TITLE PAGE

Longitudinal dose and type of immunosuppression in a national cohort of Australian liver, heart, and lung transplant recipients, 1984-2006

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Running head: Immunosuppression changes by organ type

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Abstract

Unconfounded comparative data on the type and dose of immunosuppressive agents among solid organ transplant recipients is sparse, as is data on longitudinal immunosuppressive therapy since transplantation. We addressed this issue in a population-based cohort of Australian liver (n=1895), heart (n=1220), and lung (n=1059) transplant recipients, 1984-2006. Data on immunosuppressive therapy was retrospectively collected at discharge, 3 months and 1, 5, 10 and 15 years after first transplant. We computed unadjusted and adjusted estimates for the association between the type and dose of immunosuppressive therapy and organ type. After adjustment for confounders, use of induction antibody and maintenance corticosteroids was more common in heart and lung compared to liver recipients (p<0.001), and antibody therapy for rejection more common in liver recipients (p<0.001). Liver recipients were more likely to receive calcineurin inhibitor monotherapy, with or without corticosteroids, compared to heart and lung recipients (p<0.001). Liver recipients consistently received lower doses of azathioprine than heart and lung recipients (p<0.001). These differences in immunosuppression may partly explain variations in immunosuppression-related morbidity by transplanted organ, for example malignancy risk. Longitudinal changes in the type and the dose of immunosuppressive therapy over time since transplantation also demonstrate the need for time-dependent data in observational research.

Key Words: immunosuppression, dose, transplantation, cohort

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INTRODUCTION

Immunosuppressive therapy is critical to patient survival in solid organ transplantation but is associated with a range of side-effects including toxicity, infection, diabetes, cardiovascular disease, and cancer. Immunosuppressive agents are carefully chosen and titrated to achieve a balance between graft function and risk of harm from over-immunosuppression. Patient characteristics determine the initial type and dose of immunosuppressive agents, while the occurrence of side-effects, new evidence regarding clinical efficacy, as well as the availability of novel therapies and enrolment in clinical trials influence decisions to switch agents following transplantation.

Clinical practice guidelines and OPTN/SRTR data indicate key differences in immunosuppression practice by organ type. For example, the use of induction antibody is less common in liver compared to heart and lung transplantation (1). Further, calcineurin inhibitor (CNI) monotherapy with corticosteroid withdrawal is generally only considered in highly selected low-risk heart transplant recipients, whereas it is recommended for a substantial proportion of liver recipients at one year post-transplantation(1-5). However, there has been no comparison of immunosuppressive drug regimen and dose by organ type taking into account important demographic and clinical differences between patient groups. Moreover, there is no longitudinal data showing the extent of changes in immunosuppressive therapy over time since transplantation.

To better understand the potential role of immunosuppressive therapy in immunosuppression-related morbidity, we compare the type, dosage, and combinations of immunosuppressive agents used by population-based cohorts of Australian liver, heart, and lung transplant recipients (1984-2006).

PATIENTS AND METHODS

Study population

We performed a retrospective population-based cohort study of Australian liver (n=1926), heart (n=1518), and lung (n=1200) recipients transplanted 1984-2006. Transplant recipients were registered on the Australia and New Zealand Liver Transplant Registry (1985+) or the Australia and New Zealand Cardiothoracic Organ Transplant Registry (1984+). We obtained ethical approval from all relevant institutions.

Data collection

The two registries prospectively collected demographic and some clinical data including organ type, primary transplant indication, transplant date, age at transplant, sex, and date of death. We supplemented these records with data abstracted from medical records at all Australian transplantation units (18 units at 12 hospitals). We ascertained recipient's weight and prescribed immunosuppressive agents around the time of transplantation (i.e. induction therapy), at 3 months and 1, 5, 10 and 15 years after transplantation (i.e. maintenance therapy), and during episodes of treated rejection.

We recorded use of antibodies including interleukin-2 receptor antibodies (IL-2Ra; basiliximab, daclizumab), and T-cell depleting antibodies, both monoclonal (muromonab-CD3) and polyclonal (anti-thymocyte/anti-lymphocyte globulins, ATG/ALG), during induction therapy and rejection episodes. We documented the receipt of individual immunosuppressive agents and their doses (mg/day or mg/kg/day).

