the abnormalities is shown in the Table. Abnormalities not attributed to chronic GVHD were observed most frequently in lungs, gastrointestinal tract and skin sites. The most common causes of abnormalities attributed to other causes than GVHD included: conditions prior to the transplant, sequela from prior GVHD, deconditioning, infections and medications. When scores for organs or sites with abnormalities attributed to other than chronic GVHD were rescored as zero, the global severity score shifted downward from mild (n = 7), moderate (n = 2), and severe (n = 1) to none and from moderate (n = 8) and severe (n = 1) to mild (Figure).

Conclusion: A modest downward shift of the global severity score occurs when confounders are taken into account in the 0-3 organ-specific scoring system. Our findings support the need for clarification of the NIH diagnosis and staging consensus criteria.

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### Table

<table>
<thead>
<tr>
<th>Normal N (%)</th>
<th>Abnormalities attributed only to GVHD N (%)</th>
<th>Abnormalities attributed to GVHD plus other causes N (%)</th>
<th>Abnormalities attributed only to other causes N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>55 (40)</td>
<td>63 (45)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Mouth</td>
<td>70 (50)</td>
<td>64 (46)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Eyes</td>
<td>71 (51)</td>
<td>59 (42)</td>
<td>-</td>
</tr>
<tr>
<td>GI tract</td>
<td>110 (79)</td>
<td>15 (11)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Liver</td>
<td>99 (71)</td>
<td>27 (19)</td>
<td>8 (6)</td>
</tr>
<tr>
<td>Lungs</td>
<td>97 (70)</td>
<td>18 (13)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Joint</td>
<td>97 (70)</td>
<td>32 (23)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Genital</td>
<td>123 (89)</td>
<td>10 (7)</td>
<td>2 (1)</td>
</tr>
</tbody>
</table>

### Figure

Distribution of global severity scores according to NIH chronic GVHD consensus criteria with and without confounders

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**Establishment of Xenogeneic Lung Chronic Graft-Verus-Host Disease Mice Model**


Hematology/Oncology, The Hospital for Sick Children, Toronto, ON, Canada; Developmental & Stem Cell Biology, The Hospital for Sick Children, Toronto, ON, Canada; Experimental Therapeutics, Princess Margaret Hospital, Toronto, ON, Canada; Pathology Core Centre for Phenogenomics, Toronto, ON, Canada; Department of Medical Oncology/Hematology, Princess Margaret Hospital, University of Toronto, Toronto, ON, Canada; Haematology/Oncology, The Hospital for Sick Children, Toronto, ON, Canada

NOD/SCID/IL2 receptor chain null (NSG) mice bearing human PBMCs can develop xenogeneic acute graft-versus-host disease (aGVHD), which mimics human aGVHD manifestations, including skin or liver involvement. In contrast little is known about the chronic GVHD (cGVHD) mouse model, although a recently described humanized cGVHD model using NSG mice with human thymocytes showed liver fibrosis 14 weeks after transplantation. Here, we established a humanized cGVHD model with chronic lung inflammation and fibrosis mimicking human cGVHD 8 weeks post transplantation.

**Material and Method:** NSG mice were recipients and treated with cyclophosphamide (Cy) at different doses (20-120mg/kg) at day -3 and -2 plus 200cGy X-ray at day-1 followed by injection of 1-5x10^6 G-CSF mobilized human PBMCs or 1x10^5 CD34+ cells at day 0. Samples of skin, lung, liver, spleen and small intestine were taken 8 weeks post transplantation, and fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned, slide mounted, and stained with hematoxylin and eosin and Masson's trichrome. Specimens were evaluated blinded by a pathologist. Engraftment was assessed in peripheral blood by flow cytometry and in each organ by immunohistochemistry (IHC) using anti-human CD4/8/20/68 antibodies.

**Result:** Mice that received over 60mg/kg of Cy lost weight and died earlier than those receiving 20mg/kg or less. Subsequently we used 20mg/kg Cy combined with 200cGy TBI as conditioning for the study. Mice receiving 5x10^6 PBMCs exhibited acute illness including hunching or ruffled hair and died earlier compared with mice receiving 1x10^6 PBMCs or 1x10^5 CD34+ cells at day 0. Samples of skin, lung, liver, spleen and small intestine were taken 8 weeks post transplantation, and fixed in 10% neutral-buffered formalin, embedded in paraffin, sectioned, slide mounted, and stained with hematoxylin and eosin and Masson's trichrome. Specimens were evaluated blinded by a pathologist. Engraftment was assessed in peripheral blood by flow cytometry and in each organ by immunohistochemistry (IHC) using anti-human CD4/8/20/68 antibodies.

**Figure 1.** Engraftment of human cells in NSG mice.

Lung or liver from NSG mice 8 weeks post transplantation were examined for human CD4, CD8, CD20 and CD68 engraftment by IHC. Three randomly selected sections per slide were observed under the x40 objective. Data were calculated as: % positive cells = positive nuclei cells / total cells nuclei x 100.
inflammatory cells, collagen deposition and expansion of airways in the lung despite showing no sign of acute illness or weight loss. Masson’s trichrome revealed increased fibrosis in the lung (Figure 2), but not in other organs.

