-1 receptor antagonist).

HSCT: Since no related donor was available, child underwent HSCT from UCB 6/6 HLA-compatible at the age of 2-ys-10-mos, after conditioning regimen with Busulfan and Cyclophosphamide. Cyclosporin A and antithymocyte globulin (ATG) were used as GvHD prophylaxis and defibrotide was given as veno-occlusive disease prophylaxis. He received 12.5 x 10^9/kg nucleated and 0.64 x 10^9/kg CD34+ cells. Neutrophil engraftment was observed at day 22, platelet engraftment at day 61, with complete donor chimerism. Infective complications represented by streptococcal sepsis, pulmonary mycosis and CMV reactivation occurred in the first 100 days after HSCT. He developed acute skin GvHD (stage 2) which responded to methylprednisolone, tacrolimus and extracorporeal photopheresis. 3 months after HSCT, Posterior Reversible Encephalopathy Syndrome due to tacrolimus occurred. Immunosuppressive therapy was stopped 12 mos after HSCT with no signs of chronic GvHD and in presence of persistent complete donor chimerism.

Following engraftment, MK activity in lymphocytes normalized and mevalonic acid in the urine disappeared and no fever triggered by inflammatory activation occurred.

3 years after HSCT, at the age of six, the child's clinical condition and quality of life are good. Growth curve, psychomotoric development and bone age are adequate; there is no organomegaly and no dysmorphic features. MRI of the brain showed an improvement of cerebral atrophy. He has secondary hypothyroidism in replacement therapy.

Conclusion: MA is a rare, poor prognosis, autosomal recessive inborn error of isoprene metabolism; severe life-threatening attacks of fever and inflammation with multiorgan involvement are clinical features and the main causes of death. This case shows that a prompt diagnosis and allo-HSCT performed in the first years of life can radically improve prognosis.
PP 9 – Autoimmune haemolytic anaemia following hematopoietic stem cell transplantation
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Introduction: Autoimmune haemolytic anaemia (AIHA) is an uncommon complication after hematopoietic stem cell transplantation (HSCT), which develops mainly along the first year after the transplant. Its incidence is higher in the allogeneic HSCT. Its origin is still unknown but apparently is consequence of donor antibodies reacting with donor red cells antigens. The true incidence and evolution of this complication in paediatric patients is poorly understood. Scarce reports have shown a high mortality despite treatment with steroids and intravenous immunoglobulin (IVIg).

Patients and methods: We report the evolution of 13 cases of AIHA after allogeneic HSCT in three Spanish Centers. Patients were aged between 1 and 16 years with the following hematopoietic diseases: acute lymphoblastic leukaemia (n = 5), acute myeloblastic leukaemia (n = 2), chronic myeloid leukaemia (n = 1) and non-malignant blood disorders (n = 5). Patients were submitted to allogeneic HSCT from January 2006 to November 2011 and all of them developed AIHA along the first year after transplantation.

Results: 12 out of 13 patients developed, at the same time of AIHA, graft versus host disease (GvHD) (6 acute GvHD, 3 chronic GvHD and 3 patients presented symptoms and signs of acute and chronic GvHD) and they were treated with immunosuppressive therapy. All patients presented with hemolysis and positive direct Coombs test. As first line therapy all patients presented with hemolysis and positive direct Coombs test. As first line therapy all patients were treated with steroids (n = 5) and steroids with immunoglobulins (n = 7). However, 7 patients didn’t respond and needed a second line treatment with an anti-CD 20 monoclonal treatment with an anti-CD 20 monoclonal antibody (rituximab). 6 patients achieved a complete response and one patient died due to AIHA refractory to rituximab.

Conclusions: AIHA is an inefrequent complication after allogeneic HSCT associated with high mortality due to the own haemolysis or secondary infections. In refractory patients to conventional therapy, monoclonal anti CD 20 may be a therapeutic option.

PP 10 – Double prophylaxis with oral ursodiol and intravenous glutamine is effective to prevent hepatic sinusoidal syndrome in children undergoing hematopoietic stem cell transplantation.

A single centre experience
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Background: Hepatic sinusoidal obstruction syndrome (SOS) is a well-known and potentially fatal complication of hematopoietic stem cell transplantation (HSCT) in children. Although therapeutic iv defibrotide has changed SOS prognosis, major efforts are still needed to identify SOS prophylactic agents.

