

sequence based typing (SBT) and Luminex™ sequence-specific oligonucleotide primed PCR (PCR-SSO). Both methods showed a homozygous phenotype for HLA class I loci (A, B, C) and heterozygosity for HLA class II alleles (DRB1, DQB1). After consolidation, increasing minimal residual disease (MRD) measured by a close NPM1, monitoring was observed in September 2013 and the patient was scheduled for an allogeneic stem cell transplantation. No blasts were present in the peripheral blood of the patient at the time point of molecular relapse. To initiate search for an unrelated donor a confirmatory patient HLA-typing was performed. Due to poor S2 DNA quality a new blood sample (S3) was ordered and used for secondary typing. Whereas HLA class II results were consistent, SBT and PCR-SSO showed discrepant class I typing results when compared to S1, with heterozygosity for all three loci. Consultation of the transplantation center revealed that S1 + S2 were taken while the patient had 84% blasts in the peripheral blood. Heterozygous patient HLA status was finally confirmed by typing of a saliva sample (S4) with SBT and SSO-PCR, respectively. According to the information about S1 (primary diagnosis, peripheral blasts), S3 (increasing MRD, no peripheral blasts) and S4 (blast free material) we assumed that in S1 cells with somatic HLA class I phenotype were highly outnumbered by blasts bearing a complete class I LOH.

In regard to this case we strongly recommend confirmatory typing to be carried out on blast free samples only, or by using a highly sensitive PCR-SSP in case of a substantial amount of blasts in the blood. This should help to prevent the otherwise potentially fatal selection of a highly mismatched donor. As a consequence we established a routinely feedback on the peripheral blast status of the sample on our test request form.

IMMUNE RECONSTITUTION

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The Impact of Leukocyte Dose during Autologous Stem Cell Transplant on Lymphocyte Recovery in Lymphoma Patients

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Background: CD34+ and CD3 cell-doses given during autologous stem cell transplantation (ASCT) can vary between patients (pts) depending on ability to mobilize and cell collection method. Retrospective studies suggest that immune recovery and absolute lymphocyte count (ALC) may be a predictor of overall survival (OS), progression free survival (PFS), and infectious complications in ASCT. However, It is unclear whether there is an optimal CD34+ or CD3 cell-dose that correlates with better outcomes.

Methods: A retrospective analysis of the immune recovery was performed on 24 consecutive lymphoma pts who had undergone ASCT from 1/2012 to 6/2014 at the University of Virginia to determine if a relationship existed between CD34+ or CD3 cell-dose given during ASCT and immune recovery. For each pt, number/type of infections, IgG level, T cell counts (CD4 and CD8), and ALC were collected for the first 100 days post-ASCT. OS and PFS were also assessed for each pt. Cox proportional hazard models were used to estimate the association of cell-dose infused with time to count recovery after ASCT.

Results: Median age was 57 years (range from 28–69 years), and 62% were male. Lymphoma subtypes were 62% B cell lymphoma, 21% Hodgkin and 17% systemic T cell. All Pts were with advance stages and heavily pretreated (median 2 lines). Peripheral blood stem cell grafts had a median CD34 of 6.2×10^6 , TNC of 10.9×10^8 , and T cells of 154×10^6 . Almost half of the pts (n=11) were mobilized with plerixafor with no chemotherapy. Median follow up was 417 days (102–763). The analysis revealed that a higher CD34+ cell-dose given was significantly ($p < 0.05$) associated with higher IgG at day 30 and earlier platelet recovery ($> 50,000$). Higher T cell dose ($> 150 \times 10^6$) was also associated with higher IgG level at day 30, but unlike CD34 dose, CD3 was associated with higher ALC (> 1000 cells/microL) at day 100 ($p < 0.05$). Due to sample size and different disease associated risk factors, effect of cells dose on relapse was not statistically significant. Infection rate (almost half of pts) was similar in high vs. low cell dose. Most of the infections were bacterial and happened early post transplant with the exception of two cases of influenza and CMV. Pts who were mobilized with plerixafor without chemotherapy had a higher dose of CD3 collected compare to pts mobilized with chemotherapy (239 vs. 113, $P = 0.056$).

Conclusion: These preliminary results suggest that CD3+ cell-dose $> 150 \times 10^6$ cells/kg given during ASCT for aggressive lymphoma may be associated with not only better early IgG level and but also with better ALC. However, larger prospective trials are necessary to further define optimal CD3 and CD34+ cell-dose infusion for ASCT in lymphoma pts in order to improve immune recovery and prevent relapse.

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Trends in Bloodstream Bacterial Infections and Resistance Patterns over the Past Decade in Pediatric Allogeneic Hematopoietic Cell Transplant Recipients

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Introduction: Bacterial bloodstream infections (BBSIs) contribute significantly to transplant related mortality (TRM) post allogeneic hematopoietic cell transplant (alloHCT). In 2006, a new central-line associated bloodstream infection (CLABSI) protocol was established at our institution. We report the incidence rate and sensitivities of both Gram negative rod (GNR) and Gram positive cocci (GPC) BBSIs during Pre-CLABSI era (2004-06), CLABSI era (2006-10), and post-CLABSI era (2010-13).

Methods: A retrospective chart review between 2004–2013 was conducted. 100 person-month bacterial infection incidences were calculated and compared by Poisson regression analysis. Patients did not receive prophylactic anti-bacterial antibiotics and piperacillin/tazobactam was started at the onset of fever.

Results: Between 2004–13, 302 BBSIs were identified in 190 patients (mean age 9.97 years). Malignant 111 (58.4%), Non-malignant 79 (41.6%); donor source: Marrow 71 (37.4%), Peripheral Blood Stem Cell 59 (31.1%), Cord blood 60 (31.5%). Conditioning regimens: myeloablative= 86 (45.3%), reduced