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Comparison of immune reconstitution after allogeneic vs. autologous stem cell transplantation in 182 pediatric recipients

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ABSTRACT

Background: Hematopoietic stem cell transplantation (HSCT) is a life-saving procedure for children with a variety of malignant and non-malignant conditions. However, even if immune reconstitution after HSCT has been studied in extensively, until now data on the comparison of immune reconstitution after autologous vs. allogeneic HSCT is scarce, but might provide important clinical implications.
Patient and methods: We examined immune reconstitution (T-, B-and NK-cells) at defined time points in 147 children who received 182 HSCT. Differences in the time course of immune reconstitution were analyzed in autologous vs. allogeneic HSCT.
Results: We identified a quicker immune reconstitution in the T-cell compartment, especially in the CD4 and naïve subset after autologous HSCT, whereas recipients of allogeneic transplants showed a higher TCRgd proportion. B-cell reconstitution showed a delayed immune reconstitution after allogeneic HSCT in the first two years after HSCT. However, a reconstitution of all lymphocyte subsets after HSCT could be achieved in all patients.
Conclusion: Children undergoing a HSCT show a different pattern of immune reconstitution in the allogeneic and autologous setting. This might influence the outcome and should affect the clinical handling of infectious prophylaxis and re-vaccinations.

1. Introduction

Hematopoeitic stem cell transplantation (HSCT) has become an established therapy strategy for various diseases. There are two different approaches of HSCT: autologous and allogeneic. High-dose chemotherapy followed by autologous stem cell transplantation is designed to eliminate higher numbers of chemosensitive residual tumor cells. The efficacy of this treatment modality is based on the chemosensitivity of the malignant tumor cells with a steep dose–response curve as well as the choice of drugs for which bone marrow impairment is the dose-limiting toxicity [1]. To rescue the hematopoietic system, patients receive cryopreserved autologous hematopoietic stem cells. Autologous HSCT has become an established treatment for distinct advanced pediatric tumors as brain tumors, neuroblastoma and sarcoma [2–4]. In contrast the autologous setting, allogeneic HSCT represents a unique immunotherapeutic modality in which donor-derived T-cells exert a graft versus host response, which when directed at host-derived malignancy, can result in long-lasting immunologic tumor/leukemia control [5]. However, when this phenomenon extends to normal host tissue, it results in the single most dreaded complication of this procedure, graft versus host disease (GVHD), which causes a significant morbidity and mortality after allogeneic HSCT.

Immune reconstitution after HSCT has been studied in various studies [6–8]. However, the comparison of autologous and allogeneic reconstitution seems to be of clinical interest because differences might provide (I) new insights in the pathophysiologic basis of graft versus tumor effect, (II) in the pathophysiologic basis of GvHD, (III) might be used as prognostic marker and (IV) therefore might have clinical implications. In the presented study we analyzed immune reconstitution after 182 pediatric stem cell transplantations with regard to clinical characteristics and

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differences in immune reconstitution. These will have clinical implications in tailored prophylactic regimes.

2. Methods

2.1. Flow cytometry analysis

Subsets of peripheral blood lymphocytes were analyzed by flow cytometry. Anti-CD3-phycoerythrin (PE), anti-CD19-fluorescein isothiocyanate (FITC) and anti-CD16/CD56-FITC monoclonal antibodies were used to identify T-, B and NK-cells, respectively. T-cell subsets were further characterized by 4-colour flow cytometry to measure the expression of CD4, CD8, TCRab, TCRgd, CD45RA and CD45RO. All monoclonal antibodies were purchased from Becton-Dickinson (Heidelberg, Germany). Flow cytometry was performed on a FACS Calibur, and data analyzed with CellQuest Software (Becton–Dickinson, Heidelberg, Germany).

2.2. Statistical analysis

Student's paired *t*-test for mean differences was used to analyze data for levels of statistical significance among two groups (e.g. autologous vs. allogeneic). Kaplan–Meier Plots were used for survival probability. Statistical correlations between parameters were analyzed using a bivariate fit model. Additional multivariate analysis was performed against conventional outcome predictors (age, gender, disease status, and stem cell dosage) between multiple groups by using repeated measures analysis of variance (ANOVA) and Bonferroni's multiple comparison test. For timely courses a hierarchic linear analysis were used. In all statistical applications a p-value of <0.05 was considered to indicate a statistical significant. For statistical analysis SPSS software was used.