Objective: To determine the role of combined prophylaxis (CP) with oral ursodiol and iv glutamine in the prevention of SOS in a single centre HSCT pediatric cohort.

Patients/Methods: Retrospective case-control study. We have included every consecutive patient transplanted in our Unit from February 1989 to January 2012. Case definition (SOS) was based on the modified Seattle criteria. Study group prophylaxis consisted on oral ursodiol (150mg/m²/12h, from day -7 to day +80) and iv glutamine daily continuous infusion (0,5 g/kg/day from day -7 until oral tolerance). We registered demographic, clinical and biological variables that, based on literature published data, could play a role in SOS etiology and/or evolution. Statistical analysis was performed by using a logistic binomial regression (forward stepwise) for multivariate analysis. We included in the analysis the main study variable (CP) and variables described as risk factors for SOS that could work as confounding factors: type of conditioning regimen, type of SCT and disease status at transplant.

Results: In the study period, we performed 395 HSCT (259 autologous, 109 allogeneic, 27 cord blood; 1º HSCT 333, 2º 61, 3º 1). All patients had malignant diseases (ALL 33.4 %, AML 21.3 %, Neuroblastoma 17.2 %). One hundred and ninety nine patients received CP. Thirty three children (8.3 %) developed SOS at a median time of 9 days after stem cell infusion (1–26). SOS severity was mild in 9 patients, moderate in 17 and severe in 7. Painful hepatomegaly was the most common symptom (93.7 %). TRM in SOS patients was three times higher than in no-SOS patients (30.3 % vs 10.2 %).

In the univariate analysis (first step) both, conditioning regimen (p = 0.012), and CP (p = 0.03) raised statistical significance. CP was the only independent variable significantly associated with SOS in the last step of the analysis (multivariate) (p = 0.049). Relative risk to develop SOS was 0.12 in patients without CP vs. 0.04 in CP group (Protective factor).

Conclusion: Double prophylaxis with oral ursodiol and iv glutamine significantly decreases SOS incidence in children undergoing HSCT. A prospective, randomized study is warranted to confirm our results.

PP 11 – A case of MDS/AML M6 in a 3-y. o. boy complicated by secondary hemophagocytic syndrome during post transplant period
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Introduction: Myelodysplastic syndromes (MDS) are rare clonal disorders accounting for less than 5 % of hematopoietic malignancy in children. Erythroid-rich MDS/AML M6 have similar characteristics. This, along with the infrequency with which they are encountered, makes these two entities a formidable diagnostic and therapeutic challenge.

Results: A 3-y. o. boy was referred to our hospital with a 3 month history of malaise, thrombocytopenia, anemia and splenomegaly. BM aspiration showed marked hyperplasia of erythroid lineage (79%) with dysplastic changes and myeloid blasts count 30% of non-erythroid cells (NEC). Fluorescent in-situ hybridization studies revealed Monosomy 7 and del (6q). The diagnosis of MDS/AML M6 was made and the child started treatment according to the protocol AML BFM 2004. On day 28 a complete cytromorphologic, IF, and cytogenetic remission was achieved. Since the child did not have sibling, allogeneic hematopoietic stem cell transplantation (HSCT) from a matched unrelated donor (9/10) with CD34+ cells as 5.99 ×10⁶/kg/weight was performed. He was conditioned with a busulfan-based myeloablative regimen consisting of Busilvex 1.1 mg/kg/dose × 4 doses × 4 days and Cyclofosfamide 60mg/kg/day × 2 days. GVHD prevention included Cs-A, MTX, ATG Fresenius 10mg/kg × 3 days. On day 28 post transplant,
with still no signs of engraftment, the child began to have unremitting fever unresponsive to broad spectrum anti-infective therapy. BM was aplastic with markedly increased number of histiocytes and signs of hemophagocytosis. Extensive search for causative agent revealed only positive blood culture for Staphylococcus haemolyticus with no confirmation of viral or fungal infection. With fulfillment of 5 out of 8 diagnostic criteria, the diagnosis of secondary HLH with failed engraftment was made and therapy started according to the HLH 2004 protocol (Dexamethasone, CSA). A BM check-up showed slightly decreased number of histiocytes with no signs of hemophagocytosis in otherwise aplastic bone marrow. A re-transplantation of PBSC from the same donor was performed with CD34+ cells at 10 × 10^6 kg/weight. Reduced re-conditioning with etoposide/alemtuzumab was considered to be helpful to eradicate HLH activity. He achieved leukocyte and platelet engraftment on day 33 and 39 respectively. Full donor chimerism was confirmed until 67 days after transplant when recipient chimerism appeared in 24% and BM evaluation by FISH analysis confirmed reappearance of Monosomy 7 in 70% of cells. So far, he is in a good clinical condition, transfusion-independent and, as the next step in treatment, donor lymphocyte infusions are considered.

