SUPPLEMENTARY APPENDIX

Risk factors for Epstein-Barr virus-related post-transplant lymphoproliferative disease after allogeneic hematopoietic stem cell transplantation

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Complete Methods

Patients

In total 1021 patients who underwent SCT at Karolinska University Hospital in Huddinge, Stockholm, between 1996 and 2011 were included in this retrospective analysis of risk factors and clinical outcome for PTLD. This study was approved by the regional ethical committee in Stockholm, 425/97. Some patients in the initial time period have also been included in the two studies [1, 2]. In the majority of patients indications for SCT were hematologic malignancies. Patients with various non-malignant disorders (e.g., bone marrow failures, immunodeficiencies and inborn errors of metabolism) and solid tumors were also included. 287 (28 %) of the patients were children and 734 (72%) were adults. A review of patient charts and the clinical database identified 40 cases of verified PTLD. Characteristics of patients with and without PTLD are displayed in Table 1.

EBV PTLD diagnosis and treatment

The diagnosis of PTLD was made according to the histological criteria reported for B-cell lymphoproliferative states following transplantation [3]. In three patients the diagnosis of PTLD was clinical (adenopathy, mass lesions, fever, unexplained pain etc) and corroborated with computed tomography scans. Thirty-seven patients were diagnosed with both a) PCR of EBV DNA in peripheral blood lymphocytes or sera, together with b) EBV positive biopsies (EBER+ immunohistochemistry).

From January 2003 quantitative PCR was introduced and performed sporadically on patients if suspicion of EBV reactivation. After July 2005 (total of 446 individuals) all patients considered to be high-risk for developing PTLD were screened weekly or bi-weekly for EBV during the first three months post-SCT. Patients considered to be at high-risk, were individuals with; serological EBV mismatch, umbilical cord blood grafts, primary EBV infection close to SCT and lymphoma or chronic lymphocytic leukemia. In patients at low-risk, quantitative PCR was performed on suspicion of EBV reactivation. From July 2005 all patients were treated with rituximab if EBV load was >10 000 copies/mL.

Thirty-five of the patients were treated with rituximab, 3 to 4 doses of 375 mg/m^2. The doses were administrated weekly for four weeks or shorter if serum EBV DNA became negative. EBV specific T cells from the donor or relatives were given to 8 patients and 8 patients were treated with donor lymphocyte infusions.

Conditioning regimen and GVHD prophylaxis

In 402 patients RIC was used. The different protocols applied were; **a**) fludarabine 30 mg/m^2/d for 3-6 days in combination with cyclophosphamide (Cy) 60 mg/kg/d for 2 days (n=80), **b**) 2 × 3 Gy total body irradiation (TBI) and Cy 60 mg/kg/d for 2 days (n=65), **c**) 2 Gy TBI (n=42), treosulphan 14 g/m^2 for three days (n=55) or **d**) 4 mg/kg/d busulphan (Bu) for 2 days (n=160) [4].

Myeloablative conditioning (MAC) was give to 619 patients. MAC consisted of Cy 60 mg/kg/d for 2 days in combination with either; **a**) 7.5-10 Gy single fraction TBI (n=120), **b**) 12 Gy fractionated TBI (n=191) or **c**) 4 mg/kg/d busulphan for 4 days (n=286). Sixteen patients with severe aplastic anaemia and sibling donors received only Cy 50 mg/kg/d for four days. Six patients were treated according to individualized protocols.

Anti-thymocyte globuline (ATG) was given to 705 patients as part of the conditioning with the last dose on day -1. ATG was used in all patients with an unrelated or mismatched donor and in all patients with a non-malignant disease, independent of donor. A few patients with a sibling donor treated with RIC (n=44), were also given ATG. The different types of ATG were: Thymoglobulin (Genzyme, MA, USA)(n=595) in a total dose of 4-10 mg/kg, ATG-Fresenius (Fresenius, AG, Bad Homburg, Germany)(n=22) 20-50 mg/kg, OKT-3 (Ortho Biothec, NJ, USA)(n=48) 25 mg or Alemtuzumab (Genzyme, MA, USA) 30-90 mg. In 21 patients the ATG type were changed due to side-effects.

GVHD prophylaxis consisted of cyclosporine A (CsA) alone (n=7) or in combination with **a**) methotrexate (MTX, n=767), **b**) prednisolone (n=60) or **c**) mycofenolate mofetile (MMF, n=47). Sirolimus and tacrolimus was given to 92 patients and 15 received tacrolimus and MMF [5]. Seventeen patients received a T-cell depleted graft and 7 patients with a syngeneic donor received no GVHD prophylaxis [6]. Nine patients received CsA + MTX and cyclophosphamide on days 3-4 after HSCT [7]. During the first month, blood CsA levels were kept at 100 ng/mL or 200-300 ng/mL when a sibling donor or unrelated donor was used, respectively. In the absence of GVHD, CsA was discontinued after six months in patients with malignancies and 24 months for patients with non-malignant disorders.

Stem-cell source,

The graft source was bone marrow (BM) in 361 cases, peripheral blood (PBSC) in 608 and umbilical cord blood (CB) in 52. Before aphaeresis, stem cells were mobilized with subcutaneous G-CSF daily for 4-6 days in all donors of PBSC [8].

Supportive care,

Supportive care has been described in detail previously [9].

Statistics

Overall survival was calculated using the Kaplan-Meier method and compared with the log-rank test. Survival time was calculated from the day of transplantation until death or last follow-up. The incidence of PTLD was estimated using an estimator of cumulative incidence curves. Death without PTLD was considered as a competing event. Predictive analyses for PTLD were based on the proportional hazard model for sub-distribution of competing risk. Univariate and multivariate analyses were then performed using Gray's test and the proportional sub-distribution hazard regression model of Fine and Gray[10]. A stepwise backward procedure was used to construct a set of independent predictors. All predictors with a p-value below 0.10 were considered and sequentially removed if the p-value in the multiple model was above 0.05. All tests were two-sided. The type I error rate was fixed at 0.05 for factors potentially associated with time-to-event outcomes. Several factors were analysed in the univariate analysis: patient sex and age, HLA-match (A, B, DRB1), GVHD prophylaxis (CsA+MTX vs. other), GVHD II-IV. blood group-compatibility, sex-mismatch, diagnoses (lymphoma vs. other, lymphoid vs. myeloid, malignant vs. non-malignant), disease stage (early vs. late), conditioning (RIC vs. MAC, Bu vs. others, TBI vs. others), stem-cell source (BM, PBSC, CB), G-CSF after transplantation, previous herpes virus infections, EBV-serology before SCT, EBV serological mismatch (recipient+, donor-) and (recipient-, donor+), nucleated and CD34+ cell-dose, ATG and treatment with multipotent mesenchymal stromal cells (MSC). Time-dependent variables were only included if they occurred before the diagnosis of PTLD (minimum interval >1 week). Categorical parameters were compared using χ^2 test and continuous variables were compared using the Mann-Whitney test. Analyses were performed using the cmprsk package (developed by Gray, June 2001), Splus 6.2 software and Statistica software. As there is a risk for significance by random when performing multiple comparisons we also present the results from multivariate analysis after a simple form of Bonferroni corrections.

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