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**Supplementary material**

# Association of Whole Blood Tacrolimus Concentrations with Kidney Injury in Heart Transplantation Patients

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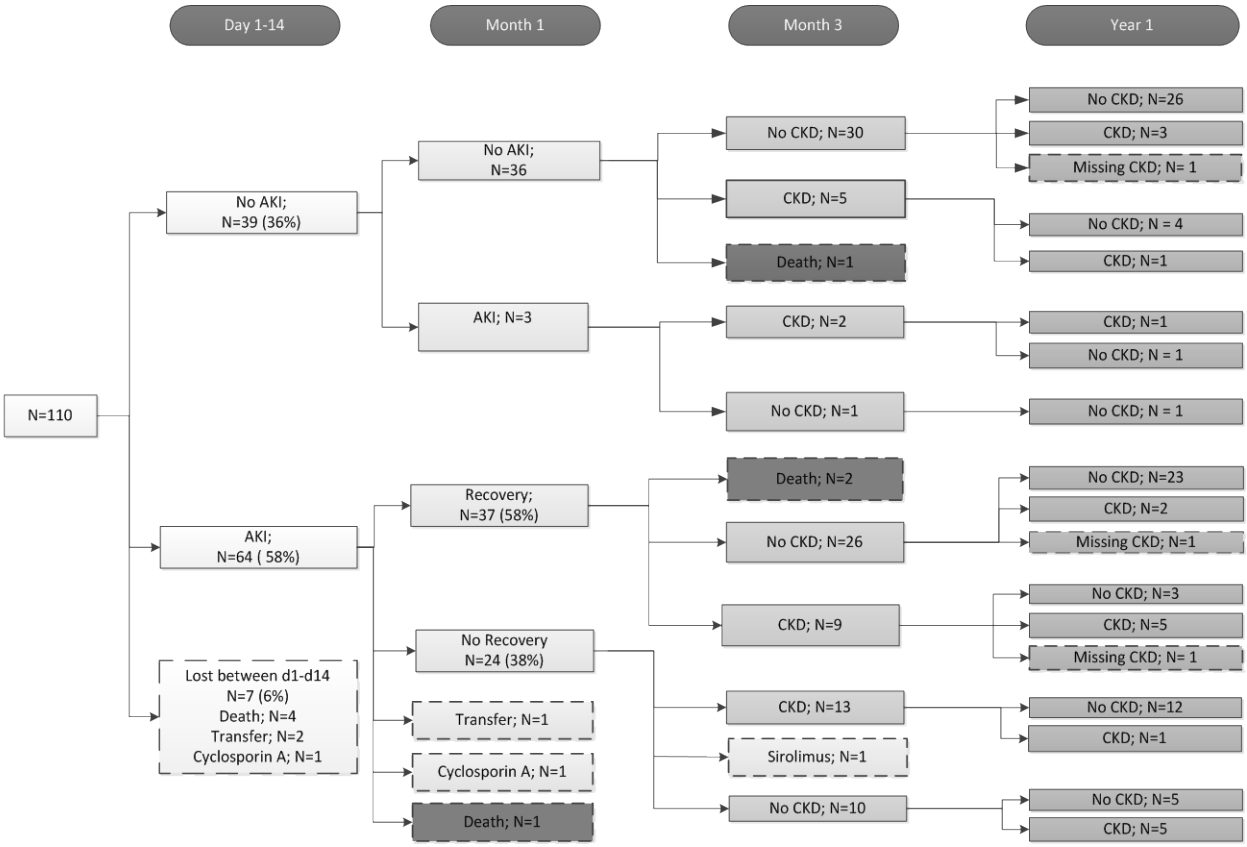


Figure S.1

Frequency of AKI and chronic kidney disease between day 1 and year 1

AKI=acute kidney injury, CKD=chronic kidney disease

Table S.1

Definitions of the covariates

| **Covariate** | **Definition** |
|---|---|
| AKI | "No AKI"; No increase in serum creatinine from baseline or serum creatinine <354 μmol/L. AKI stage 1; Increase in serum creatinine ≥26 μmol/L or 150-200% from baseline. AKI stage 2; Increase in serum creatinine >200% and ≤300% from baseline. AKI stage 3; Increase in serum creatinine >300% or ≥354 μmol/L with an acute increase of minimally 44 μmol/L or initiation of renal replacement therapy |
| Recovery of AKI | A reduction in peak AKI stage within 3 days and consistent for 48 hours between day 1 to day 14 |
| Recurrent AKI | Two periods of AKI within 14 days with a minimum of 48 hours in between |
| CKD | GFR categories; G1=normal GFR: ≥90 ml/min/1.73 $m^2$, G2=mildly decreased: 60–89 ml/min/1.73 $m^2$, G3a/b=mildly to severely decreased: 30-59 ml/min/1.73 $m^2$, G4= severely decreased: 15-29 ml/min/1.73 $m^2$, G5= Kidney failure: <15 mL/min/1.73 $m^2$. GFR = 141 x |

