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Modélisation conjointe pour données longitudinales et données de survie: analyse des facteurs prédictifs du devenir de la greffe rénale

Joint modelling of longitudinal and time-to-event data: analysis of predictive factors of graft outcomes in kidney transplant recipients

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“All models are wrong; some are useful.”

George Edward Pelham Box (1919-2013)

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Droits d’auteurs

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Glossary

ABMR	antibody-mediated rejection
AIC	akaike information criterion
AR	acute rejection
AUC	area under the concentration-time curve
AZA	azathioprine
BIC	bayesian information criterion
CKD	chronic kidney disease
CNI	calcineurin inhibitor
CsA	cyclosporine A
CV%	coefficient of variation percentage
DSA	donor-specific antibodies
ECD	expanded criteria donor
ESRD	end-stage renal disease
GFR	glomerular filtration rate
HLA	human leukocyte antigen
HR	hazard ratio
IgG	immunoglobulin G
IQ	inter-quartile
IST	immunosuppressive treatment
MMAS	morisky medication adherence scale
MMF	mycophenolate mofetil
m-TOR	mammalian target of rapamycin
OFV	objective function value
OR	odds ratio
ROC	receiver operating curve
SCD	Standard criteria donor
SCr	serum creatinine
SD	standard deviation
TAC	tacrolimus
TDM	therapeutic drug monitoring
VPC	visual predictive check
WHO	world health organization
WPV	within-patient variability

CHAPTER I: Kidney transplantation and kidney graft survival

Chronic kidney disease (CKD), defined as kidney damage or decreased kidney function over 3 or more months, is a worldwide recognized health problem leading progressively to end-stage renal disease (ESRD) and kidney failure and requiring initiation of renal replacement therapy (RRT) (1). Kidney transplantation remains the preferred RRT option for people with ESRD, offering numerous advantages over hemodialysis or peritoneal dialysis including reduced morbidity and mortality, improved quality of life, and better cost-effectiveness (2,3). However, due to shortage of kidneys available for transplantation in this population, this privileged procedure is not available to all patients with ESRD who would like to benefit from it. In practice, the procedure that should be followed from the moment of ESRD diagnosis to the moment of receiving a new kidney graft is often long and very complex. For majority of patients it includes at least several steps: initialization of RRT with hemodialysis or peritoneal dialysis, placing a patient on a waiting list for a kidney transplant, testing patient's compatibility with potential donor and grafting the kidney.

The number of patients with CKD who develop ESRD is constantly increasing. According to data published by European Commission on organ donation and transplantation, over 63000 patients were officially placed on waiting lists for organ transplantation in Europe on 31 December 2013, of which some 50000 were patients on waiting lists for kidney transplantation (4). In France, the number of new yearly registered patients on waiting list for kidney transplantation, the total number of patients placed on waiting list for kidney transplantation at the beginning of year and the number of patients yearly transplanted with kidney evolved disproportionally from 2006 (newly registered: 3301, total: 5946, transplanted: 2731) to 2015 (newly registered: 4735, total: 11794, transplanted 3486) (5). Added to this, the proportion of kidney grafts of marginal quality also increased over the past years mainly as a consequence of increase in donor age and in the number of accompanying comorbidities.

For a patient who undergoes kidney transplantation, the optimal function of received organ is assured only thorough regular intake of immunosuppressive treatment, and this treatment is required for as long as the graft functions. Herein, the main objective is to maintain the level of immunosuppression high enough to prevent the episodes of graft rejection but not to induce over-immunosuppression which can potentially lead to drug toxicity and infectious diseases. This goal, however, is not always easily achievable in particular given the narrow therapeutic range of these drugs and different levels of immunosuppression which might be required at different time after transplantation. According to the mechanism of action, three main groups of drugs used for the maintenance of immunosuppression in kidney transplant recipients include calcineurin inhibitors (CNI) i.e. cyclosporine A (CsA) and tacrolimus (TAC),

mammalian target of rapamycin (m-TOR) inhibitors i.e. sirolimus and everolimus and anti-proliferative agents such as mycophenolate mofetil (MMF) and azathioprine (AZA). Currently the most frequently used immunosuppressive maintenance regimen involves a triple therapy composed of one calcineurin inhibitor (predominantly tacrolimus), one anti-proliferative agent (mostly MMF) and corticosteroids (6–8).

Despite significant improvement in short-term kidney graft survival driven by introduction of new immunosuppressive agents which were able to reduce the rate of early graft rejection, the long-term survival has only marginally improved within the past two decades (9,10), with significantly higher graft survival in Europe compared to the United State (11). Yet, as reported by Meier-Kriesche *et al.* (9) and Lamb *et al.* (10), this modest improvement in long-term kidney graft survival was mainly due to decrease in the rate of graft failure in the high-risk populations such as repeated transplants, black recipients or Expanded Criteria Donor (ECD) recipients. Now that the first-year kidney graft survival rate exceeds 90%, the question remains if any further improvement in long-term kidney graft survival is possible.

Clearly, maintaining healthy patient and viable graft requires the consideration of multiple factors associated with graft failure. These include donor- and recipient-specific factors, transplantation-related and immunological factors, biomarkers of graft function collected repeatedly over time and factors relative to immunosuppressive treatment. Even though these risk factors are described in literature and well-known today, their predictive utility for kidney graft failure is usually modest, especially when they are used alone.

Thus, we commence this section by introducing the major factors associated with kidney graft failure, followed by a more detailed review of methods available for analysis of time-to-event (survival) and longitudinal data, with focus on studies conducted in the domain of kidney transplantation. We continue by describing the advantages of joint-modelling of time-to event and longitudinal data over separate analysis of these two types of data and we end-up this section by presenting some of the scores developed for prediction of graft failure after kidney transplantation.

I.1. Factors associated with kidney graft survival

I.1.1. Donor-specific characteristics

Donor-specific factors that are known to be associated with kidney graft survival include donor age and type of donation (i.e. living vs. deceased donor), while there is less evidence for donor ethnicity and gender.

In the group of first kidney transplant recipients with a functioning graft for at least 6 months and who were followed-up for median of 7.5 years, living donation was the only significant predictor of better graft survival (β=-0.750, p=0.046) in the final multivariate time-dependent Cox model adjusted for serum creatinine (SCr), decline in SCr, and the interaction of SCr with both time since transplantation and time since last observation (12).

More frequently, however, the impacts of donation type (i.e. living vs. deceased), donor age and accompanying donor comorbidities on kidney graft survival are evaluated together though a composite criterion of Expanded Criteria Donor (ECD), defined as any deceased donor older than 60 years or between 50 and 59 years old with at least two of the following: SCr > 1.5 mg/dl (132.6 µmol/L), death caused by cerebrovascular accident or history of hypertension. In a large population study evaluating evolution of kidney graft survival in the United States according to transplantation year, Lamb *et al.* reported that in 2005, graft-survival half-lives in recipients of living donation, deceased donation and expanded criteria donation were 11.9, 8.8 and 6 years, respectively (10). A recent meta-analysis performed by Querard *et al.* found that the adjusted HR was significantly higher in the recipients of ECD kidneys compared to recipients of Standard Criteria Donor (SCD) kidneys whatever the outcome studied (pooled HR for patient-graft survival for ECD group: 1.68, 95% CI: 1.33-2.12; pooled HR for patient-survival only: 1.25, 95% CI: 1.12-1.40; pooled HR for death-censored graft survival only: 1.81, 95% CI: 1.60-2.06) (13).

In the prospective cohort study of kidney transplant recipients Aubert *et al.* compared long-term outcomes of kidney transplantation between SCD and ECD according to presence of circulating donor-specific antibodies (DSA) on day 0 (14). Overall, patients receiving ECD kidney had higher risk of graft loss compared to those receiving SCD kidney (HR: 1.87, 95% CI: 1.50 - 2.32). When the 7-years graft survival was compared in groups of ECD patients according to their DSA status, the ECD/DSA$^+$ group was associated with 4.4 fold increased risk of graft loss compared to the ECD/DSA$^-$ group. In addition, ECD/DSA$^+$ recipients showed remarkably worse 7-year graft survival (44%, P<0.001) compared to three other groups: SCD/DSA$^+$ (73%), ECD/DSA$^-$ (85%) and SCD/DSA$^-$ (90%).

Schnitzler *et al.* reported that, compared to living donation, the deceased donation in the group of SCD kidney recipients was significantly associated with higher risk of graft failure, whatever the cause of death (anoxia HR: 1.21, 95% CI: 1.06-1.39; cerebrovascular accident HR: 1.19, 95%CI: 1.07-1.34; head trauma HR: 1.22, 95% CI: 1.09-1.36, other HR: 1.35, 95% CI: 1.10-1.66) (15). In addition, recipients of kidney grafts from African American donors had higher risk of graft failure compared to recipients of grafts from white donors (HR African American donor: 1.13, 95% CI: 1.04-1.22) (15).

