Conditioning regimen was myeloablative for 92% of patients. It included TBI in 39%, and ATG in 55%. GVHD prophylaxis was tacrolimus-based (except for 2 patients) with methotrexate (75%) or MMF (19%). 44 patients (39%) received double CB units and 70 (61%) single units. Units were ex-vivo expanded for 20/44 and 9/70 patients, respectively. Median number of infused total nucleated cells (TNC) was 3.5×10^7/kg (1–7) and 4.7×10^7/kg (0.6–56) in the adult and pediatric groups, respectively. cGVHD was defined according to the recent NIH consensus criteria. Risk factors were evaluated by Cox's regression analysis including age, gender, disease status at transplantation, a prior autologous transplant, conditioning regimen, use of ATG in conditioning, GVHD prophylaxis, number of CB units received, number of infused TNC, HLA-A, B, or DRB1 mismatch, and early withdrawal of immunosuppression. **Results:** With a median follow up of 9 months, 21/114 patients developed cGVHD at a median of 126 days post CBT (range 100–276). 62% of these cases (n = 13) were de Novo. Recipient age was the strongest risk factor with a significantly higher 1-year cumulative incidence in adult patients (31%) compared with pediatric patients (n = 4, 8%), p = 0.002), despite a comparable incidence of grade II-IV (38% and 34%) and III-IV (14% and 10%) acute GVHD; and a superior disease free survival in the pediatric group. In adult patients, a prior autologous transplant (n = 16) was the only significant risk factor, and was associated with a higher incidence (50% versus 23%, p = 0.02). cGVHD was extensive in the majority of adult cases (12/17), yet it was associated with a lower rate of relapse (HR = 0.1, p = 0.07) and mortality (HR = 0.4, p = 0.06) when evaluated as time dependent variable in a landmark analysis starting on post SCT day +100. **Conclusions:** Recipient age is a significant predictor of cGVHD following CBT. In adult patients, the impact of prior autologous transplant deserves further evaluation.

363
EARLY DETECTION OF SEVERE ACUTE GRAFT-VERSUS-HOST DISEASE USING PLASMA MARKERS OF T-CELL ACTIVATION

August, K.J.[1], Chiang, K.-Y.[1], Bostick, R.M.[2], Waller, E.K.[3], Langston, A.[3], Khoury, H.J.[3], Moore, K.F.[4], Worthington-White, D.[1], Rowland, P.[1], Horan, J.T.[1]. [1] Emory University, Atlanta, GA; [2] Rollins School of Public Health, Emory University, Atlanta, GA; [3] Winship Cancer Institute, Emory University School of Medicine, Atlanta, GA; [4] Emory University School of Medicine, Atlanta, GA.

Severe acute graft-versus-host disease (GVHD) is a significant cause of transplant related mortality in patients that receive allogeneic hematopoietic stem cell transplantation (HSCT). Treatment of grade III and IV GVHD is frequently ineffective. Preemptive treatment based on the early detection of cytokines associated with T-cell activation and subclinical GVHD might be a more effective strategy, similar to the monitoring of CMV infection after HSCT.

Various plasma markers of T cell activation, soluble IL-2 receptor (sIL-2R) in particular, have been shown to correlate with cellular immune responses in several diseases, including GVHD. Building on this work, as the first step toward developing an early treatment approach for severe GVHD, we conducted a prospective, observational study evaluating three plasma markers of T-cell activation. sIL-2R, sCD40L and sCD8 were evaluated as screening tests with the goal of detecting GVHD in its incipient stages prior to clinical manifestations.

We measured plasma levels of these markers on days 5 and 10 following HSCT. Testing was performed in 50 transplants (49 patients). The median age of patients was 14 (range: 0–67); 19 received genotypically matched related transplants, 4 received mismatched related transplants, 20 received unrelated transplants, and 7 received unrelated cord blood transplants. This population included patients with both malignant and non-malignant diseases and who received myeloablative and reduced intensity conditioning regimens. Nine patients developed grade III or IV GVHD. Receiver operating curves were generated for each of the markers at both time points. This analysis showed sIL-2R and sCD8 to be more accurate markers than sCD40L. Day 10 levels correlated more closely with outcome than those measured on day 5. A parallel testing strategy combining sIL-2R and sCD8 yielded results superior to using either marker alone.