3. Results

3.1. Patient characteristics

We analyzed 2566 immune states of 147 patients with 182 stem cell transplantations with regard to relapse free und overall survival. Furthermore, we compared autologous and allogeneic transplanted patients according to patterns of immune reconstitution. The study was approved by the ethics committee of the University Hospital Würzburg (study #133/04)). Informed consent was obtained from all parents according to institutional guidelines.

Patients' immune states were analyzed to defined time points: before HSCT, day 0–30, 30–60, 60–180, 180–365, 1–2 years, 2–5years and over 5 years. This time points are commonly used land marks in clinical practice.

The median age of the patients was 3077 ± 2433 days. Of these patients 40% were female, 60% male. 58% (106 patients, median age 8.9 ± 7.0 years) of the HSCT were allogeneic, 42% (76 patients; median age 6.2 ± 6.3) were autologous. 76% (138) of patients received one transplantation, 20% (36) two, 4% [7] three and 0.5% [1] four HSCT. 30% (55 patients; 100% allogeneic) of the patients were transplanted because of leukemia, 6% (11 patients; 6 allogeneic) because of lymphoma, 24% (43 patients; 13 allogeneic) because of solid tumors, 23 (41 patients; 98% autologous) because of CNS tumors and 18% (32 patients; 31 allogeneic) because of other, non-malignant diseases as immune deficiency, MDS or congenital blood abnormalities.

We did not find significant differences between allogeneic and autologous transplanted patient regarding sex, age, follow up and total number of transplanted cells as well as for remission status before HSCT, outcome and days until relapse. The distribution between stem cell sources (peripheral stem cells vs. bone marrow) was significant different as almost all patients with autologous stem cell transplantation received peripheral progenitor stem cells. However, as only a minority of autologous grafts was manipulated a statistical sub-analysis of these patients was not possible, but an exclusion of those patients does not change the described results. Furthermore, there was no difference between the first and the second autologous reconstitution regarding T-cell recovery. A prior autologous HSCT before allo-HSCT did not influence significant the T-cell recovery after allo-HSCT.

Regarding survival we found a slightly higher relapse rate in patients who had received autologous transplants and could not find differences between event-free-survival and overall survival between those two groups. However, age could be defined as a risk factor for relapse and death. In the cox regression analysis we could show that increasing age (every 1 year) increased the risk of death at about 5% (HR: 1.052, CI: 1.014–1.092; $p = 0.007$). For further details regarding patient characteristics see Table 1.

3.2. Immune reconstitution

By comparing immune reconstitution between autologous and allogeneic hematopietic stem cell recipients we could document a faster increase of lymphocytes in the autologous group, until 60 days after HSCT.

Regarding T-cells, we detected higher CD3 and CD4 positive cell counts (absolute and relative) until day 60 in autologous transplanted patients. The timely course of CD3-reconstitution in patients with autologous HSCT differed significantly from allogeneic transplanted patients ($p < 0.01$, suppl. Fig. 1). Furthermore, significant more naïve T-cells and consecutively less memory T-cells were found until one year after HSCT in patients after autologous transplantation, which implicate a faster regeneration of the naïve compartment in autologous transplantations. Interestingly, we could also confirm a higher proportion of TCRgd in allogeneic patients in the first post-HSCT year.

Regarding NK-cells, in allogeneic HSCT, but not in autologous HSCT, NK-cell reconstitution takes place before reconstitution of B- and T-cells (during the first year post-HSCT). Absolute NK cell counts are not different between the groups, though. But the timely course of reconstitution differed between the groups: autologous transplanted patients showed an earlier increase ($p < 0.01$).

As to B-cells, from day 30 until two years after transplantation autologous transplanted patients showed a much faster reconstitution of B-cells, which approximate after two years only. This could be confirmed by the linear hierarchic analysis, which showed also an earlier peak in autologous HSCT patients. For further details see Table 2 and Supplementary Fig. 1.

In addition, we performed a multivariate analysis and could not detect an association between immune reconstitution and superior survival in the both cohorts. In the allogeneic cohort, we could confirm common T-cell recovery aspects, which are already described in the literature as slower recovery of T-cell subsets after T-cell depletion and after BM source as transplantation as well as the influence of immunosuppressive medication in case of GvHD. As it was not the focus on this study, data are not shown, however it confirms its representative character.

4. Discussion

Over the last decades immune reconstitution has emerged as a general concern in HSCT. Interest in immune development after transplantation is due to the high rate of susceptibility to opportunistic (life threatening) infectious complications and the relationship of delayed immune recovery to relapse of malignant diseases [8–11]. The problem of delayed or impaired immune

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