I.1.2. Transplantation-related factors

Among transplantation-related factors, the two which are most frequently studied as risk factors of graft and patient survival are cold ischemia time (i.e. the period of kidney preservation between harvesting and grafting during which kidney is perfused with a cold flush) and retransplantation.

Acknowledging decrease in cold ischemia time over the past decade, Debout *et al.* investigated its' effects on graft and patient survival in the large multicentre French cohort of the first heart-beating deceased donor kidney recipients (16). In multivariate analysis, each additional hour of cold ischemia time was associated with 1.013 fold higher risk of graft failure (95% CI 1.001-1.025) and 1.018 fold higher risk of death (95% CI 1.002-1.035). Compared to the recipients of kidneys with less than 16 hours of cold ischemia time whose absolute risks of graft failure and death at 1-year, 5-years and 10-years post-transplantation were 4 % and 1%, 6% and 6% and 11% and 11%, respectively, the recipients of kidneys with cold ischemia time between 16 and 24 hours had higher risks of graft failure and death (risk of graft failure: 5%, 13% and 24%; risk of death: 3% ,7% and 13% at 1, 5 and 10 years post-transplantation) and similar to risk of kidney recipients with cold ischemia time between 24 and 36 hours.

In the previously mentioned study of Aubert *et al.*, increased cold ischaemia time (reference: <12 h; HR for cold ischemia time 12-24h: 1.46, 95% CI: 1.04-2.04; HR for cold ischemia time >24h: 1.73, 95% CI: 1.18-2.52) and graft rank higher than 1 (HR: 1.54, 95% CI: 1.13-2.05) were the main predictors of graft loss in the multivariate analysis adjusted for deceased donor, donor diabetes, ECD, number of HLA A/B/DR mismatches and circulating DSA on day 0 (14).

I.1.3. Recipient-specific factors

Recipient characteristics that are frequently assessed as potential predictors of kidney graft failure include age, gender and ethnicity. However, confusing results have been reported in literature regarding these factors.

In the multivariate Cox regression model adjusted for fixed-time covariates (panel reactive antibodies, number of acute rejections, SCr at M6, dipstick proteinuria and level of cross-reactive groups of major histocompatibility complex class 1 molecules), higher recipient age was significantly associated with better graft survival (β par year increase in recipient age =-0.038, $p<0.005$) (12). On the other hand, Schnidler *et al.* reported that the 10-year increase in recipient age was significantly associated with higher risk of graft failure in recipients of both SCD and ECD kidneys (ECD HR: 1.03, 95% CI: 1.01-1.06; SCD HR: 1.02, 95% CI: 1.00-1.05) (15).

Lepeytre *et al.* evaluated the impact of recipient sex on death-censored graft failure in the large population of first deceased-donor kidney recipients. The authors hypothesed that the effect would differ with respect to donor sex and recipient age-groups (i.e. 0-14, 15-24, 25-44 and ≥45 years) (17). In case of male donor, a consistently and significantly higher risk of graft-failure was found for female compared to male recipients, whatever the age group (adjusted HR 0-14 years: 1.51, 95% CI: 1.19-1.90, aHR 15-24 years: 1.37, 95% CI: 1.18-1.59, aHR 25-44 years: 1.14, 95% CI: 1.03-1.26; aHR ≥45 years: 1.05, 95% CI: 1.01-1.09). In contrast, when female were donors, only the group of female recipients aged 15-24 years had significantly higher risk of graft failure compared to their male counterparts (aHR: 1.06, 95 % CI: 1.06-1.53), while the group of female recipients aged more than 45 had significantly lower risk of graft failure compared to male recipients from the same age group (aHR: 0.95, 95% CI: 0.91-0.99).

I.1.4. Variables collected over clinical follow-up

Many variables collected in the period after transplantation and over patients' regular follow-up visits were shown to be associated with graft survival. Classically, these include repeatedly collected biomarkers of graft function such as serum creatinine (SCr) and proteinuria or the onset of acute rejection (AR).

In the group of kidney transplant recipients with a functioning graft for at least 6 months, De Brujine *et al.* evaluated the prognostic ability of different variables related to SCr longitudinal measurements for kidney graft failure: the time elapsed since the last SCr measure, the 1000 times reciprocal of the serum creatinine concentrations (RC), the last recorded RC, the ratio

between last measured RC and RC at month 6 and the time elapsed from the last observation (12). In the final multivariate regression model adjusted for donation type (living vs. cadaveric), decrease in RC (i.e. increase in SCr, β=-1.383, p<0.001), and steeper decline of in renal function (RC/RC_6, β = -5.057, p=0.001) were the independent predictors of graft failure. The interaction between RC and the time since the last observation was also significantly associated with graft failure (β=8.847, p<0.001) indicating that the prognostic value of RC decreased with increase in the time elapsed since its measure. Similarly, Kasiske *et al.* reported that in the population of 1663 kidney transplant recipients, first decline of 30 % in inverse SCr from baseline (the maximum inverse SCr level in the first 3 months after transplantation was considered as baseline) was strong independent predictor of graft failure (HR:2.56, 95% CI: 2.12-3.09), death-censored graft failure (HR: 6.07, 95% CI: 4.36-8.45) and death (HR: 1.99, 95% CI: 1.57-2.52) (18).

Proteinuria is another important and frequently used marker of kidney damage repeatedly collected in kidney transplant recipients over their follow-up. Cherukuri *et al.* explored in kidney transplant recipients the association between early protein excretion, on one hand, and the onset of death-censored graft loss, death with a functioning graft and composite vascular end-point, on the other. Participants were divided into four groups according to median of all protein creatinine ratio (PCR) measurements obtained in the 3rd month post-transplantation, and the three groups with higher median PCR were associated with significantly higher risk of death-censored graft failure compared to the group with the lowest median PCR (reference group: median PCR below 0.15 or equivalent of <0.15 g/24h; group with median PCR between 0.15 and 0.5 HR: 7.1, 95% CI: 1.7-29.3; group with median PCR between 0.5 and 1 HR: 10.5, 95%CI: 2.4-45.7; group with median PCR higher than 1 HR: 16, 95% CI: 3.5-72). However, the early proteinuria did not impact the risk of death with functioning graft or the risk of composite vascular endpoint consisting of fatal/nonfatal myocardial infraction, unstable angina, congestive cardiac failure, cardiac arrhythmia, transient ischemic attack, cerebrovascular accident, limb revascularisation or limb amputation during the graft lifetime. When studying the longitudinal effect of PCR throughout the first post-transplant year in the largest PCR group with 51.4% of participants (i.e. the group with median PCR between 0.15 and 0.5), a significantly higher risk of graft failure was observed in patients whose median PCR increased over 0.5 at M12 compared to patients whose median PCR remained below 0.5 at M12 (HR: 2.6, 95% CI 1.2-5.3).

In the large population of first kidney transplant recipients, Lentine *et al.* evaluated the relative risk of graft loss associated with onset of acute rejection (AR) according to the timing of AR (i.e. within the first 6 months after transplantation, from M6 to M12, from M13 to M24 and from M25 to M36) and the risk period after AR onset (i.e. <90 vs. ≥90 days) (19). Graft

loss was defined as death or renal allograft failure and separate analysis was performed for groups of recipients with respect to donation type (i.e. SCD, ECD or living donation). Acute rejection was categorized to Ab-treated AR (i.e. more severe AR) and non-Ab-treated AR (less severe AR) and regardless of donation type, both categories of AR were associated with significantly higher risk of graft loss compared to the absence of AR. In general, the relative risk of graft loss (adjusted for donor age, hypertension, presence of cytomegalovirus, patients' weight and race, cause of death and delayed graft function) increased when AR occurred later after transplantation for both Ab-treated and non-Ab-treated AR regardless of donation type. Also, whatever the time of AR onset, the risk of graft loss was in general higher within the 89 days following AR than thereafter. For example, in the group of SCD recipients with Ab-treated AR, the relative risk of graft loss within the 89 days following AR increased from 2.75 (95% CI: 1.78-4.28) for AR occurring within the first 6 months after transplantation to 4.90 (95% CI: 1.16-1.58) for AR occurring between M25 and M36 when compared absence of rejection. Similarly, the relative risk of graft loss 90 days or later after AR occurrence increased from 1.35 (95% CI: 1.16-1.58) for AR occurring within the first 6 months after transplantation to 2.60 (95% CI: 1.89-3.58) for AR occurring between M25 and M36 after transplantation. Last, after adjustment for eGFR at 1-year post-transplantation, Ab-treated AR and non-Ab-treated AR within the first year were associated with 58% (aHR: 1.58, 95% CI: 1.43-1.75) and 43% (aHR: 1.43, 95% CI: 1.34-1.53) increase in relative risk of graft loss, respectively, compared to absence of AR.