Conclusion: Despite recent advances in understanding the biology of MDS, it still poses a great diagnostic and therapeutic challenge in children, with the HSCT remaining the treatment of choice. Further study is needed to determine the most effective therapy for children with MDS especially for those who fail allogeneic HSCT.

PP 13 – Molecular chimerism as a nonspecific marker in the post-transplant period

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Introduction: Chimerism is a unique state when cells from genetically different individuals coexist in one body. Allogeneic haematopoietic stem cell transplantation (allo-HSCT) leads, in the recipient, to the development of chimerism. Persistence of recipient haematopoiesis in proportional representation (mixed chimerism – MC) augments the risk of relapse, which is one of the reasons for mortality after allo-HSCT.

Methods: The gold standard for quantitative characterisation of chimerism is the short tandem repeat polymerase chain reaction (STR-PCR). Currently, we are also using length polymorphisms of VNTR type (Variable Number of Tandem Repeats), sex-specific loci and a method based on SNP (Single Nucleotide Polymorphism). The ability to detect the recipient haematopoiesis depends on the used methods (VNTR 1–5 %, STR 1 %, SNP 0.035 %). We have regularly received the samples of paediatric patients after allo-HSCT from The Department of Paediatric Haematology and Oncology of The University Hospital Motol since 1992.

Results: To date we have determined informativity in 356 paediatric patients indicated for allo-HSCT, including 112 cases of acute lymphoblastic leukaemia (ALL), 49 cases of acute myeloid leukaemia (AML), 58 cases of aplastic anaemia, 26 cases of chronic myeloid leukaemia (CML), 40 cases of immunodeficiency, 41 cases of myelodysplastic syndromes (MDS), 13 cases of metabolic disorders, 8 cases of lymphoproliferation and 9 cases of other diagnoses. We also analysed 23 investigations of maternal engraftment and 2 detections of twins zygosity. In total, 67 % of transplantations were unrelated and 33 % were related. Patients achieved CC in a median of 21 (range, 7–543) days after allo-HSCT, 9 % of all patients never reached CC and they still have MC. Altogether, 77 % of all monitored patients continue to survive and are constantly monitored, while 23 % of all patients died. Five years after allo-HSCT 80 % of patients with CC and 74 % of patients with MC continue to survive. The cause of death was relapse in 37 %, infection in 23 %, graft versus host disease (GVHD).

Background: Parents donating G-CSF stimulated PBSC to their child, lacking any alternative donor option, are a unique population of donors. They fulfill the dual role both of parents (“care giver”) and donor (“life saver”). Parents see their donor role as an extension of their care giving. As such they feel they have no choice, other than to donate (1). Long term follow up may highlight both physical and psychosocial (mal-) adaptation, related to the donor procedure. Our hypothesis is that compared to non parental family and unrelated donors, parents are more likely to have long term negative outcome. This may be related to the outcome of transplantation.

Primary objectives: We have developed a specific data base aimed at including haploidentical donor cohort from major centers in Europe in order to investigate the specific experience of parents donating stem cells to their child. The one time questionnaire will also investigate physical outcome and health related quality of life (HRQoL) of parents who have donated G-CSF stimulated peripheral blood stem cells to their child.

Study design: A retrospective multicenter cohort study of parents who have donated G-CSF stimulated PBSC to their child from 1995 to 2010, will be included. Expected minimal recruitment is 200 subjects. Informed written consent will be obtained. Epidemiological data sets will be derived from EBMT and national registries. Single time point self reported evaluation questionnaires (SF36) to assess general health and psychosocial adaption will be sent by post from the treating centers. Individual centre collaborating organizers will be responsible for the distribution of relevant questionnaires to centre participants. All results will be sent to the LUMC, Leiden for further analysis.

Controls: Non parent donors will be requested to complete similar datasets to act as a control group. In addition, using identical questionnaires, control data will be obtained from the ongoing study of adult family (non-parental) and unrelated donors, who donated G-CSF PBSC in the Leiden University Medical Centre from 2005 to 2009. Based on present rates, projected recruitment will be 200 eligible controls. Data will be analysed by specifically designed computer software and statistical analysis undertaken using SPSS v 16.0.

