Description of statistical methods

Baseline characteristics

Continuous variables are described by mean and standard deviation and compared between groups by analysis of variance, or, in case of skew distributions, described by median, 25th and 75th percentiles and compared by Kruskal-Wallis-tests. Categorical variables are described by frequencies and percentages and compared by chi-square tests.

Survival analysis

Our study aimed at assessing the interaction of the associations of donor age and immunosuppressive therapy (IT) at day 90 after engraftment (including calcineurin inhibitors: CNI+, not including calcineurin inhibitors: CNI-) on actual graft survival (treating graft loss or death as event), functional graft survival (censoring patient death) and patient survival (censoring graft loss). For all types of survival analysis, we considered only the period from engraftment until graft loss (censoring for later death) or death with functioning graft. The distribution of these outcomes was illustrated by Kaplan-Meier curves, stratified for immunosuppressive therapy and categorized donor age (group I: 35 years or less, group II: 35-49 years, group III: 49-65 years, group IV: 65 years or more). In our study we were primarily interested in patients older than 65; therefore this relatively small number of patients constituted a separate group (group IV). The remaining patients were divided into three approximately equally-sized age groups.

Multivariable Cox proportional hazards regression was used to adjust the interaction effect of donor age and immunosuppressive therapy at day 90 after engraftment for the potentially confounding covariates year of engraftment, time on dialysis before engraftment, recipient age, presence of cardiac or vascular disease, serum cholesterol level, mean arterial blood pressure, and diabetes status as well as type of donor (cadaveric or living). All these covariates entered the analysis with their values measured at time of engraftment, since a confounder is required to be causally independent of the effect of interest. In a second analysis, we additionally included biopsy confirmed acute rejection, delayed graft function and log serum creatinine level at day 90 after engraftment. Since these variables are measured after engraftment, we cannot rule out that they are already affected by donor age and immunosuppressive therapy. Therefore, these variables are not considered as confounders but rather assumed to mediate the effect of our target variables on the outcome. For the analysis of functional graft survival, we additionally included sum HLA mismatches, panel reactive antibodies (categorized into 0-10, 10-30 and 30-100) in our model. Since we are studying the interaction of donor age and type of IT, results are presented by adjusted hazard ratio (AHR) estimates referring to the

comparison of patients with different donor age within each IT group, and also by hazards ratio referring to the comparison of CNI+ and CNI- patients within each donor age group.

Potential interactions of any variables with donor age or immunosuppressive therapy were checked for statistical significance. The assumption of proportional hazards was assessed by evaluating interactions of each variable with log of time for statistical significance. Significant interactions with log of time led to the inspection of scaled Schoenfeld residuals, partitioning of the time axis based on the course of the Schoenfeld residuals and the subsequent estimation of two piecewise constant hazard ratios for such variables. The non-linearity of the donor age effect was taken into account by first, creating four groups according to donor age as outlined above, and second, by depicting the hazard ratio of donor age on survival graphically using restricted cubic splines using the RCS macro available at www.meduniwien.ac.at/msi/biometrie.
Median follow-up time (25^{th}, 75^{th} percentiles) was computed using the Kaplan-Meier method with inverse status indicator.

Handling of missing data

Missing values are a problem commonly encountered in studies like ours. Particularly in the analysis of multivariable models one may end up with 30-40% complete observations even if each variable has no more than 5-10% missing values. Therefore, we applied multiple imputation to our data set, which is currently the state-of-the-art technique to deal with missing values. We used SAS/PROC MI to generate 20 completed data sets and SAS/PROC MIANALYZE to properly combine Cox regression results from each of the completed data sets (http://support.sas.com/rnd/app/da/new/dami.html). Generally, results obtained by multiple imputation can be biased if covariate values are not missing just randomly. Therefore, we assessed the sensitivity of our initial results on the assumption of randomly missing data by artificially doubling the amount of missing data in each covariate in two ways: first, purely at random and second, only in subjects with covariate values higher than the median. The results from both analyses were then compared.
All statistical analyses were done using the SAS System V9.1 (SAS Institute Inc., 2003, Cary, NC, USA). P-values lower than 0.05 were considered as indicating statistical significance. Kaplan-Meier curves were drawn using the R/survplot program of Frank Harrell's Design package (cran.r-project.org).