I.1.5. Immunological factors

Everly *et al.* explored the impact of *de-novo* donor-specific anti-human leukocyte antigen (HLA) antibodies (*dn*DSA) on the long-term graft survival (20). In total, 47 of 189 patients developed *dn*DSA and the actual cumulative incidence of *dn*DSA development was 20 % at 5 years post-transplantation, with the majority of *dn*DSA occurring within the first post-transplant year (cumulative incidence of 11 % at 1 year post-transplantation). Compared to the group of patients who developed *dnDSA*, the 10-years survival was significantly higher in the group of patients without *dn*DSA ($p<0.01$). Chronic rejection was identified as the primary cause of graft loss (72% of patients with loss) and 56% of patients who lost their graft due to chronic rejection had previously developed *dn*DSA. In the group of patients who developed *dn*DSA, 11 (24%) lost their graft within the 3 years from time of the *dn*DSA detection (20).

Lefaucheur *et al.* evaluated in kidney transplant recipients the association between different characteristics of DSA developed over the first year post-transplantation (i.e. specificity, HLA class, mean fluorescence intensity, C1q-binding, IgG subclass and graft injury phenotype) and 4-year graft survival. In the final multivariate Cox regression, the presence of

IgG3 and C1-binding antibodies were associated with significantly higher risk of death-censored graft failure (HR for IgG3 Abs: 4.8, 95% CI: 1.7-13.3; HR for C1q-binding Abs: 3.6, 95% CI: 1.1-11.7) (21).

Gonzales and colleagues previously reported that the higher cumulative mean fluorescence intensity (MFI) of class 2 DSA was associated with significantly higher risk of death-censored graft failure (HR for cumulative MFI ≥800: 4.34, 95% CI: 1.89-11.1) in the multivariate Cox regression model adjusted for recipient factors (estimated GFR, acute rejection, proteinuria, age, sex, race and serum albumin) and histological factors (glomerulitis score and chronic interstitial fibrosis score) at 12 months post-transplantation (22).

Cooper *et al.* evaluated the impact of *dn*DSA development on kidney graft survival in the population of 244 consecutive kidney or kidney-pancreas recipients who were prospectively screened for *dn*DSA over the first 2 post-transplant years (23). In 63 patients who developed *dn*DSA, 90% were detected for *dn*DSA within the first 6 months after transplantation. Compared to patients without *dn*DSA, significantly higher proportion of patients with *dn*DSA experienced graft failure within the 2 years after transplantation (9.5% vs. 19%, $p<0.001$). However, after exclusion from analysis of *dn*DSA positive patients who experienced AR, there was no significant difference in 2-year graft-survival between the remaining *dn*DSA-positive patients and the patients who did not develop dnDSA ($p=0.45$). The authors thus concluded that in patients with stable kidney function without concomitant AR, development of *dn*DSA was not associated with impaired graft survival.

I.2. Multivariate models for survival data and analysis of factors associated with graft survival after kidney transplantation

The primary goal of many clinical and epidemiological studies in transplantation is to study the time until the event of interest. In such circumstances, the random variable studied is the time until the event, also denoted as survival time, failure time or event time. An important characteristic of survival times is that they are only partially observed: the entity we seek to identify (which is mainly the exact time of death) is not available for all patients and for majority of subjects we only know that it occurred before or after a certain time point. The event (outcome) usually corresponds to death, but it can as well be any other irreversible transition between two fixed states. The events which are frequently analyzed in kidney transplantation include the onset of graft rejection (24–26), graft loss (10,27), patient death (16,18), the development or recurrence of some disease (28), transplantation (for patients on waiting lists) and retransplantation (29) or the combination of 2 or more specific events (i.e. composite outcomes) (28,30).

I.2.1. Cox proportional hazards model

The use of Cox proportional hazards model (also known as relative risk model or Cox regression) in clinical and epidemiological research has become pervasive since a while. This model evaluates the impact of explanatory variables on the hazard associated with onset of specific event (31). In kidney transplantation, the proportional-hazards model is typically used to quantify the effect of different pre-transplant or post-transplant covariates on relative risk for graft failure or patient death (9,10,13,14,18,20,29).

Using Cox proportional-hazards model, Gonzales *et al.* investigated the impact of clinical and histological factors measured at 1 year post-transplantation on overall and death-censored 5-year kidney graft survival (22). In the final multivariate model adjusted for recipient factors (age, sex, ethnicity, renal function, proteinuria and prior acute rejection) at 1 year post-transplantation, glomerulitis score was significantly associated with higher risks of overall graft loss (HR per unit increase: 1.83, 95% CI: 1.44-2.31) and death-censored graft failure (HR: 2.74, 95% CI: 1.77-4.25). Chronic interstitial fibrosis score (HR per unit increase: 1.90, 95% CI: 1.27-2.85) and mean fluorescence intensity (MFI) of class 2 DSA (MFI>800; HR: 4.57, 95% CI 1.89-11.1) were independent predictors of death-censored graft loss.

In the group of 74 kidney transplant recipients who all experienced an episode of antibody-mediated rejection (ABMR) in the first post-transplant year and were followed-up for a median of 54 months thereafter, Loupy *et al.* investigated whether more accurate predictions of kidney graft loss could be obtained by combining traditional approaches based on histology and presence of DSA with gene expression profiling (i.e. ABMR molecular score and endothelial DSA-selective transcript set) (32). After adjustment for donor age (HR for group ≥60 years: 3.84, 95% CI: 1.48-9.96) and humoral histologic score defined as the sum of Banff's score humoral parameters (i.e. glomerulitis, peritubular capillary, vasculitis, transplant gromerulophaty and C4d, HR per unit increase in score: 1.43, 95%CI: 1.09-1.90), AMBR molecular score was an independent predictor of graft loss in the multivariate Cox proportional-hazards model (HR: 2.22, 95% CI: 1.37-3.58). A similar result was observed for endothelial DSA-selective transcripts (HR: 3.02, 95% CI: 1.00-9.16) in the Cox model adjusted for donor age. Compared to the Cox model without gene expression profiling, significant improvement in discriminative ability was observed after inclusion of AMBR molecular score (increase in C-statistics from 0.77 to 0.81, continuous net reclassification index of 1.0135, the integrated discrimination improvement of 0.1579, $P<0.001$).

Application of Cox model with outcomes other than patient or graft survival after kidney transplantation is not unusual. Everly *et al.* investigated the predictors of *dn*DSA development in a cohort of 189 primary kidney transplant recipients without circulating DSA at transplantation (20). Forty seven patients (25%) developed *dn*DSA over the 10 years of follow-

up, and the predictors significantly associated with *dn*DSA development in the multivariate Cox model were DQ-locus mismatch in HLA system between donor and recipient (DQ mismatch >0, HR: 3.48, 95% CI: 1.37-8.87), younger age (18-35 years old at transplantation, HR: 2.62, 95% CI: 1.39-4.94), presence of non-DSA before transplantation (HR: 2.31, 95% CI: 1.12-3.64) and receiving a deceased-donor transplant (HR: 2.02, 95% CI: 1.12-3.64).

In a group of 125 kidney transplant recipients with circulating DSA at the first post-transplant anniversary, Lefaucheur *et al.* explored predictors of death-censored graft survival according to different characteristics of DSA, including specificity (pre-formed vs. *de novo*), HLA class specificity (class I vs. class II), mean fluorescence intensity (MFI), C1q-binding status, IgG subclass (1 to 4), and graft injury phenotype in time of *sera* evaluation for DSA (i.e. acute antibody mediated graft rejection vs. subclinical graft rejection vs. absence of rejection) (21). In the final multivariate Cox proportional-hazards model, anti-HLA IgG3 positivity (HR: 4.8, 95% CI: 1.7-13.3) and C1q-binding capacity (HR: 3.6, 95% CI: 1.1-11.7) of immune-dominant (i.e. with the highest MFI) DSA were independently and significantly associated with increased risk of death-censored graft failure.

Foucher *et al.* used Cox regression model in combination with time-dependent receiver operating curves to develop Kidney Transplant Failure Score (KTFS) by taking into account multiple pre-transplant and 1-year post-transplant risk factors of graft loss (33). The developed score was calculated as the sum of each risk factor value multiplied by the corresponding logarithm of hazard-ratio from the final multivariate Cox model which included SCr at month 3 (HR: 0.96, 95% CI: 0.93-0.99), square root of SCr at month 12 (HR:1.55, 95% CI: 1.43-1.69), donor creatinine value, recipient age (HR:0.37, 95% CI: 0.23-0.61) recipient gender (reference female, HR for male: 0.42, 95% CI: 0.28-0.63), number of previous transplantations (HR: 2.94, 95% CI: 1.68-5.16), proteinuria at M12 (HR: 1.73, 95% CI: 1.19-2.51), square of proteinuria at M12 and the interaction of recipient gender with last two terms. Predictive ability of the KFTS (time dependent ROC AUC: 0.78, 95% CI: 0.73-0.80) was significantly higher when compared to predictive abilities of 1-year serum creatinine (ROC AUC: 0.73), 1-year eGFR (ROC AUC: 0.70) or the evolution of SCr between 6 months and 1 year post-transplantation (ROC AUC: 0.60) in both training (n=2169) and validation set (n=317). Sensitivity and specificity of the developed score were 0.72 and 0.71, respectively, and the KTFS threshold of 4.17 was used to classify patients from training set in the group of lower (65% of patients, 8-years graft-failure rate of 8%) and the group of higher risk for graft failure (35% of patients, 8-years graft failure rate of 29.8%).