We collected use of the CNIs cyclosporine and tacrolimus, the antiproliferatives azathioprine, mycophenolate (mycophenolate mofetil or enteric-coated mycophenolate sodium), and the mTOR inhibitors (mTORi) sirolimus and everolimus. We did not distinguish the different formulations of ATG/ALG, cyclosporine or tacrolimus. We collected use of maintenance oral corticosteroids and intravenous corticosteroid pulse therapy at induction and at up to three rejection episodes, but we did not differentiate the type (cellular or humoral) or severity of acute rejection.

Data preparation

We followed-up organ recipients from the date of first transplant until re-transplantation, 80 years of age, death, or the end of follow-up (31 December 2006), whichever occurred first. In Australia, tacrolimus and mycophenolate were approved by the Therapeutic Goods Administration in 1997, and sirolimus and everolimus in 2002 and 2005, respectively. However, tacrolimus and mycophenolate were used from 1995, and sirolimus from 1998, in clinical trials in Australia. We therefore categorized transplant period according to the broad availability of immunosuppressive agents for our cohort; 1984-1994, 1995-1997, and 1998-2006. We included all drug use, whether it was approved use, approved only under a special access scheme (Section 100 of the Australian Pharmaceutical Benefits Scheme), or in clinical trial.

Recipient weight was used to standardize the dose of individual agents to mg/kg/day. As weight was not recorded at all observation times we imputed the missing values from a linear mixed model including age at transplantation, sex, and weight at other observation times for the same individual (6, 7).

We converted all doses of mycophenolic acid to equivalent doses of mycophenolate mofetil (8). We reviewed the immunosuppressive therapy data and identified potential outlying dose values (i.e. >1.5-times the interquartile range (IQR) from the lower or upper quartile dose). Such values were changed to missing, unless there was a clear and logical pattern within the individual record, in which case they were retained. As the type and dose of individual immunosuppressive agents was not recorded at all observation times the missing data were imputed, where possible, by carrying the last observation forward to the next follow-up point. This is the conventional method for imputing longitudinal medication data, especially a combination of binary (drug type) and continuous (drug dose) data where most recipients received more than one immunosuppressive agent, with the dose of one agent likely to be related to the dose of the other due to drug-drug metabolic interactions (9).

Data analysis

We compared the use of antibodies for induction, antibodies for rejection, corticosteroid therapy, and each maintenance immunosuppressive agent by organ type, and other recipient subgroups (e.g. age, sex) using Pearson's chi-square test or Fisher's exact test as appropriate. We used the Kruskal-Wallis non-parametric test to compare the median dose of each immunosuppressive agent for recipient subgroups; we applied a post-hoc multiple comparison procedure for non-parametric pairwise differences following a significant result (10). We demonstrated the change in dose by time since transplantation by plotting box-and-whisker plots; time trends were evaluated using the Mann-Kendall trend test.

We wanted to study the association between organ type and the receipt of induction antibody, antibody rejection, each maintenance immunosuppressive agent (both type and dose) and the number of agents (monotherapy vs. combination therapy). We therefore needed to consider potential confounding factors that also varied by organ type and affected immunosuppression, such as recipient age at transplantation, sex, race, history of dialysis, history of diabetes, and transplant year. Using logistic regression we computed unadjusted and adjusted estimates for the receipt of immunosuppression by organ type, and retained covariates with p-values less than 0.20. For corticosteroids the maximum likelihood estimation did not converge due to quasi-complete separation of data, thus adjusted estimates were not possible. We modelled the adjusted median dose of each immunosuppressive agent by organ type using quantile regression (11). Analyses were carried out based on both original and imputed data.

Analyses were performed using SAS 9.3 (SAS Institute, Inc., Cary, USA), STATA 11.0 (StataCorp LP, College Station, Tex) or R statistical software version 3.1.3 (R Development Core Team, 2014).

RESULTS

Cohort characteristics

We excluded recipients with no retrievable transplantation medical records (n=89) and those with some clinical but no immunosuppression therapy data (n=381; Figure 1). The eligible cohort consisted of 4174 transplant recipients, 1895 (46%) liver, 1220 (29%) heart, and 1059 (25%) lung, of whom 405 (302 liver, 83 heart, and 20 lung) were pediatric (0-15 years at transplant). The median follow-up time was 5.6 years (IQR 2.4-10.2). We found significant differences in the distribution of transplant year and recipient and donor characteristics among organ types (Table 1).

