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Methods and biomarkers for outcome prediction after allogeneic hematopoietic stem cell transplantation

AKADEMISK AVHANDLING

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ABSTRACT

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potent immunotherapeutic procedure but its usability is limited by a high risk of serious complications. A prerequisite for timely initiation of preventive measures is the availability of predictive methods. This thesis aims to evaluate techniques that may potentially be used to assess the risk of some of these complications on the individual level.

Defective function of the pattern recognition receptor NOD2, due to naturally occurring gene polymorphism, has been indicated as a risk factor for graft-versus-host disease (GVHD). We investigated the potential influence of NOD2 on clinical outcome after HSCT in a retrospective study of 198 patients. Contrary to previous reports, we found no association between NOD2 mutations and acute GVHD, transplant-related mortality (TRM) or overall survival. We conclude that NOD2 genotyping is not a pertinent analysis before HSCT.

Leukemic relapse is a major cause of death after HSCT. Donor lymphocyte infusion (DLI) is one of the few therapeutic options remaining in these situations. Previous studies have shown varying results regarding treatment efficacy against acute leukemia. We aimed to investigate if the use of molecular techniques for relapse monitoring could improve the clinical outcome after DLI. Through retrospective analysis of 118 patients treated with DLI we showed that those with acute leukemia or myelodysplastic syndrome, who had received DLI based on the result of molecular methods, had a better survival rate than those treated during hematologic relapse (16% vs. 43%, $p < 0.006$). Non-hematological relapse and chronic GVHD were identified as independent predictors for response to DLI in multivariate analysis. The overall incidence of severe acute GVHD was only 8.5% and was acceptable (14%) in the cohort treated before 100 days post-HSCT. Our conclusion is that early administration of DLI to patients with acute leukemia, based on changes in cell lineage-specific chimerism and MRD analysis can significantly improve relapse-free survival after HSCT.

Adaptive immunity is compromised after HSCT, mainly due to defective T-cell function. Reconstitution of the T-cell population is dependent on thymic function. We quantitatively assessed thymic function in 260 patients during a two-year period following HSCT. Levels of T-cell receptor excision circles (TRECs) in separated T-cells were measured with real-time quantitative PCR and used as a surrogate marker for thymic function. We found that low TREC levels 3-6 months after HSCT was correlated to inferior survival, increased TRM, and higher incidence of cytomegalovirus reactivation. We could also for the first time show that the use of bone marrow grafts and anti-thymocyte globulin had a negative effect on TREC levels, as did mesenchymal stromal cells when co-infused with umbilical cord blood grafts. We conclude that TREC analysis appears to have a high predictive value concerning outcome parameters after HSCT, and that factors related to the transplant procedure may significantly affect thymic function.

Finally, we present the results of a prospective pilot study in which we sought to design a functional, individualized strategy for assessing the risk of acute GVHD. Peripheral blood mononuclear cells were collected from patients and donors before HSCT and co-cultured in a mixed lymphocyte reaction (MLR) in the GVHD direction. Cells were phenotypically characterized by flow cytometry before and after MLR. We found that donors corresponding to patients who later developed acute GVHD grades II-IV had significantly higher levels of $\gamma\delta$ T-cells and NKT-cells in peripheral circulation. We could also demonstrate a possible correlation between a high proportion of naïve CD4⁺ T-cells in the allogeneic MLRs and occurrence of acute GVHD *in vivo*. We conclude that flow cytometric analysis of donor cells for phenotype and allogeneic reactivity may be used to predict acute GVHD before HSCT.