Two crucial assumptions are to verify when Cox model is used in survival analysis. First, it is assumed that the ratio of hazards (hazard ratio (HR)) for an event of interest given different modalities of an explanatory variable does not change over time (i.e. hypothesis of

hazard-proportionality). Second, the relationship between independent explanatory variables and hazard function is assumed to be log-linear (i.e. log-linearity hypothesis).

Despite the widespread application of Cox model in different clinical settings related to kidney transplantation, in many previous studies considered death with a functioning graft as a non-failure (17,22,30,34). In addition to this, the use of Cox proportional-hazards model is limited on covariates whose value is known only at one specific time-point (e.g. time-fixed covariates known at the moment of transplantation or at one year post-transplantation) and accordingly, it cannot handle time-dependent explanatory variables (i.e. variables whose value for a given individual can change over time). This limitation can be partially overcome by using the extended (time-dependent) Cox model which will be discussed in the following sub-section.

I.2.2. Statistical methods for inclusion of time-dependent covariates in time-to event models

I.2.2.1. Extended Cox model for time-varying covariates

There are many cases in clinical practice where it may be useful to evaluate whether the information that is collected repeatedly over time is associated with the risk for an event. Herein, it is important to distinguish between two main types of these repeatedly collected (also called time-varying) covariates.

Exogenous time-dependent covariates are those for which the value at any time *t* is known infinitesimally before *t* and is not influenced by onset of event (i.e. the measurement of these covariates does not require the existence of subjects under study, it can be performed even once the patient has passed away). Some classical examples are the air pollution, the season of the year or temperature. On the other hand, biomarkers of disease progress (i.e. serum creatinine, proteinuria) and clinical parameters (acute rejection, *de novo* DSA) are typically *endogenous time-varying covariates* measured on subjects under study. As a consequence, the change in their value is hardly predictable and is often related to modified risk for an event.

In kidney transplant patients, it may be reasonable to assume that the instant risk of death is the highest immediately after transplantation and declines progressively thereafter. In this context, Rabbat *et al.* used time-dependent Cox model to evaluate this non-proportional hazards effect of transplantation on patient mortality and compare it to mortality in the group of patients who remained wait-listed for transplantation over the same time-period (29). In the multivariate model controlling for donor age, race, gender and time elapsed from start of end-stage renal disease therapy until wait-listing, the relative risk for mortality in transplanted group

of patients was significantly higher up to M1 post transplantation (HR: 2.91, 95% CI: 1.34-6.32), not different over the first year and significantly lower thereafter (HR: 0.25, 95% CI: 0.14-0.45) compared to the corresponding relative risk of patients who remained wait-listed for kidney transplantation over the same time-period.

Kasiske *et al* used time-dependent Cox model to evaluate the association between time of occurrence of decline in different functional measures of serum creatinine (relative decline from baseline in inverse creatinine (*Δ1/Cr*), creatinine clearance *(C_{Cr})* and slopes of inverse serum creatinine according to Cr measurements at 1 week, 1, 3, 6, 12, 18, 24 and 36 months and annually thereafter) and acute rejection episode measured as time-dependent covariate, on one hand, and graft-failure, death-censored graft failure or death with a functioning graft, on the other. (18). In their final multivariate model that included acute rejection, baseline graft function and other covariates (depending on studied outcome), decrease from baseline (defined as the maximum inverse Cr of three consecutive measures in the first 3 months post-transplantation) of -30% in *Δ1/Cr* was an independent risk factor of graft failure (HR: 2.56, 95% CI : 2.12-3.09), death-censored graft failure (HR: 6.07, 95% CI: 4.36-8.45) and death (HR: 1.99, 95% CI : 1.57-2.52).

Nevertheless, there exist some important shortcomings which can limit the use of extended Cox model. First, the value of time-dependent explanatory variable must be known for all at-risk subjects whenever the event of interest occurs for any of patients. Second, this approach does not account for measurement error in time-dependent variable and should not be used with endogenous time-dependant covariate or with events that can occur repeatedly over time (i.e. multiple graft rejection, undesirable drug effects).

I.2.2.2. Joint models for longitudinal and time to event data

In longitudinal studies related to kidney transplantation, two different types of outcomes are typically collected: (i) repeated measures of a marker over time (i.e. longitudinal data) and the time to an event of interest (i.e. survival data). A classic example of these are repeated measures of serum creatinine and time to graft failure in kidney transplant recipients (34–36).

An important feature that makes longitudinal data particular in comparison with survival data presented earlier in this section is the correlation between repeated measurements: it seems reasonable to assume that measurements taken on the same individual are more correlated between themselves then with measurements taken on other subjects under study. Different parametric (mixed-effects models) and non-parametric approaches (K-means) are available today for analysis of such correlated data. They may, in addition, account for the

measurement error in longitudinal biomarker. Our primary interest being the analysis of risk for kidney graft failure, we will now focus on the methods available for simultaneous analysis of continuous longitudinal outcomes and time-to-event data in kidney transplantation.

These approaches, called joint models for longitudinal and time to event data, have recently become very popular in the area of biostatistics. The concept of joint modelling approach consists in (i) describing the longitudinal marker trajectory usually with a linear mixed effects model (37), (ii) defining the risk for an event of interest mainly through Cox proportional-hazards model and (iii) linking these two parts using a shared structure. In comparison to previously-described time-dependent Cox model, the use of joint model presents some important advantages. First, it does not require the value of time-dependent covariate to be known at each time the event of interest takes place. Second, it accounts for the measurement error in longitudinal marker through random-effects part of mixed-effects model and it accounts as well for the correlation between repeated measurements of longitudinal marker.

According to the model structure, two main types of joint models are in use: shared random-effects model (26,34,36,38) and latent class joint model (35,39–41).

I.2.2.2.1. Shared random-effects model

The idea behind this type of joint models is to include the characteristics of the longitudinal marker defined as a function of random effects as a covariate in the survival model (42). Thus, the same random effect captures the correlation between repeated measurements of longitudinal marker and the association between the longitudinal marker and the time to event.

Utility of shared random effects models for analysis of longitudinal and time to event data in kidney transplant recipients have been demonstrated in several studies (26,34,36).

For instance, while some factors can be considered as directly associated with increased risk of kidney graft failure, the others may indirectly impact graft survival, through modifying the evolution of SCr. Recently, Fournier *et al.* used shared random-effects multivariable joint model to evaluate the association between logarithmic transformation of SCr time-evolution and graft failure (defined as return to dialysis or death with a function) after the 1st post-transplant year (34) as well as their predictors. Higher SCr at M6 and older donor were significantly associated with higher 1-year SCr whereas higher SCr levels at M6 and M12, older donor, history of diabetes and donation after cerebrovascular cause of death were significantly associated with SCr increase over 5 years. Graft failure depended on both current value of SCr (HR for an increase of 25%: 1.92, 95%CI: 1.75-2.11) and the current slope the SCr (HR for an increase of 25%: 1.89, 95%CI: 1.17-3.06). Factors associated with both SCr

and graft failure included recipient age (for a 10-year increase in recipient age: 2.04% lower SCr at M12 post-transplantation [95%CI: 1.31%-2.7%], 5.57% lower SCr at 5 years post-transplantation [95%CI: 4.20%-6.95%], HR for graft failure: 1.35, [95%CI: 1.25%-1.46%]), SCr at month 3 (for a 50 µmol/L increase in SCr at M3: 8.08% higher SCr at 1 year [95%CI: 6.83%-9.32%], HR for graft failure: 0.85, [95%CI: 0.75%-0.95%]), acute rejection in the first post-transplant year (5.65% higher SCr at M12 [95%CI: 3.65%-7.71%], HR for graft failure: 1.46 [95%CI: 1.17%-1.83%]), history of cardiovascular disease (HR for graft failure: 1.39, 95% CI: 1.14-1.69) and pre-transplant immunization. In a cause-specific joint model, current SCr (HR for return to dialysis: 2.57, 95%CI: 2.22-2.84) and onset of acute rejection (HR for return to dialysis: 1.63, 95%CI: 1.20-2.20) showed significantly stronger association with return to dialysis than with time to death, while the opposite effect was seen for history of cardiovascular disease (HR for death: 2.01, 95%CI: 1.49-2.70) and recipient age (HR for death: 2.36, 95%CI: 1.95-2.86). Finally, when parameter estimates from the developed joint model were compared with their corresponding estimates obtained with separate use of linear mixed model and time-dependent Cox model, no relationship between history of cardiovascular disease or donor type with SCr evolution was observed and no association between higher recipient age or diabetes and increased hazard ratio for death failure was found.