Conclusion: We have shown that in the NSG mouse, a combination of Cy/TBI with a low number of G-CSF mobilized human PBMCs causes chronic lung inflammation and fibrosis that can serve as an important pre-clinic model of lung cGVHD.

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GRAFT-VERSUS-HOST DISEASE CLINICAL PROFILE AND DURATION OF IMMUNOSUPPRESSION AMONG PATIENTS WHO RECEIVED CORD BLOOD STEM CELL TRANSPLANT: A SINGLE CENTER EXPERIENCE

Vaneuza Araujo Moreira Funke1, Diogo Kloppel2, Andreia Melo1, Lisandro Ribeiro1, Carmem Bonfin2, Elaneide Coutinho Nunes Sr.3, Caroline Sola3, Daniela C. Setubal4, Samir Nabhan4, Michel Michels Oliveira4, Ricardo Pasquini4, Mariester Malvezzi4, 1 Hematology, Federal University of Parana, Curitiba, Brazil; 2 Federal University of Parana, Curitiba, Brazil; 3 Bone Marrow Transplantation Service, Federal University of Parana, Curitiba, Brazil; 4 Nossa Senhora das Graças Hospital, Curitiba, Brazil; 5 Stem Cell Transplantation, Hospital De Clínicas Da Ufpr, Curitiba, Brazil; 6 BMT, Federal University of Parana, Curitiba, Brazil; 7 Internal Medicine, Federal University of Parana, Curitiba, Brazil

Introduction: Transplants from cord blood stem cells is known to have lower incidence of graft-versus-host disease (GVHD). However, in patients who undergo cord blood transplant (CBT) and develop GVHD, its features are not well studied.

Objectives: Determine clinical features of GVHD and duration of therapy in patients who received CBT.

Patients and Methods: From 1993 to 2013, 196 patients received CBT were retrospectively analysed and divided into two categories. Group 1: 64 patients who developed GVHD. Group 2: 132 patients without this complication. Acute GVHD was graded according to Glucksberg criteria and Chronic Graft versus Host Disease was graded by NIH consensus criteria. Statistical analysis: Kaplan Meier (survival) and Fisher test (comparison of categorical variables). P level of significance was <0.05.

Results: Thirty three percent of patients developed GVHD (40 males and 24 females). Median age was 6 years old (1-31). 61 patients received CBT from a mismatched donor. Thirty (48%) were transplanted for malignancies. Five transplants were from a related and 59 from an unrelated donor. Conditioning: Reduced intensity (RIC) in 6 cases and myeloablative in 58. Engraftment was complete in 48 cases (75%). Median survival in group 1 was 1832d (27-7283) versus 201d in group 2 (1-6242). Twenty nine patients have died. Forty one patients developed acute GVHD (aGVHD), 6 patients classic chronic GVHD (cGVHD) and 17 had an overlap syndrome. Grade II-IV aGVHD was seen in 49 cases (84.4%). Among cGVHD patients 9 (39.2%) were mild, 6 (26%) moderate and 8 (34.8%) severe. Median time for the onset of aGVHD was 23d (7-227) and cGVHD was 176d (64-659). The main sites of aGVHD were skin: 55(86%), gut: 22 (34%) and liver:14 (21%). Among cGVHD patients, 14 had skin (21%), liver:12 (18%), mouth:9 (14%), gut and lung (BO): 6(9%) each. Median time of cyclosporine therapy was 923d (7-3365). Steroids were used for a median time of 290d (8-4303). GVHD was less common in patients with a full match donor (p=0.001), those who used thymoglobuline (p<0.0001) and methotrexate (MTX) (p=0.0133). In contrast, GVHD rates were higher in patients who had an early (p=0.0111) and complete (p<0.0001) engraftment and had bacterial (p=0.0133) or viral (p=0.0086) infections during the pre-engraftment period. Survival rates were higher in patients who developed GVHD (p=0.0256), had a myeloablative conditioning regimen (p=0.048), children <14 yo (p=0.0002), patients who used cyclosporine for at least one year (p<0.0001) and full chimerism (p<0.0001).

Conclusions: We conclude GVHD can be frequent and even serious in CBT recipients. Risk factors included early and complete engraftment, mismatched donor, viral or bacterial infection during the pre-engraftment period, use of RIC and lack of MTX. Risk factors for survival were absence of GVHD, RIC, older age, and lack of full engraftment.

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Vitamin D Deficiency Predicts Acute Cutaneous Graft-Versus-Host Disease in Reduced-Intensity Allogeneic Hematopoietic Stem Cell Transplantation

Alex Gametsky1, Lee P. Richman1, Noelle V. Frey2, Robert H. Vonderheide3, David L. Porter3, Ran Reshef4.

1 Hospital of the University of Pennsylvania, Philadelphia, PA; 2 Abramson Family Cancer Research Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 3 Blood and Marrow Transplantation Program, Abramson Cancer Center, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

Background: Acute graft-versus-host disease (GVHD) remains a leading cause of morbidity and mortality in allogeneic hematopoietic stem cell transplant (HSCT) recipients.