Recipient weight was not recorded at 27% of observation times. We found no difference in median recipient weight at each observation time before and after imputation (data not shown). The type and dose of individual immunosuppressive agents was missing at 18% and 31% of all observation times, respectively. The prevalence and median dosages of individual agents were comparable before and after imputation (data not shown). Although imputation attenuated the results obtained based on the original data, the statistical significance of the findings remained and the conclusions were unchanged (data not shown). The results presented are based on imputed data. Seventy-two percent of the cohort (n=3019) had complete data on immunosuppressive agents for the entire follow-up time.

Induction therapy

Induction antibody was most common for heart recipients (42%), adults (23%), and those transplanted in the earliest period (1984-1994; 37%). Induction antibody use was also more common in liver recipients with alcoholic liver disease compared to other primary indications and in those with a history of dialysis and intravenous corticosteroids (data not shown). Use of ATG/ALG and muromonab-CD3 decreased substantially after 1984-1994 and IL-2Ra was the most common induction antibody therapy in the latest period (1998-2006; 11%). After adjustment by the above confounders, induction antibody therapy during this recent period was significantly more common in

heart (odds ratio, OR 8.22, 95%CI 5.18-13.2) and lung (OR 5.87, 95%CI 3.78-9.21) compared to liver recipients.

Therapy for acute rejection

Treatment for acute rejection was most common for liver recipients (44%), pediatric patients (49%), and those transplanted in the earliest period (1984-1994; 50%). Intravenous corticosteroid pulse was the most frequent therapy (86% of those treated and 29% of all transplant recipients); only 8% of transplant recipients received antibody therapy, most commonly liver recipients (10%). The use of antibodies decreased significantly over time, from 17% in 1984-1994, to 9% in 1995-1997, and 4% in 1998-2006 ($p<0.001$). Antibody rejection therapy was more common in recipients who were younger, female, had no intravenous corticosteroids, and those who did receive antibody induction therapy (data not shown). After adjustment by the above confounders, antibody rejection therapy was significantly more common in liver (OR 1.84, 95%CI 1.27-2.70) and lung (OR 2.19, 95%CI 1.29-3.68) compared to heart recipients.

Maintenance immunosuppressive therapies

At least 85% of the cohort received oral corticosteroids 3 months after transplantation. Receipt of corticosteroids decreased over time from transplantation only for liver recipients; 34% of liver recipients were corticosteroid-free 5 years after transplantation, compared to 28% of heart and 1% of lung recipients. In unadjusted analyses, compared to heart and lung, liver recipients were significantly less likely to use corticosteroids at all follow-up times ($p<0.001$, data not shown).

We observed the expected changes in maintenance immunosuppressive regimens by transplant era, from predominantly cyclosporine and azathioprine in 1984-1994 to a more varied mix of regimens and single agents in 1998-2006 (Supplementary Figures 1-4). We found marked differences in maintenance immunosuppressive regimen by organ type, year of transplant, and time since

transplantation (Figures 2-4). Overall, the use of cyclosporine declined over time after the introduction of tacrolimus. Similarly, azathioprine use decreased and mycophenolate increased over time, although the extent of these changes was greater than for the CNIs.

Across all periods, a greater proportion of liver recipients received CNI monotherapy than heart and lung recipients at all follow-up times ($p<0.001$, data not shown). CNI monotherapy was less common in recent years and during 1998-2006, 35-50% liver, 3-4% heart and 10% lung recipients received monotherapy. There was also variation by age, sex, race, primary indication, dialysis history, diabetes history, antibody induction therapy, and recipient CMV serostatus at transplantation (data not shown). After adjustment by these confounders, liver recipients were more likely to receive monotherapy compared to heart and lung recipients at 3 months, 1 year and 5 years post-transplantation ($p<0.001$).