In a previous study of our group, shared random-effects model with Weibull baseline risk function was used to investigate the association between longitudinal exposure to mycophenolic acid (MPA AUC) and acute rejection in the first year following kidney transplantation on one hand, and to determine time-dependent MPA AUC thresholds which minimize the risk of graft rejection on the other (26). The model included polynomial function with a quadratic term to describe trajectories of MPA time-exposure which was adjusted for dose-adjustment strategy (i.e. concentration-controlled vs. fixed dose) while the survival part of the model was adjusted for recipient age. An increase in MPA AUC was associated with a significant decrease in the risk for AR over the first post-transplant year (coefficient of association: α=-0.044, p=0.0081) and the determined thresholds for MPA AUC increased significantly with time post-transplantation (from 35 mg*h/L around week 2 to 41 mg*h/L after month 6).

I.2.2.2.2. Joint latent-class mixed models

The joint-latent class mixed models are the second group of joint models. They consider that within a heterogeneous population of subjects with respect to a specific longitudinal marker there exist a finite number of homogenous subgroups, so-called latent classes because they are not directly observed (40,43). The subjects within each latent class share the same class-specific marker trajectory, and class-specific risk of the event. The joint-latent class mixed model consists of three main sub-models: (1) a multinomial logistic regression sub-model which, for a given patient, calculates his/her probability of belonging to a given latent class, (2) a latent class mixed effect model, which is an extension of classical linear mixed model, describing class-specific time-trajectories of longitudinal marker and (3) a survival sub-model aiming to describe the class-specific risk of an event. A brief mathematical rational of these three sub-models will be provided in the following paragraphs while the more detailed description is not the subject of current work and can be found elsewhere (40,43,44).

For subject *i*, latent class membership is defined by a discrete random variable c_i that equals *g* if the subject belongs to latent class *g (g = 1, …, G)*. Thus, the variable c_i is latent and for subject *i*, the probability of belonging to a latent class *g* is given with a multinomial logistic regression with respect to covariates X_{ci} as follows:

$$\pi_{ig} = P(c_i = g|X_{ci}) = \frac{e^{\xi_{0g}+X_{ci}^T\xi_{1g}}}{\sum_{l=1}^{G} e^{\xi_{0i}+X_{ci}^T\xi_{1i}}}$$

where ξ_{0g} is the intercept for class *g* and ξ_{1g} is the q_1-vector of class-specific parameters associated with the q_1-vector of time-independent covariates X_{ci}. Each subject can be allocated to one and only one latent class.

For each subject *i* in a sample of *N* subjects, let us consider a vector of n_i repeated measurements of longitudinal marker $Y_i = (Y_{i1}, \dots, Y_{ij}, \dots, Y_{in_i})^T$ where Y_{ij} is the outcome value at occasion *j*. The *G* mean profiles of longitudinal marker (i.e. SCr time-profiles in our first study) are defined according to time and covariates through latent class-specific mixed models. Herein, both fixed effects and the distribution of the random-effects are allowed to be class-specific contrary to standard linear mixed model. For a Gaussian outcome, latent class mixed effects model can be defined for class *g*:

$$Y_{ij}|_{c_i=g} = X_{L1i}(t_{ij})^T\beta + X_{L2i}(t_{ij})^T v_g + Z_i(t_{ij})^T u_{ig} + \omega_i(t_{ij}) + \epsilon_{ij}$$

where $X_{L1i}(t_{ij})$ and $X_{L2i}(t_{ij})$ are two vectors of covariates at time t_{ij} of respective length p_1 and p_2 associated with class-common fixed effects β and class-specific fixed effects v_g, $Z_i(t_{ij})$

is the vector of covariates associated with vector of random-effects $u_i|_{c_i=g}$ called u_{ig} whose distribution is now class-specific. In class *g*, the vector u_{ig} of *q* random-effects has a zero-mean multivariate distribution with variance covariance matrix $\omega_g^2 B$ where B is an unspecified variance covariance matrix and ω_g is a proportional coefficient. The measurement errors ϵ_{ij} are independent Gaussian errors with variance σ_ϵ^2 i.e. $\epsilon_{ij} \sim N(0, \sigma_\epsilon^2)$. Finally, $\omega_i(t_{ij})$ is the zero-mean Gaussian stochastic process.

Last, lets denote T_i^* the time-to-event of interest, $\widetilde{T}_i$ the censoring time, $T_i = \min(T_i^*, \widetilde{T}_i)$, and $E_i = \mathbb{1}_{T_i^* \leq \widetilde{T}_i}$. The class specific risk of event in latent class *g* is then described with a proportional-hazard model as follows:

$$\lambda_i(t)|_{c_i=g} = \lambda_{0g}(t) e^{X_{Si1}^T v + X_{Si2}^T \delta_g}$$

where λ_{0g} is a class specific baseline hazard defined according to a vector of parameters ζ_g which can be stratified on the latent class structure ($\lambda_{0g}(t) = \lambda_0(t, \zeta_g)$) or proportional in each latent class ($\lambda_{0g}(t) = \lambda_0(t, \zeta^*) e^{\zeta_g}$). Numerous families of parametric baseline risk functions parameterized by a vector ζ are available of which Weibull, piecewise constant and cubic M-splines are most frequently in use; all three of them restrict parameters to positive values. X_{Si1} and X_{Si2} are vectors of covariates associated with the vector of parameters common over all classes v and the vector of class-specific parameters δ_g, respectively.

In order to avoid the constraints of (i) testing the normality hypothesis for random effects and error term and (ii) linear relationship with longitudinal marker (SCr in the experimental part of current work), the observed data for longitudinal marker can be transformed using different mathematical functions (i.e. rescaled cumulative distribution function of a beta distribution, quadratic I-splines with a different number of knots, thresholds). This transformation is called latent process and accordingly, the model does not take into account the observed data of longitudinal marker but their transformation.

The choice of the optimal number of latent classes is based on Bayesian Information Criteria (BIC) and each patient is *a posteriori* allocated to the class for which he/she has the highest probability of belonging. In contrast to shared random-effects model which may be more appropriate when the interest is in exploring specific assumptions with respect to longitudinal marker trajectory and the influence of longitudinal marker time-evolution on the risk of an event, the joint latent-class mixed model is more useful when aiming to investigate

the longitudinal marker evolution without specific assumptions on its time-trajectories or when developing tools for individual dynamic predictions (39,40). Joint latent-class mixed model relies on the conditional independence assumption of the longitudinal marker and the time-to-event of interest given the latent class.

Boucquemont *et al.* recently used the latent class mixed model approach to identify subgroups of renal function trajectories over time with respect to measured (mGFR) and estimated (eGFR) glomerular filtration rate in 1957 patients with chronic kidney disease (45). Five latent classes of patients characterized with different profiles of renal function time-evolution were identified according to the final covariate-free model: two classes had high mGFR value at inclusion followed by a strong non-linear decline (class 1: “strong decline”, n=11) or a non-linear improvement of mGFR over time (class 2: “Improvement”, n=94) while the three other classes were characterized with a slow and nearly linear decline in mGFR at different levels (class 3: “slow decline at high level”, n=820; class 4: “slow decline at intermediate level”, n=744; class 5: “slow decline at severe level”, n=298). Patients in classes with high baseline mGFR were on average younger (Class 1 and 2) and more frequent male and of African origin (class 1, 2 and 3). The proportion of patients with diabetes, cardiovascular disease or vascular nephropathy was higher in class 4 and 5 while the proportion of those with glomerular nephropathy was the highest in class 1. The proportion of patients with uncontrolled hypertension was the highest in classes 1, 4 and 5 while higher median protein creatinine ratio was observed in classes 1 and 5. When the analysis was repeated (i) including only patients with at least 2 mGFR assessments or (ii) using absolute change in mGFR (iii) or eGFR as longitudinal marker of renal function, all three models identified subgroups of patients with time-trajectories of renal function similar to those of original model, with optimal number of latent classes being four, three and five respectively. The authors reported that the use of joint latent class mixed model with start of renal-replacement therapy as an event of interest was tested with current data set, but it did not ended-up in successful convergence.

I.3. Predictive tools of graft survival

In a group of 651 adult kidney recipients with a functioning graft at 1st post-transplant year, Shabir *et al.* developed predictive scores for 5-year death-censored and overall graft survival based on demographic and clinical information collected at 12 months post-transplantation (Birmingham score) (46). After confirmation of significant association with death-censored and overall graft survival in multivariate Cox regression, the variables included in the final scores were recipient age, sex and race, acute rejection, estimated glomerular

filtration rate (eGFR) and proteinuria. The developed score indicated good to excellent discrimination in case of death-censored graft failure (area under the ROC curves [C-statistics]: 0.78-0.90) and moderate to good discrimination in case of overall graft failure (C statistics: 0.75-0.81) in three validation cohorts with 736, 787 and 475 kidney transplant recipients, respectively. Compared to use of eGFR alone as a risk factor for graft loss, application of scores significantly improved risk reclassification for death-censored graft-failure (net reclassification improvement [NRI]: 36.1-83.0%, P<0.001 in all validation cohorts), and overall transplant failure (NRI: 38.7%-53.5%, P<0.001 in all validation cohorts). In addition, both scores showed good discrimination (Hosmer-Lemeshow P>0.05 in all validation cohorts).