This study will be supported by the EBMT Pediatric Diseases and Late Effects working parties and will be open to recruitment as of summer 2012.

References

Aimed at recruiting and informing potential participating pediatric centers in the study
PP 14 – Low levels of receptor chimerism, determined by real-time PCR at the time of granulocytic engraftment, predicts the onset of acute graft vs. host disease in transplanted leukemic paediatric patients

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**Background and objectives:** Acute graft vs. host disease (aGVHD) represents one of the main causes of death in patients who receive allogeneic stem cells transplantation (SCT). Quantification of hematopoietic chimerism by real-time PCR allows the detection of residual receptor cells with a high sensibility (0.01%). Since aGVHD is supposed to be produced by reactivity of donor cells, this study was designed to relate the appearance of aGVHD with the amount of receptor cells remaining early after SCT.

**Patients and methods:** Since January 2009, six patients who underwent myeloablative allogeneic SCT for acute leukaemia (67% lymphoblastic) could be included in the study (a minimum of 100 days follow-up was required). In 100% de donor was identical. The 84% (5) the donor was non-related. Conditioning with total body irradiation (TBI) was performed in 4 cases (67%). All the patients received the same GVHD prophylaxis with cyclosporine and methotrexate.

Chimerism was determined by quantitative real-time PCR amplification of null alleles or insertion/deletion polymorphisms (indels), as previously described (Leukemia. 2005; 19: 336–343). Granulocytic engraftment, defined by the presence of more than 0.5 x 10^5/L granulocytes in two consecutive determinations, appeared at a median of 17 days (11–27) in our series. At least one sample had to be collected in the next week after granulocytic engraftment.

Risk factors related to aGVHD were recorded and analyzed using Chi square for qualitative variables and non parametric tests for quantitative variables.

**Results:** Four patients (67%) presented aGVHD (grade II–IV) in our series. Median of receptor chimerism at the time of engraftment was 0.08% (0.01–2), showing significantly lower levels in patients who developed aGVHD (0.04 vs 1.8%, p = 0.001 Mann-Whitney test).

A chimerism value of 0.1% at the time of granulocytic engraftment was used to classify patients. COR curves were plotted previously for a better selection of the cut-off point.

At 0.1% or lower, four patients (100%) developed aGVHD, whereas none the patients above this value presented aGVHD signs.

Univariate analysis showed only 2 significant variables in relation to the development of aGVHD: TBI conditioning (p = 0.001, log rank test) and low level of receptor chimerism (p = 0.001).

In the multivariate study, only chimerism remained as a significant variable.

**Interpretation and conclusions:** Our data show that early monitoring of chimerism may be useful for identification of patients at risk of developing aGVHD after SCT.

With the quantitative method used, a low chimerism receptor level at the time of engraftment (0.1% or lower) predicts the onset of aGVHD and can anticipate it in more than one week. This could allow the use of measures destined to reduce aGVHD in patients at higher risk.

PP 15 – Value prognostic of total blood chimerism by real time PCR in patient who underwent stem cell transplant for non malignant disease

Hematology department, Carlos Haya Hospital, Malaga, Spain

**Background and objectives:** Allogeneic hematopoietic cell transplantation (HCT) is a procedure to replace an abnormal hemopoiesis by a healthy hemopoiesis, constituting a therapeutic option not only in hematological malignancies but also in non-neoplastic diseases. Chimerism study has become an important tool to evaluate and monitor these patients. The new techniques of hematopoietic chimerism analysis by real-time quantitative PCR (RT-PCR) of insertion and deletion null alleles in whole blood, can detect up to 0.01 % of receptor cells in post-transplantation. Our objective was to assess the technique’s sensitivity and related it with the non-malignant diseases evolution in the post transplantation.

**Patients and methods:** Since January 2007 we have studied six patients who underwent allogeneic SCT for non malignant disease (1 hemophagocytic lymphohistiocytosis, 1 aplastic anaemia, 1 osteopetrosis and 2 congenital immunodeficiency). In 4 cases (67%) the donor was non related and the 100% was HLA identical.