The unadjusted frequency of use of individual immunosuppressive agents and drug combinations over time since transplantation by organ type for the three periods (1984-1994, 1995-1997 and 1998-2006) are shown in Figures 2-4. Overall, regimens were more likely to change with increasing time since transplantation. For patients transplanted in the most recent period (1998-2006), after adjustment for age, sex, race, transplant year, primary indication, dialysis history, diabetes history, antibody induction therapy, and recipient CMV serostatus at transplantation, lung and heart recipients were more likely to receive cyclosporine at 3 months (OR (95%CI): 17.0 (8.67-35.2) and 11.2 (5.57-23.6), respectively), 1 year (9.45 (4.84-19.1) and 9.08 (4.49-18.9)) and 5 years (3.34 (1.21-9.62) and 7.17 (2.58-21.4)) compared to liver recipients. Heart recipients were also more likely to receive mycophenolate (6.04 (3.01-12.2), 6.76 (3.27-14.2) and 9.13 (3.26-26.9) at 3 months, 1 year and 5 years, respectively) and lung recipients azathioprine (2.52 (1.43-4.54), 3.30 (1.78-6.17) and 4.48 (1.57-13.4) at 3 months, 1 year and 5 years, respectively) than liver recipients. Liver recipients, on the other hand, were more likely to receive tacrolimus compared to heart and lung recipients at 3 months,

1 year and 5 years (p<0.001). There were no differences between heart and lung transplant recipients. All of these differences were maintained when only adult recipients were considered.

Dose of immunosuppressive agents by time since transplantation

The unadjusted median dose of several immunosuppressive drugs decreased with increasing time since transplantation, particularly the first 5 years (Supplementary Figures 5-6). For the entire cohort the reduction in median dose over 5 years was around 50% for CNIs and 20-30% for antiproliferative agents. Dosages were stable between 5 and 10 years, except for cyclosporine, which continued to decline (p<0.05). Between 3 months and 10 years after transplantation the median dose of cyclosporine and mycophenolate significantly decreased for all transplanted organs (p<0.05), the median dose of azathioprine significantly declined for heart and lung transplant recipients (p<0.001), but not liver, and the median dose of tacrolimus significantly declined for liver and lung recipients (p<0.05) but not heart (Supplementary Figures 5-6).

Dose of immunosuppressive agents by recipient subgroup

The unadjusted median dose of several individual immunosuppressive agents differed significantly by recipient age, sex, year of transplant, and primary indication (data not shown). Overall, higher dosages per kg were received by pediatric compared to adult recipients (and their corresponding primary indications), females compared to males, and those transplanted during the early compared to the latest period.

Figures 5-7 and Supplementary Table 1 show the median dose of immunosuppressive agents by transplanted organ before and after adjustment for potential confounders at 3 months, and 1 and 5 years after transplantation. After adjustment, compared to heart transplant recipients, liver recipients received a lower dose of azathioprine at all follow-up times (p<0.001), a lower dose of mycophenolate at 3 months and 1 year (p<0.001), a higher dose of cyclosporine at 5 years (p<0.001), and no

difference in tacrolimus dose. After adjustment, compared to lung transplant recipients, liver recipients received a lower dose of azathioprine ($p<0.05$) at all follow-up times, a lower dose of tacrolimus at 1 year ($p<0.05$), a higher dose of cyclosporine at 5 years ($p<0.05$), and no difference in mycophenolate dose. Compared to lung recipients, heart recipients received a lower dose of cyclosporine at 1 year ($p<0.05$), a higher dose of azathioprine at 1 and 5 years ($p<0.05$), and no difference in mycophenolate or tacrolimus dose.

DISCUSSION

We have documented the longitudinal use and dosage of immunosuppressive agents for a national population-based cohort of solid organ transplant recipients. As expected, with increasing time since transplantation the median dose of most individual agents declined, and regimens were more likely to change. Liver recipients were less likely to receive corticosteroids than heart and lung recipients. Our novel approach, adjusting for potential confounders, demonstrated significantly lower use of induction antibodies in liver compared to heart and lung recipients, and significantly more common antibody rejection therapy in liver and lung compared to heart recipients. Adjusted models also showed liver recipients were more likely to receive CNI monotherapy, with or without corticosteroids, compared to heart and lung recipients. Moreover, liver recipients consistently received lower doses of azathioprine compared to heart and lung recipients. These statistically and clinically significant differences in iatrogenic immunosuppression among organ recipients may contribute to differences in post-transplantation morbidity by transplanted organ, for example, a lower incidence of cancer in liver compared to heart and lung transplant recipients, as observed in this cohort (12).