Gonzales *et al.* evaluated if the previously developed Birmingham score for 5-year graft survival based on clinical factors available at 1-years after kidney transplantation could be improved by incorporating the histological findings or DSA data (modelled as presence vs. absence of DSA, the number of DSA and highest MFI for individual antibody or cumulative MFI for all antibodies in the category). (22) Taking into account histological findings such as presence of glomerulitis or chronic interstitial fibrosis on 1-year surveillance biopsy resulted in higher predictive utility for death-censored graft failure (improved c-statistics: 0.9 vs. 0.84, improved calibration and net reclassification improvement (NRI) of 29% compared to original score) and overall graft failure (improved c-statistics: 0.81 vs. 0.78, improved calibration, NRI of 30.8%). No significant improvement in predictive ability of the score was observed after inclusion of DSA data available at 1 year post-transplantation.

Our group recently developed conditional and adjustable score (AdGFS) for prediction of kidney graft failure up to 10 years post-transplantation using Random survival forest approach to identify and rank covariates predictive of graft failure and include them in the conditional survival tree (47). The final score included 5 baseline variables (pre-transplant NDSA, donor age, serum creatinine and proteinuria at 12 months post-transplantation, k-means cluster for SCr measured over the first 12 months post-transplantation) and two factors collected over patient's follow-up (development of *dn*DSA and onset of first acute rejection). Inclusion of *dn*DSA and first acute rejection developed over time resulted in significant improvement of the predictive ability compared to the score accounting for variables available at baseline only (time-dependent ROC AUC at 10 years: 0.83 (95%CI: 0.76-0.89) vs. 0.75 (95%CI: 0.58-0.82)) and improvement in survival prediction beyond 5 years (p=0.02). Significant difference in 10-years graft-survival (p<0.0001) was observed between four main risk-groups that were identified with respect to the AdGFS value: low risk (AdGFS = 0), intermediate risk (AdGFS: 2 to 4), high risk (AdGFS: 6 to 8) and very high risk (AdGFS: 10 to 12).

Dynamic predictions

As it was seen in previous sub-section, most of developed predictive tools for graft survival in kidney transplantation to date were based on characteristics collected before transplantation, at baseline (usually at the end of the first post-transplant year) or were able to be updated for occurrence of *dn*DSA or AR afterwards. However, when a longitudinal biomarker of disease progression is considered as a potential predictor of an event of interest, as in case of SCr and graft failure, taking into account its whole trajectory over time (i.e. dynamics) is more pertinent over considering its value at a single time-point.

One of approaches that can produce such individual predictions that may be dynamically updated over patients follow-up is the previously described joint latent class model. Typically, based on the measurements of longitudinal marker up to time *s*, the model provides individual dynamic probabilities of experiencing an event of interest for at a horizon of time (*s+t)* after the last available observation of longitudinal marker. The motivating example of such predictions developed from a joint-latent class model is the dynamic prognostic tool for prediction of prostate cancer recurrence using the longitudinal trajectories of Prostate-Specific Antigen (PSA) (39) or the prediction of dementia/death in elderly population using two longitudinal tests of semantic memory (Isaacs Set Test and Wechsler Similarities test) (48), recently proposed by Proust-Lima *et al*. To date, the use of this type of predictions in kidney transplantation has not been reported.

CHAPTER II: Adherence to immunosuppressive medication in kidney transplant recipients

While in everyday speech the term “adherence” is used to denote that someone behaving according to a particular rule, agreement or belief, this term is used in medicine to refer to “extent to which the patient follows medical instructions”. This definition of adherence given by the World Health Organisation (WHO) in 2001 was later updated in order to take into account other types of interventions used in treatment of chronic diseases. According to the newly proposed definition, adherence to long-term therapies is “the extent to which person’s behaviour (taking medication, following a diet or executing lifestyle changes) corresponds with agreed recommendations from a health-care provider” (49,50). Compliance is often used as a synonym for adherence. Whereas adherence refers to patient who actively participates in his or her own health-care management through collaboration and communication with health professionals, compliance refers rather to patient’s passivity in relation to his care. Therefore, the term “adherence” should be preferred over “compliance”.

After solid organ transplantation, the recipient’s immune system would naturally get activated and act against the new organ. Immunosuppressant therapy (IST) is thus necessary to prevent episodes of graft rejection (acute and chronic), which could further lead to impaired graft and patient survival and/or return to dialysis. Calcineurin inhibitors (CNI), i.e. cyclosporine A (CsA) and tacrolimus (TAC), are nowadays used by the majority of transplant centres as the bases of maintenance immunosuppressive regimen, alone or in combination with mammalian target of rapamycin (m-TOR) inhibitors (sirolimus and everolimus) or antiproliferative agents such as mycophenolate mofetil (MMF) and azathioprine (AZA). Although the introduction of CsA in the mid-1970s and TAC during the early 1990s led to reduction in rejection episodes and increase of short-term survival (i.e. within the first year post-transplant) (8), there has been only a marginal improvement in long-term kidney survival over the past decades (10).

Non-adherence remains one of rare modifiable factors that can still be addressed not only to assure better long-term graft survival but also to limit the need for retransplantation and thus increase the number of grafts available for patients on waiting lists. However, the full benefit of kidney transplantation compared to other types of treatment for end stage renal disease (e.g. haemodialysis or peritoneal dialysis) can only be assured with correct intake of prescribed treatment but this usually becomes a serious challenge for a patient who should take daily treatment throughout his/her life. Among the potential factors of poor clinical outcomes after kidney transplantation, the non-adherence to IST is of particular interest. Non-adherence to IST may result in periods of insufficient immunosuppression and/or over-immunosuppression

which may affect both graft and patient survival. Insufficient immunosuppression increases the risk of graft rejection and graft failure, while over-immunosuppression increases the risk of infections and malignancies as well as drug-specific toxicity (24–26,51–54). As reported in meta-analysis by *Dew et al.* (55), kidney transplant recipients have shown the highest rates of non-adherence to IST when compared to other types of organ transplants (i.e. liver, heart and lung).

The prevalence of non-adherence to IST in kidney transplantation is still debated, with studies reporting very different values, ranging from 2% to 67% (50,55–59). However, this discrepancy in reported values of non-adherence is not unexpected given the absence of gold standard of adherence assessment – a unique tool that would be suitable for measuring adherence whatever the condition. As a consequence, not only the methods of adherence assessment vary from one study to another, but also the way in which data are collected and analyzed, the study design and the criteria used to assign a patient as non-adherent are different.

Therefore, a brief summary of methods for adherence assessment and statistical approaches used for analysis and modelling of adherence will be presented followed by the outcomes associated with poor adherence to IST and a short reflection on the limitations of previous studies. Finally, objectives of this work will be presented and discussed later in this chapter.

II.1. Methods for assessing adherence to immunosuppressant treatment

In clinical studies, different measurement techniques of studied outcome are sometimes available to a researcher; the decision on which one to use is often based on multiple factors. In majority of cases, we might intuitively opt for one which provides the biggest accuracy with respect to a measured entity. This, however, does not always stand in reality

In particular, when the measured entity is non-adherence to IST after kidney transplantation, the desired entity we would like to know is “when exactly each prescribed medication was taken”. As it will become evident later in this section, the answer to this question is often complex and requires considering all available experience from previous studies for at least two reasons: first, there is currently no gold standard of adherence assessment (49,50,60) and depending on the specific settings of each study, every method disposes of its own advantages and disadvantages compared to others. Second, non-adherence is notoriously sensitive to method of measurement (61).

For instance, several tools for assessing adherence to IST have been described in literature. According to the WHO (49), all of them fall into one of the three following groups: *(i) subjective ratings of adherence* (physician-reported non-adherence, direct patient interview, self-reported questionnaires, patient-kept diary); *(ii) objective methods of adherence assessment* (remaining pill count, electronic monitoring devices, prescription-refill recording) and *(iii) biological measurements* (determination of drug concentrations, of its metabolites or specific biological markers in blood or urine). Alternatively, methods of adherence assessment can be classified as *direct* which provide the physical proof of drug intake (i.e. all biological measurements or direct observation of patient's medication intake) and *indirect*. These methods are presented further in this section.

II.1.1. Subjective ratings of non-adherence

II.1.1.1. Physician reported non-adherence

Transplant physician, nephrologist or other member(s) of transplant/health care team can be asked to rate/evaluate patient's adherence (62–67). In kidney transplantation, these ratings are typically based on clinical evidence from patient's medical-record history: for example, physician may estimate adherence according to patients IS serum levels or graft rejection episodes, but he/she normally remains blinded to results of other methods of adherence assessment if used in the same study.