Conditioning regimen was: BuCy in 3 cases (50%), Fludarabine + Melphalan + ATG in 2 cases (33%) and Fludarabine + cyclophosphamide + Alemtuzumab in 1 case. All the patients had received GVHD prophylaxis with cyclosporine and methotrexate. Chimerism was determined by quantitative real-time PCR amplification of null alleles or insertion/deletion polymorphisms (indels), the chimerism was study at certain time-point on days +21, +60, +90 and +120 post-allogeneic stem cell transplant.

**Results:** Median time of receptor engraftment was 16 days (range 11–29). At diagnosis all the patients presented normal karyotype. In our series 50% developed acute graft versus host disease (GVHD) grade II–IV. Median time of GVHD appearance was day +46 post-allo SCT (range 35–65). None patient developed chronic GVHD. Failure engraftment was observed in one cases and failure rejection in other patient.

Median of receptor chimerism in the patients who developed acute GVHD was 15% at day +21, 6% at day +60, 2% at day +90 and 3.8% at day +120 post-allo SCT while was 30% at day+2, +26, +90 and 5% at day+120 in these patients who did not develop (p 0.02).

Median of receptor chimerism at days +21, +60, +90 and +120 was related significantly with the engraftment failure and the autologous recuperation, observing lowers levels in these patients with a stable graft (0.72% vs. 90%).

Median overall survival was 26 months (range 4.4–72 months). Mortality rate was 16% (1 case): Infection complication.

**Interpretation and conclusions:** We obtained high sensibility on residual cellular receptor analysis in malignant diseases with RT-PCR in whole blood. However there are not studies witch
validate its use in non-malignant diseases. In our experience we have found this technique could be used with suitable sensitivity in those patients to prevent the engraftment failure and the recurrence disease, however it would be necessary to validate these findings in others series.

**PP 16 – Case report: Allogeneic 1-antigen mismatched umbilical sibling cord blood transplantation in an 8-year old boy with chronic granulomatous disease**

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Chronic Granulomatous Disease (CGD) is a rare, inherited disease caused by insufficient forming of reactive oxygen forms. This dysregulation of immune system leads to severe, recurrent infections and granuloma formation in different organs. The diagnosis is based on clinical symptoms, medical history and results of functional tests like oxidative burst or NBT test (with nitroblue-tetrazolium). Here we report an 8-year old boy with CGD who was diagnosed at age of 22 months with pneumonia, otitis, laryngitis and chronic lymphadenopathy. Multiple granulomas in lungs, small and large intestine were found. The patient was initially admitted to Children’s Memorial Health Institute in Warsaw, where the CGD diagnosis was confirmed. The boy was referred to the Department of Pediatric Oncology, Hematology and Bone Marrow Transplantation in Wroclaw for of allogeneic umbilical cord blood transplantation from 9/10 antigen HLA matched brother.

The patient underwent high-dose chemotherapy with busulphan (4 × 0.95 mg per kg BW), fludarabine (4 × 40 mg per m²) and MabCampath-1H (3 x 0.1 mg per kg BW). The transplant material contained 5.82 × 10⁶ NC per kg BW. The patients post transplant recovery was uneventful, with exception of one asymptomatic CMV reactivation only.

The ANC recovery was achieved on day +26, and a mixed chimerism (MC) with 28% cells of recipient origin was diagnosed. During observation the increasing autologous chimerism in CD3⁺ subpopulation reached 81% in T cells and 21% in peripheral blood (PB), respectively. On day +67 the patient was given a donor lymphocyte infusion (1 × 10⁶ CD3 cells/kg of the recipient body weight), and after 2 months the autologous chimerism decreased to 49.8% in T-lymphocytes and to 2.2% in MNC’s of the PB, respectively. Oxidative burst test done on day +63 was absolutely normal, which confirmed the cure of the disease. No signs of aGVHD were observed.

Allogeneic stem cells transplantation is the ultimate therapeutic option in patients with CGD. 1 antigen mismatched umbilical cord blood from a sibling provides a safe and curative option for children with CGD. The use of low-dose CAMPATH-1H may be required for smooth GVHD-free engraftment in high risk CGD patients. On the other side, low dose DLI may prove useful in gentle modulation of posttransplant chimerism.