There are no prior published comparisons of immunosuppressive drug dosages by organ type. We comprehensively mapped the use of immunosuppressive agents over time since transplantation and by period, by organ type. Minimizing the degree of immunosuppression is the goal in long-term maintenance therapy, and this is achieved by reducing the number and/or the dosage of

immunosuppressive agents. We found that monotherapy was more commonly achieved in liver compared to heart and lung transplant recipients. We showed liver recipients received lower doses of azathioprine compared to heart and lung recipients at all follow-up times. There was no consistent pattern for CNIs, but liver recipients used a higher dose of cyclosporine compared to heart and lung transplant recipients at 5 years. On balance, liver recipients were shown to use a lower overall degree of immunosuppression than heart and lung transplant recipients.

We found maintenance immunosuppression regimens used in liver transplantation were consistently different to those used in heart and lung transplantation. CNI monotherapy, first cyclosporine and increasingly tacrolimus, was used by around 40% of our liver cohort at any point in time. CNI-free regimens were rarely observed. Furthermore, prior to 1998 the uptake of tacrolimus in liver transplantation exceeded that in heart and lung transplantation, and after 1998 tacrolimus-based regimens were used by the majority of liver recipients. Corticosteroid withdrawal was also more common in liver transplantation. These patterns and trends are in alignment with data from the OPTN/SRTR in the US (1, 3), however, we did not see a decline in azathioprine in favor of mycophenolate.

We showed a decline in the use of cyclosporine and azathioprine for heart transplantation since 1998, and an increase in tacrolimus- and mycophenolate-based regimens, consistent with international clinical practice trends (1, 13). In agreement with United States practice (1), we found changes in regimen during the latest period (1998-2006) occurred throughout follow-up but predominantly during the first year.

Maintenance immunosuppressive therapy was very similar for lung and heart transplant recipients prior to 1998. Since 1998, use of tacrolimus-based therapies increased, more so in lung compared to heart recipients, in line with international trends (1, 14). During the latest period (1998-2006), the

most common regimen for lung transplant recipients at 1 year was cyclosporine-based, in contrast to international data during 2002-2011 showing majority use of tacrolimus-based regimens (14). However, by year 5, there was approximately equivalent use of cyclosporine- and tacrolimus-based regimens. In agreement with United States practice (1), during the latest period we observed the greatest variation in regimens over time since transplantation among lung transplant recipients, reflecting their higher incidence of late infections and chronic rejection, and the implementation of trial evidence on clinical efficacy.

Our finding of higher adjusted rates of antibody induction in heart and lung compared to liver transplant recipients is consistent with unadjusted international and United States transplant registry data (1, 3, 15, 16). Overall, one third of recipients experienced at least one treated episode of acute rejection, more commonly in liver compared to heart and lung recipients. These observations are also comparable with international data for rejection during the first year after heart (30%; 2003-2008) (13), lung (34%; 2004-2011) (14), and liver transplantation in the United States (43%; 1998-2003) (17). In addition, we found a higher rate of rejection in pediatric heart recipients, as previously reported (13). In our data the timing of the acute rejection was not known, but the majority are expected to have occurred within one year of transplantation (3, 13, 18-20). As reported internationally (1, 3, 13), we observed a reduction in the incidence of acute rejection over time, a trend attributed to a wider range of immunosuppressive therapies, allowing tailoring of therapy to the individual. Whilst a number of factors related to transplantation including surgery, selection of donors and recipient management have changed over time, reductions in complications and mortality are predominantly attributed to improvements in immunosuppressive regimens and dosing over time.

The key strengths of our study are the population-base and the adjusted comparison of immunosuppressive agents and dosages by organ type. The transplant registries included all recipients, thereby avoiding selection bias. As we had data on recipient and donor characteristics that

may influence the choice of immunosuppressive agents, we were able to compare organ groups taking into account potential confounding factors. On the other hand, several limitations must also be considered. Being a retrospective study, we were reliant on the availability and quality of medical records. We had missing data, to a greater extent for heart and lung compared to liver transplant recipients. As we used the last observation carried forward approach to missing data, we are likely to have underestimated changes in immunosuppressive regimen over time, and overestimated the dose with increasing time since transplantation. Reassuringly however, we observed no notable differences between the analysis carried out with the original and imputed data. We did not identify clinical trial participants or collect immunosuppression trough levels, thus the doses do not represent actual drug concentrations. In addition, we are unable to exclude residual confounding as an explanation for the differences by organ type because we did not have information on every characteristic that may influence the choice and dosage of immunosuppression after transplantation, such as infection, renal dysfunction, skin cancers, vasculopathy and bronchiolitis obliterans.