In order to evaluate the accuracy of physician-reported non-adherence, Pabst *et al.* explored in a single centre cross-sectional study of kidney transplant recipients its association with other measures of adherence (patient self-reporting, IS serum level variability and biopsy-proven graft rejection). The prevalence of physician-reported non-adherence was relatively low with 22 of 238 patients (9.2%) being rated as non-adherent and there was no association between physicians' ratings of patients' non-adherence and any other method whatsoever (63). The authors concluded that true non-adherence was underestimated when measured with physician rating and acknowledged that physicians tend to use observable cues such as sex and language skills to make inferences about patient's adherence, emphasizing in particular their unintentional discrimination towards female gender (63).

Schafer-Keller *et al.* studied diagnostic value of different methods of adherence assessment, including collateral report of patient's adherence at 1 month after transplantation (estimated by 7 physicians, 4 nurses and 2 medical assistants) and two composite adherence scores (CAS 1: physician report and/or self-reported non-adherence; CAS 2: physician report

and/or self-reported non-adherence and/or non-therapeutic blood-assay variability) using adherence measured electronically as a reference standard (64). Aside from low inter-method correlation which was also confirmed in this study, the results reviled that higher sensitivity can be obtained when collateral-report is combined with other methods of adherence assessment (i.e. 72.1% for CAS 1 and 62.8% for CAS 2) than when used alone (57.9%). However, the increase in sensitivity in this study was counterbalanced by decrease in specificity.

II.1.1.2. Self-reported questionnaires, patient-kept diary and direct patient interview

Self-reporting is the method which is most often used for assessing non-adherence to IST in practice, usually in form of patient interview, adherence questionnaire or patient-kept diary. This approach is simple, not expensive and easy to administer, which consists its main advantages over the other methods of non-adherence assessment and explains its wide use. A special feature of this method, which is not always seen with other methods of adherence assessment, consists in helping to explain patient's behaviour, concerns and attitudes towards medication use, and this can be useful to tailor appropriate and individualized intervention program when non-adherence is suspected. However, reliability is the main question of concern when self-reporting is used to assess adherence. Patients who report poor adherence with IST tend to be honest and describe their behaviour accurately, which may not always be the case for patients who claim to be adherent and to follow their treatment as prescribed (68). Another issue that can affect the accuracy of self-reporting is the way in which the questions are asked. Some patients could feel reluctant to honestly answer the questions that seem to blame him/her for not being perfectly adherent and rather answer in a socially desirable manner which would result in increased number of false negatives. For instance, if a patient is asked the question "Did you take your medication as physician instructed you to do", he or she may respond with "yes" even when the true answer is the opposite. In attempt to overcome these shortcomings, several self-reporting questionnaires were used in kidney transplant patients, including (i) the Morisky 4-items Medication Adherence Scale questionnaire (MMAS-4) (69) and the Morisky 8-items Medication Adherence Scale questionnaire (MMAS-8) (70), (ii) Basel Assessment of Adherence to Immunosuppressive Medication Scale (BAASIS) (60,66,71), (iii) self-reported Immunosuppressant Therapy Adherence Scale (ITAS) (72,73), (iv) Transplant Effects Questionnaire (TxEQ) (63), Compliance Evaluation Test (CET) (65) and Medication Adherence Report Scale (MARS) (74).

Using MMAS-4, in a French prospective cohort of 312 kidney transplant recipients Couzi *et al.* evaluated over 2 years after transplantation the rate and risk factors of non-adherence measured with MMAS-4 (75). In a final multivariate analysis, younger age, lower

number of tablets per day, adverse effects reported by patient and failing to use the developed computer learning software were independent predictors of non-adherence at different times post-transplantation (M3, M6, M12 and M24). Non-adherence (i.e. MMAS 4 score > 0) progressively increased over time from 17.3% at M3 to 34.6% at M24 and there were 6% (n=13) of never-adherent patients (i.e. non-adherent at all four occasions). These results are in line with previous findings of our group where non-adherence to IST (i.e. majority of patients were on combination of tacrolimus and MMF) was measured with MMAS-4 questionnaire (76). Patients were categorized to adherent (MMAS4 = 0), medium-adherent (MMAS-4 = 1 or 2) or non-adherent (MMAS-4 = 3 or 4) according to their score and the percentage of medium-adherent patients increased from 7% at M1 to 20 % at 2 years after transplantation. Finally, the increase over time of non-adherence measured with self-reporting was also reported by Massey *et al*. (60) and De Geest *et al.* (77) separately, using BAASIS score over 18 months and 3 years post transplantation, and by Tsapepas *et al.* using ITAS questionnaire over 4 years after transplantation.

Butler *et al.* compared self-reporting according to MARS and Morisky questionnaire with other methods of adherence assessment (clinician rating, cyclosporine serum levels end electronic monitoring as reference standard) in order to determine the best method for clinical practice. The authors argued that the combination of these two self-reporting measures provides better understanding with respect to dosing and timing dimension of non-adherence and reported that self-reporting of late taking (i.e. timing dimension, categorized to occasionally, quite often and very often) provided the highest sensitivity (85.7%) and specificity (72.5%) among the methods that were studied.

II.1.2. Objective methods of adherence assessment

II.1.2.1. Remaining pill counts and electronic monitoring devices

Pill counts are another method of choice for assessing non-adherence to IST. A strong *a priori* assumption made with this method is that the number of dosage units returned by patient corresponds to the number of unused dosage units. When this is the case, the percentage of non-adherence is calculated by subtracting the number of dosage units which are returned from the number of units issued to the patient with his last prescription over a defined period of time. For instance, the percentage of adherence is 80.0 % for a given patient who has returned 24 tablets of Prograf® (twice-daily formulation of tacrolimus) out of 120 tablets that were issued to him for a 60 days period. While some authors reported that the

actual adherence is overestimated when measured with pill counts as patients might deliberately not bring all unused pills (78), the others argued in favour of its underestimation (79). Pill counts used to be much more popular as the reference standard for validating other adherence measurement tools but they are today over-performed by electronic monitoring which can provide more precise information on adherence with each individual dose and can help to discern adherence time-patterns (79). Therefore, pill counts are nowadays still in use mainly due to its simplicity and low cost (80,81).

Electronic devices called “medication event monitoring systems” (MEMS) with a specific purpose of monitoring medication non-adherence have been recently introduced for assessment of non-adherence to IST after kidney transplantation (82–88). The principle is simple: medication is dispensed in a bottle (or container) whose cap is equipped with a micro-processor which records the number of bottle openings, date and time of each opening. As mentioned before, these devices are in particular useful (i) for overcoming the limitations of other methods with regard to measurement of adherence dosing and timing dimension with each single dose of medication and (ii) for analysis of adherence time-patterns. However, use of these devices in large population studies and over extended time periods is hampered their high cost and bulkiness.

In spite of providing continuous and reliable data on bottle opening, two strong hypothesis have to stand when MEMS are used: first, that one dose of prescribed medication is removed from the bottle at each opening, and second, that the time of bottle opening corresponds to the time of medication ingestion. Accordingly, the true adherence can be underestimated with regard to dosing (i.e. if more than one dose is removed with a single bottle opening) or timing (i.e. the medication is taken off the bottle but not ingested at the time of bottle opening). In contrast, adherence is overestimated if the bottle is opened deliberately or accidentally without taking any medication. By being able to provide higher sensitivity compared to all other methods of adherence assessment, electronic monitoring is nowadays mostly used as a reference standard when different methods are compared with respect to the accuracy of adherence assessment (64,87).

In a longitudinal study of 121 kidney transplant recipients Russel *et al.* examined patterns, predictors and outcomes of immunosuppressive non-adherence measured electronically and corrected for data from MEMS diary (i.e. diary filled-in by patient to document date and time when MEMS were opened without taking any medication) in order to improve data validity (83). On a daily basis, patients were given a score of 0.5 for each morning and evening dose of immunosuppressant taken within 3 hour-window, 0.25 for each dose taken within 12 hour window and 0 elsewhere (i.e. the total score of 0, 0.25, 0.5, 0.75 or 1 per day) and adherence was expressed as the average score over 330 days of follow-up. The authors

identified four main types of adherence patterns: the *highly adherent pattern* with time-average score of 0.99, the *somewhat adherent pattern* with time-average score of 0.70 (i.e. either morning or evening dose taken more than 3 hours earlier/later than prescribed) and two *low-adherence patterns* with average scores of 0.5 and 0.3 respectively. Older age (Spearman's r = 0.25; p=0.005) and self-efficacy (r = 0.31, p=0.0006) were positively correlated with adherence but there was no correlation whatsoever between adherence and outcomes investigated at one year (i.e. serum creatinine, number of infections, acute rejection, chronic rejection and death).

II.1.2.2. Reviewing of patients' prescription refills

Adherence to IST can be assessed based on the history of *patient prescription-refills* issued over a time period, which, for practical reasons, might be of particular interest for centralised and computerized health-care systems from which the prescriptions can be easily reviewed. The number of days that a patient has a medication in his possession is calculated based on the total number of refills and is divided by the total number of days of follow-up period. This fraction is denoted as the Medication Possession Ratio (MPR) (27,89,90). Reviewing of prescription-refills is usually used for assessing adherence in large population studies.