**PP 17 – Hepatopulmonary syndrome as a late event following allogeneic stem cell transplantation in a boy with refractory cytopenia finally revealing dyskeratosis congenita – case report**

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Dyskeratosis congenita (DC) is a rare inherited syndrome exhibiting marked clinical and genetic heterogeneity. It is characterized by presence of mucocutaneous abnormalities (nail dystrophy, lacy reticular pigmentation and oral leukoplakia). Patients with DC are at high risk of bone marrow failure (BMF), myelodysplastic syndrome (MDS) and cancer, as well as pulmonary fibrosis, liver disease, neurological, ophthalmic and other abnormalities. Patients have very short germline telomeres and approximately half have mutations in one of seven genes encoding proteins that maintain telomere function. We report a case of a boy treated due to MDS who five years after stem cell transplantation (SCT) developed fatal hepatopulmonary syndrome and was finally identified to have DC.

Patient at the age of 6 years was referred to our centre with progressive pancytopenia and MDS (RC) was diagnosed. At the time of diagnosis nail dystrophy was present but this finding was evaluated in the context of family occurrence of psoriasis. He underwent uncomplicated bone marrow transplantation from unrelated donor after non-myeloablative regimen consisting of fludarabine and thiotepa. No acute graft versus host disease (GVHD) occurred and immunosuppressive therapy was discontinued 9 months after SCT. At the age of 11 years exertional dyspnea and severe hypoxemia were observed for the first time and he was referred to respiratory department for evaluation. Severe hypoxemia, liver disease and intrapulmonary vascular dilatations were found as signs of hepatopulmonary syndrome of unknown origin. At the age of 15 years the boy underwent an orthotopic liver transplantation with transient improvement and died four months later due to unresponsive progressive hypoxemia. Significant lung fibrotization on autopsy was identified. Genetic analysis of DC was performed and finally mutation (c.844C>T) the TINF2 gene was found.

The clinical presentation of the mucocutaneous triad of DC as well as other related complication (BMF, pulmonary fibrosis, liver abnormalities) may not develop at the same time different individuals. DC may be an under – recognized cause of BMF or MDS. In our patient only nail dystrophy of the typical clinical triad was present at the time of SCT. Successful SCT can correct haematological complications but does not improve some other DC – related manifestations. Reduced intensity conditioning (fludarabine and cyclophosphamide) is recommended. Progressive and lethal pulmonary fibrosis has been repeatedly observed in individuals with DC irrespective of SCT. Lung transplantation in such patients should be considered and if indicated also early performed.

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**PP 18 – Successful treatment of childhood hypereosinophilic syndrome**

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**Background:** Hypereosinophilic syndrome (HES) in children is a rare disorder defined, according to the recently published criteria, as blood eosinophilia greater than 1 500/mm³ on
at least 2 occasions, or evidence of prominent tissue eosinophilia associated with symptoms and marked blood eosinophilia after exclusion of secondary causes of eosinophilia.

**Aim:** To summarize a case report of a child with lymphocytic variant of HES and to discuss our diagnostic and therapeutic experiences.

**Methods:** We describe a case of a 13-year-old female with a history of hypereosinophilia as well as eczema since her fifth year of life. Clinical symptoms showed severe generalized eczema with persistent pruritus (which severely decreased quality of patient’s life), followed by development of fever, mild splenomegaly and lymphoadenomegaly. Biological findings included hyperleukocytosis (WBC 50.1 × 10^9/L) and hypereosinophilia (25.1 × 10^9/L). Bone marrow aspiration revealed absence of blast cells with marked eosinophilia (37%) and lymphocyte immunophenotypical analysis showed increased values of atypical T lymphocytes CD4+CD3negCD5+CD45RO+. Serum IL-5 level was increased and mutation FIP1L1/PDGFRα was negative. Immunophenotype indicated origin from memory T cells and was typical to published cases with lymphocytic variant of HES. We identified monoclonal incomplete TCR beta rearrangement and complete TCR gamma rearrangement, which was also used for minimal residual disease monitoring later during the therapy. We also excluded secondary causes of eosinophilia and our diagnostic conclusion was a lymphocytic variant of HES. Treatment with corticosteroids (16 months) achieved mild clinical improvement, and further combination with hydroxyurea (3 months) was without effect. Next, alemtuzumab (12 months) was used with good effect, but remission was not achieved. Finally, patient underwent transplantation (allo- genic PBSC from unrelated donor, HLA 9/10 matched) which resulted in remission. Busulphan, cyclophosphamide and thymoglobuline were used as conditioning. Patient is now 3.5 years after transplantation and in remission without any pruritus and with an excellent quality of life.

**Summary:** We describe a case, where transplantation proved to be a good therapeutic approach in a patient with lymphocytic variant of HES. So far this is first published case of pediatric lymphocytic variant of HES successfully treated by allogenic stem cell transplantation.