Changes in immunosuppression regimens over time and across individuals indicate that observational research based on immunosuppression data at discharge after organ transplantation may misclassify recipients' type of immunosuppressive therapy at later stages, potentially leading to spurious associations between immunosuppression and side-effects. Furthermore, given changes in the dose of immunosuppression with increasing time since transplantation, associations based on the discharge dose of immunosuppression may also be biased if the temporal changes differ for those on low compared to high discharge doses. The issue of potential misclassification and bias is an area of future study.

We have used population-based data to address a question of long-standing interest in transplantation research. Our data reveals clear differences in immunosuppression therapies for Australian liver, heart and lung transplant recipients. We provide the first empirical evidence of a lower degree of immune

suppression in liver compared to heart and lung transplant recipients. We also demonstrate marked changes in immunosuppression over time since transplantation, both the dose and type of immunosuppression. These temporal changes should be taken into account in observational studies examining the relationship between iatrogenic immune suppression and post-transplantation outcomes. An important future extension of these findings will be an examination of post-transplantation outcomes in relation to longitudinal immunosuppression.

AUTHORS' CONTRIBUTIONS

Renhua Na, PhD: Conduct of study, data analysis, and drafting and approval of article; Maarit A. Laaksonen PhD: Conduct of study, data analysis, and critical revision and approval of article; Andrew E. Grulich PhD: Concept, secured funding, and critical revision and approval of article; Angela C. Webster PhD: Drafting, and critical revision and approval of article; Nicola S. Meagher MPH: Conduct of study and critical revision and approval of article; Geoffrey W. McCaughan PhD: Secured funding and approval of article; Anne M. Keogh PhD: Secured funding and critical revision and approval of article; Claire M. Vajdic PhD: Concept, secured funding, conduct of study, and drafting and approval of article.

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FIGURE LEGENDS

Figure 1. Study cohort exclusions and losses to follow-up

Figure 2. Immunosuppressive drug combinations by time since transplantation for Australian liver, heart, and lung transplant recipients, 1984-1994

Figure 3. Immunosuppressive drug combinations by time since transplantation for Australian liver, heart, and lung transplant recipients, 1995-1997

Figure 4. Immunosuppressive drug combinations by time since transplantation for Australian liver, heart, and lung transplant recipients, 1998-2006

Figure 5. Unadjusted and adjusted median dose of immunosuppressive agent by organ type 3 months after transplantation

Figure 6. Unadjusted and adjusted median dose of immunosuppressive agent by organ type 1 year after transplantation

Figure 7. Unadjusted and adjusted median dose of immunosuppressive agent by organ type 5 years after transplantation

SUPPLEMENTARY ONLINE INFORMATION

Supplementary Table 1. Unadjusted and adjusted median doses (mg/kg/day) of individual immunosuppressive agents at 3 months, 1 year and 5 years after transplantation in Australian heart, lung and liver transplant recipients

Supplementary Figure 1. Immunosuppressive drug combinations 3 months after transplantation in Australian liver, heart, and lung transplant recipients, by era

Supplementary Figure 2. Immunosuppressive drug combinations 1 year after transplantation in Australian liver, heart, and lung transplant recipients, by era

Supplementary Figure 3. Immunosuppressive drug combinations 5 years after transplantation in Australian liver, heart, and lung transplant recipients, by era

Supplementary Figure 4. Immunosuppressive drug combinations at 10 years after transplantation in Australian liver, heart, and lung transplant recipients, by era

Supplementary Figure 5. Change in median cyclosporine and tacrolimus dose (q1-q3, mg/kg/day) by time since transplantation and type of organ

Supplementary Figure 6. Change in median mycophenolate and azathioprine dose (q1-q3, mg/kg/day) by time since transplantation and type of organ

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