| | |
|---|---|
| | [min(Scr/κ),1)α x max(Scr/κ),1)-1.209] x Age-0.993 x 1.018 [if female] x [1.157 if Black] α is 0.329 for females and 0.411 for males; min indicates minimum of Scr/κ or 1, and max indicates maximum of Scr/κ or 1 |
| SIRS | Presenting 2 or more of the following criteria: body temperature <36 °C or >38 °C, heart rate >100 /min, respiratory rate >20/min, PaCO2 <32 mmHg, mechanical ventilation and leucocyte count <4 $X10^9$ /L or >12 $X10^9$ /L |
| Shock | Mean arterial pressure <60 mmHg or use of norepinephrine, epinephrine, phenylephrine, vasopressin, dopamine, dobutamine or milrinone |
| Liver injury | Bilirubin >34 μmol/L or an ALT >90 U/L for men and >70 U/L for women |
| Nephrotoxic drugs | Amoxicillin/clavulanate, flucloxacillin, benzylpenicillin, piperacillin/tazobactam, tobramycin, vancomycin, (val)aciclovir, (val)ganciclovir, trimethoprim/sulfamethoxazole, furosemide |
| Drugs increasing tacrolimus blood concentrations by inhibition or substrate | Tobramycin, erythromycin, neomycin, trimethoprim/ sulfamethoxazole, |

| | |
|---|---|
| competition of the CYP3A4/5 and Pgp enzymes | fluconazole, voriconazole, (es)omeprazole, amlodipine, nicardipine, diltiazem, haloperidol, amiodarone |
| Drugs potentially decreasing tacrolimus blood concentrations by induction of CYP3A4/5 or Pgp enzymes | Corticosteroids and rifampicin |
| Anemia | Ht <0.35 or Hb <7.0 mmol/L |
| Low protein concentration | Albumin <20 g/L or total protein concentration <45 g/L |
| *AKI= acute kidney injury, CKD= chronic kidney dysfunction, GFR=glomerular filtration rate, SIRS=systemic inflammatory response syndrome, Scr=serum creatinine, min=minute, CYP=cytochrome P450, Pgp=p=glycoprotein, Ht=hematocrit, Hb=hemoglobin* | |

Table S.2

Type of analysis used for the different outcome variables with the potential confounders

| Type of analysis | Outcome variable | Tested variable(s) | Potential confounder(s) |
|---|---|---|---|
| GEE analysis[a] | AKI between day 2-6 | Supra-therapeutic whole-blood tacrolimus trough concentrations day 2-6 prior to AKI event | Other nephrotoxic drugs<br>Shock<br>SIRS<br>Surgery time >400 min<br>Preoperative VAD<br>Postoperative ECMO<br>Ischemic or non-ischemic heart failure<br>DM<br>Age<br>Contrast administration |
| | AKI between day 2-14 | Supra-therapeutic whole-blood tacrolimus trough concentrations day 2-14 prior to AKI event | Surgery time >400 min<br>Preoperative VAD<br>Postoperative ECMO<br>Ischemic or non-ischemic heart failure<br>DM<br>Age<br>Contrast administration |
| Kaplan-Meier analyses | CKD up to 1 year | Logrank test comparing the group of patients with AKI between day 1 and day 14 versus the group of patients without AKI between day 1 and day 14 | |
| Linear Mixed model | Whole-blood tacrolimus trough concentration[b] | Liver injury<br>Other drugs increasing tacrolimus concentration<br>Other drugs decreasing tacrolimus concentration[c] | |

[a]*A binary logistic model was selected, with a logit link and an exchangeable working matrix. The outcome variable was "AKI", with two categories: "normal" corresponding to no AKI and "abnormal" corresponding to the KDIGO classes 1, 2, and 3 together. The significance of the variables was tested by Wald chi-square tests.*

[b]*The tacrolimus whole-blood concentration was log transformed for fitting purposes.*

[c]*Fixed factors were: liver injury, included as categorical variables; day (linear and quadratic terms), the number of drugs possibly decreasing the tacrolimus concentration and the number of drugs possibly increasing the tacrolimus concentration included as continuous variables. The effect of drugs on the tacrolimus concentration was tested one day after their initiation. Observations were clustered within individuals (patients' identification number as subject variable) and the time (expressed in day) was the "repeated" variable, entered as a within-subject variable. A quadratic term for day had to be included in the model for fitting purposes as the relationship between whole-blood tacrolimus concentration and day was non-linear.*

*GEE=generalized estimating equation, AKI= acute kidney injury, SIRS= systemic inflammatory response syndrome, VAD= ventricular assist device oxygenation, ECMO=extracorporeal membrane, DM=diabetes mellitus, min=minute*

Table S.3: Linear mixed model to test the variables influencing whole-blood tacrolimus concentrations

| **Fixed effect** | **Estimate [a, b]** | **P value[c]** |
|---|---|---|
| Liver injury | -0.15 | 0.03 |
| Drugs increasing tacrolimus | -0.008 | 0.86 |
| Drugs decreasing tacrolimus | -0.09 | 0.07 |
| Day | 0.58 | <0.0001 |
| Day squared | -0.05 | 0.0002 |

[a] *Estimate = regression coefficient in linear mixed model, with log(tacrolimus concentration) as outcome variable*

[b] *Estimate of intercept = 0.70*

[c] *P value of 0.005 is significant*