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**PN 1 – Pharmacokinetic study in the treosulfan (Ovastat®) based conditioning regimen in children prior the hematopoietic stem cell transplantation**

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Achievement of therapeutic doses of chemotherapy is essential for successful treatment of malignant diseases in pediatric patients. But the use of chemotherapy in children is associated with a number of difficulties: lack of proper dosage, narrow therapeutic range, high risk of severe toxicity and often even the indication for the use of specific drug. All these issues are related to the absence of pharmacokinetic studies in children. Dosage in children is derived from pharmacokinetic studies performed on adult patients although the pharmacokinetics in children can be different.

In collaboration with children’s transplant center in Hannover and Essen laboratories, we are performing pharmacokinetic study of treosulfan (Ovastat®, Medac). Treosulfan is a chemotherapy drug that is used in combination with other drugs such as a conditioning regimen in patients prior the hematopoietic stem cell transplantation. Treosulfan is less toxic than busulphan and the use of treosulfan is primarily indicated for patients with immunodeficiency. Treosulfan is also a second-line chemotherapy drug in conditioning regimen for patients prior the re-transplantation. Dosage in children, especially in children under 2 years of age, has not been sufficiently verified in passed pharmacokinetic studies.

Between 2009 and 2011 four patients were included in the treosulfan pharmacokinetic study. Three patients had congenital immunodeficiency (2 patients had SCID and 1 patient CGD) and one patient had acute myeloid leukemia. All patients were transplanted using matched unrelated donor. Blood samples withdrawal and urine collection were performed in all patients on 1st and 3rd day of treosulfan administration. A total of about 20 blood samples have been withdrawn at fixed intervals from central vein catheter by push-pull method and approximately 45 bags of urine were collected during 72 hours urine collection from each patient. All samples were then stored at minus 20 degrees of Celsius and shipped by parcel service to the central laboratory in Essen, Germany.

Implementation of pharmacokinetic studies in pediatric patients is always difficult. Nursing staff has to deal with medicinal (one-way CVC or urinary catheter in small child) and formal (parents, informed consent) aspects of pharmacokinetic studies in children which put high demands on the staff.

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**PN 2 – Towards improvement of nurses knowledge and competency in HSCT practice:**

A Leonardo da Vinci educational project with emphasize on pediatric issues

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An interactive educational material is being developed through a Leonardo da Vinci project in order to contribute to improvement of nurses’ efficiency in HSCT practice by increasing their knowledge and awareness in critical fields and critical times of transplantation. Because of the paucity of data in pediatric HSCT nursing special attention to pediatric issues has been addressed. Besides topics on early post-transplant complications consisting of acute graft versus host disease and veno-occlusive disease (sinusoidal obstruction syndrome) general topics explaining transplant dynamics, laboratory-related issues concerning nursing practice are also included. Nursing care specifics in transplant related complications are addressed in detail in each chapter. In addition, late complications of HSCT and long term follow up issues are addressed to improve outpatient nurses knowledge particularly in the pediatric field with
increased life expectancy. Another topic chosen to be useful for HSCT nurses is the use of scoring systems in patient assessment including common toxicity scores (BMT specific issues), performance scores and risk factors for transplantation in different diseases or states. A chapter for laboratory tests is included which will contribute to understanding of the rationale and interpretation of the laboratory test results (daily and/or frequently requested tests and HSCT-specific ones). Finally, quality issues and quality indicators in HSCT practice are presented in a topic in the training material.

The methods used in this interactive electronic training material include text articles accompanied by visual materials, pictures, step by step nursing care approaches in certain conditions, video presentation of case discussions and critical nursing issues. Questions for each chapter are prepared and a question bank is being finalized to be used as a measurement and assessment tool. Explanation for wrong answers and automatic directing to the related chapters are provided. Such training activities supported by the web portal and forum are expected to promote active participation of nurses in HSCT activities and increase the willingness to contribute to better nurses practice in HSCT field. In this presentation, the interactive e-training material will be presented and examples from each category of training will be provided and training methods will be discussed.

All these information are available in English, Turkish, German, Czech and Spanish languages.