An elevation of sIL2r above 20 ng/mL or sCD8 above 4400 units/mL on day 10 was seen in 8 out of 9 patients that developed grade III or IV GVHD yielding a sensitivity of 0.89. For the specificity calculation, patients with grade II GVHD were considered to be cases, since these patients routinely receive systemic treatment. This method produced a specificity of 0.57.

The results of this pilot study demonstrate the feasibility of using biomarkers of T-cell activation for the early detection of severe acute GVHD. Plans are underway for a larger, more definitive observational study.

364
THE RELATIONSHIP BETWEEN TACROLIMUS SERUM CONCENTRATIONS AND ACUTE GRAFT-VERSUS-HOST DISEASE IN ALLOGENEIC STEM CELL RECIPIENTS

Trifilio, S.M., Flickner, S., Sheldon, S., Pi, J., Evens, A., Frankfurt, O., Gordon, L., Singhal, S., Tallman, M., Williams, S., Winter, J., Mehta, J. Northwestern University, Chicago, IL.

Tacrolimus is commonly used as prophylaxis of GVHD following allogeneic HSCT. While there is an association between increasing serum tacrolimus levels and the incidence of renal toxicity, there is no established relationship between tacrolimus levels and the incidence of GVHD. We studied the outcome of 92 consecutive allogeneic blood HSCT recipients receiving tacrolimus-based GVHD prophylaxis. 55 had received conventional-intensity conditioning (usually busulfan-fludarabine; 34 unrelated donors and 21 sibling donors) where tacrolimus was combined with short-course methotrexate, and 36 had received reduced-intensity conditioning (melphalan ± cyclophosphamide; all unrelated donors) where tacrolimus was combined with mycophenolate mofetil. For each patient, a weekly average tacrolimus level was determined for each of the first 7 weeks post-transplant by averaging all the levels available for that week. The relationship between the average weekly tacrolimus levels and the occurrence of any grade of acute GVHD was examined. The ANOVA test was used to compare the weekly average tacrolimus levels between patients who developed GVHD and those who did not. The cumulative incidence of acute GVHD was compared by tacrolimus level categories each week. A total of 1385 tacrolimus levels were available (6–45 per patient; median 14). The cumulative incidence of acute GVHD at 60 days was 37% (95% CI: 29–49%). As the table below shows, the average tacrolimus levels amongst patients going on to develop acute GVHD tended to be lower than those not developing acute GVHD; significantly so for week 4 and showing a trend towards significnace for weeks 5 and 6. The cumulative incidence of acute GVHD amongst patients whose week 3 average tacrolimus was <10 was 51% (95% CI: 39–68%) compared with 24% (95% CI: 14–41%) for those with a level of ≥10 (P = 0.015). Similarly, the cumulative incidence of acute GVHD amongst patients whose week 4 average tacrolimus was <10 was 43% (95% CI: 32–59%) compared with 20% (95% CI: 10–41%) for those with a level of ≥10 (P = 0.046). The relationship between higher acute GVHD incidence and lower tacrolimus levels was observed for other weeks and tacrolimus cut-off levels, but the differences were not statistically significant. Our data suggest that there is a relationship between tacrolimus levels and acute GVHD. How this affects relapse rates, transplant-related mortality, and survival remains to be determined.

Median average tacrolimus levels in patients with or without acute GVHD

Week	Acute GVHD	No acute GVHD	P
1	13.6	13.1	0.97
2	11.1	12.1	0.21
3	8.9	10.9	0.20
4	7.3	8.5	0.031
5	8.6	10.2	0.057
6	7.8	9.1	0.06
7	7.5	8.1	0.25