**PN 3 – Questionnaire on the Training Needs of Pediatric Bone Marrow Transplantation Nurses**


eScience Institute, University of Bremen

**Objectives:** To develop an interactive electronic questionnaire on the training needs of BMT nurses as a measurement and assessment tool. Explanation for wrong answers and automatic directing to the related chapters are provided. Such training activities supported by the web portal and forum are expected to promote active participation of nurses in HSCT activities and increase the willingness to contribute to better nurses practice in HSCT field.

**Methods:** All in all the results of the evaluation turned out to be highly informative and provided a measurement and assessment tool. Explanation for wrong answers and automatic directing to the related chapters are provided. Such training activities supported by the web portal and forum are expected to promote active participation of nurses in HSCT activities and increase the willingness to contribute to better nurses practice in HSCT field.

**Results:** Even though the evaluation team had to deal with a relatively modest participation, the evaluation brought some significant results, mostly relating to the different educational systems in each participating country. While most of the Turkish BMT nurses for example can provide a College/High School qualification, German BMT nurses are mostly attending vocational school. Furthermore it has shown that German BMT nurses have a higher average age which has a direct impact on the prefered method of learning: Compared to the Turkish Nurses, German BMT nurses prefer to learn from their older colleagues on mostly every working field. Also the working definitions in these countries are diverging, since the Turkish BMT-nurses work on a wider field of working competences, including those of a re-search nurse or home care nurse for example.

In addition it turned out, that the least areas of knowledge, relating to answers from all participating countries, are understanding of cell dose, donor lymphocyte infusion, late effects-endocrine follow up and granulocyte transfusion blood product infusion.

The least areas of competencies are management of a patient treated with elimination methods, respiratory physical therapy in BMT patient, insertion of PIC-Line and supporting the family of a child with Fanconi Anemia.

All in all the results of the evaluation turned out to be highly informative and provided a measurement and assessment tool. Explanation for wrong answers and automatic directing to the related chapters are provided. Such training activities supported by the web portal and forum are expected to promote active participation of nurses in HSCT activities and increase the willingness to contribute to better nurses practice in HSCT field.

**Conclusion:** The results of the present study has suggested that the psychological support given to children and adolescents during the transplant period may assist them to cope with the emotional challenges and may contribute to hastening of the healing process.

**PN 4 – Psychological support to alleviate depression and anxiety in children undergoing hematopoietic stem cell transplantation (HSCT)**


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2. Hacettepe University Faculty of Literature, Psychology Department, Ankara, Türkiye

**Objectives:** Going through the hematopoietic stem cell transplantation (HSCT) process is a stressful time for children. Based on the fact that psychosocial factors are important determinants of the physical and emotional healing process; here, in the present study we aimed to investigate the effects of psychological support on anxiety and depression levels of children and adolescents undergoing HSCT at our unit (ACHO Hospital).

**Methods:** The study includes 21 children with the age of 8-16 years. Twice weekly interviews were held with each patient. Children’s Depression Inventory (CDI) and State-Trait Anxiety Inventory for Children (STA) were applied at 3 time points. Data were analyzed by SPSS program, 18.0 version. ‘One way ANOVA’ was used to determine the significance of three readings at 3 time points. Paired t test with Bonferroni correction was used to detect the source of difference.

**Results:** Mean depression score decreased significantly in a linear manner by time Wilk’s Lambda = 0.3, F(2,19) = 20.9 (p < 0.001). The decrease in depression score was more pronounced in the post transplant time point t(20) = 5.8 (p < 0.016).

When the mean anxiety score was analyzed, a similar trend was detected, demonstrating a significant and linear decrease in mean scores of both trait anxiety and state anxiety Wilk’s Lambda = 0.49, F(2,19) = 10.0 (p < 0.001), Wilk’s Lambda = 0.37, F(2,19) = 16.44, (p < 0.001), respectively. The decrease in both types of anxiety scores was again more prominent in the post-transplant time point t(20) = 3.1 (p < 0.016); t(20) = 5.1 (p < 0.016), respectively.

**Conclusion:** Children undergoing HSCT experience emotional stress during the hospitalization period which is maximum at admission and continues till day 0, the day of stem cell infusion. The depression and anxiety levels show a steady decline after the first week of transplantation. The mean scores have shown a linear decline by time pointing out the favorable emotional state in the post transplant period.

The results of the present study has suggested that the psychological support given to children and adolescents during the transplant period may assist them to cope with the emotional challenges and may contribute to hastening of the healing process.
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**Indikace**

- **Tělesná**
  - Léčba invazní kandidózy
  - Profylaxe kandidových infekcí

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