Spivey *et al.* investigated the effect of adherence to CNIs (tacrolimus and cyclosporine) measured as MPR on graft survival and its association with patient characteristics in a population of 31913 adult kidney transplant recipients (89). Median MPR reported in the study was 0.58 with median time post-transplant of 1215 days, and participants were categorized according to MPR quartiles. In addition to these, *Pinsky et al.* also evaluated the economic consequences of non-adherence to CNIs and antimetabolites measured as MPR in a retrospective cohort of kidney transplant patients (27). Adherence at 1, 2 and 3 years post-transplantation was categorized to excellent, good, fair and poor according to quartiles of MPR distribution. Of 11119 participants for whom the cumulative 3-years adherence was assessed, 23.1% were reported as having overall low compliance (i.e. fair or poor adherence at all 3 measures) while only 6.3% had overall high compliance (I.e. excellent compliance at all 3 measures).

Nevertheless, there are several limitations to using patient prescription-refills in assessment of adherence. First, the possession of medication over a time period does not necessarily assure that the medication was used over this period. Second, all missed IST medication is treated equally with this approach.

II.1.3. Biological measurements for adherence assessment – determination of drug concentrations, drug metabolites or specific biological markers in blood and urine

Measurement of drug concentration and drug metabolites in biologic fluids (i.e. blood and urine) or *measurement of specific markers* can be used for assessment of non-adherence. For immunosuppressant drugs, these measurements can be easily implemented as the part of their regular therapeutic drug monitoring (TDM). For instance, calcineurin inhibitors TDM is commonly based on *trough concentrations* (i.e. measurement of drug concentration in blood at its lowest value, just before the next scheduled dose) for tacrolimus and either through concentrations or 2-hour post-dose concentrations for cyclosporine. By assuming that presence of drug and its metabolites in blood is a valid proof of drug intake, the non-adherence can be suspected according to (i) the sub-therapeutic drug levels (i.e. percentage of levels below the target concentration range) (51,64,74,90), (ii) the levels under the lower limit of quantification (64) or (iii) the variability in drug exposure (24,25,51,53,54,91–95). Depending on drug half-life, this method can determine medication intake over a certain time period prior to measurement of concentration, but it is incapable to detect *white coat adherence*, that is, those non-adherent patients who purposefully proceed to correct medication intake before their scheduled clinical visits in order to avoid being blamed for non-adherence (96). It is important to mention that drug levels can also be influenced by numerous factors other than non-adherence, some of which are prescribed individual dose-adjustment, genetic polymorphism of proteins included in its absorption and metabolism, rate of excretion, diet and drug-drug interactions (97). Despite the aforementioned drawbacks, some authors consider that calculating the standard deviation of drug exposure can become the new gold standard for assessing non-adherence to tacrolimus (98). A more thorough reflection on the association of exposure to IST with other methods of adherence assessment and clinical outcomes will be given later in this chapter.

The most often used criteria to discriminate between adherent and non-adherent patients based on exposure to IST include achievement of target concentration (64,74,90), the intra-individual variability of IS exposure (i.e. measured by standard deviation or coefficient of variation in IS exposure) (24,53,54,71,95), difference between the maximum and the minimum measured serum concentration of the last six or the last three measurements between (87,92) or the combination of these criteria (57).

In a group of paediatric kidney recipients Hsiau *et al.* explored the utility of variability in TAC and MPA blood levels (measured as CV% and SD over the first post-transplant year) as a potential marker for medication non-adherence and their association with acute rejection

(54). Significantly higher variability in TAC blood levels was observed in group of patients with rejection compared to the group without rejection with respect to both measures of variability (SD: 5.3 vs. 3.5 p=0.031, CV%: 53.4% vs. 30% p=0.005), but this was not the case for MPA. Using receiver operator curve analysis, the authors established the clinically relevant threshold of 41% for TAC CV% associated with a 10-fold increased risk of acute rejection (OR: 9.7, ROC AUC 0.79, p=0.005).

In a previously mentioned study of Schafer-Keller *et al.* which compared diagnostic accuracy of different methods of non-adherence assessment in 249 adult kidney transplant recipients, adherence with respect to immunosuppressant blood concentration was explored using individual immunosuppressant trough blood levels (i.e. for TAC, MMF and CsA) as well as combination of blood trough levels (64). Non-adherence was defined according to three different criteria: the percentage of sub-therapeutic blood levels variability (the number of sub-therapeutic blood levels divided with the total number of measurements per patient), the percentage of supra-therapeutic blood levels variability (the number of supra-therapeutic blood levels divided with the total number of measurements per patient) and the percentage of non-therapeutic (i.e. the sum of sub- and supra-therapeutic) blood levels variability. The prevalence of non-adherence was 33 % when the non-therapeutic blood levels variability criteria with respect to all three immunosuppressive drugs was used (cut off > 52%). Although different criteria were used in this study for definition of non-adherence according to blood levels of immunosuppressant, all of them showed in general very low sensitivity (i.e. between 30% and 60%) in comparison to electronic monitoring of non-adherence. Nevertheless, overall non-therapeutic assay variability was significantly correlated to self-reported non-adherence measured with the Siegal scale (Spearman's rho: -0.140, $p<0.05$), indicating that the combination of self-reporting methods and methods based on blood concentrations variability might be considered as a potential tool of adherence assessment in future studies.

II.1.4. Combination of different methods of adherence assessment – recommendation from literature

In absence of adherence assessment method that can be applied universally (i.e. gold standard), a combination of two (or more) approaches may be useful. Although more complex, this strategy has recently started to gain popularity in studies with focus on adherence to IST (51,53,62–64,66,71,74,87,90). By selecting two (or more) methods of adherence assessment, the strengths of one method can help to compensate the weaknesses of the other. As a result,

more realistic assessment of non-adherence can be obtained and different components of non-adherence (i.e. irregularity in drug taking, skipping a dose, drug holidays, dose reduction and discontinuation of drug taking) can be evaluated.

The combination of one subjective (i.e. self-reporting or physician-reporting) and one objective method of adherence assessment (i.e. immunosuppressant drug levels) which is currently recommended by the WHO (49) is frequently used in practice (51,53,71,74,92).

Griva *et al.* explored the association between sub-target IS levels and self-reported non-adherence measured with Medication-Adherence Report Scale as total, intentional and unintentional non-adherence (MARS-Total, MARS-Intent, MARS-Forget) assessed as both continuous (score range: 5 to 25) and dichotomized variable (score < 24), where higher adherence is represented by higher score (74). Three measurements of TAC and CsA serum concentrations (i.e. the measure at study entry and the one most recent preceding/following measure) were used to assess non-adherence according to serum IS levels and patients were classified as *"achieving target"* if at least two of three measurements reached clinical targets and *"not achieving target"* elsewhere. The authors reported higher overall non-adherence rate when measured with self-reporting compared to serum IS levels (51% vs 25%), higher unintentional compared to intentional non-adherence (62.4% vs.13.8%, McNemar X^2, p<0.001) and significant association of non-adherence according to IS serum levels with total (p=0.005) and unintentional self-reported non-adherence (p=0.003). Interestingly, patients on CsA were more likely to have sub-target concentrations than patients on TAC (37.8% vs. 18.2%, p=0.005).

In a cross-sectional study of 209 first kidney recipients, Liu *et al.* investigated the association between MMAS-4 score and serum concentration fluctuations of CNIs (TAC and CsA) and the relationship between time post-transplantation and non-adherence (92). At inclusion, patients were between 3 months and more than 10 years post-transplantation (categories 3 to 6 months, 6 months to 1 years, 1 to 5 years, 5 to 10 years and more than 10 years), and concentration fluctuation was calculated as the difference between the maximum and the minimum serum concentrations among 3 consecutive measures for each CNI separately. Patients who gave an affirmative answer to at least one of four items of MMAS-4 questionnaire (i.e. total with MMAS-4 score > 0) were considered as non-adherent. According to self-reporting, the prevalence of non-adherence was 31.3% and varied significantly between patients with respect to period post-transplantation (the highest: 44.2 % for period 6 months-1 year, the lowest: 5.9% for period after 10 years, p=0.003). A significant difference was observed in TAC serum concentration fluctuations according to post-transplantation period (the highest fluctuations [SD]: 3.11 ng/ml [5.61] for period 3 months -6 months; the lowest: 0.34 ng/ml [0.38] for period after 10 years, one-way ANOVA: p=0.004) and was confirmed in

regression analysis controlling for sociodemographic confounders (age, gender, education, marital status, income and donor type, p<0.001). Last, the significant association between higher fluctuations of blood concentrations and higher self-reported non-adherence was reported for both TAC (Mann-Whitney U-test, p<0.001) and CsA (p<0.001), which remained significant for TAC (p<0.001) but not for CsA in regression analysis.

Tielen et al. explored the attitudes towards immunosuppressive regimen measured with Q-methodology at 6 weeks post-transplantation and their relationship with self-reported adherence assessed with BAASIS interview and within-patient variability (WPV) in whole blood TAC concentrations (i.e. median of 5 measurements per patient, % of WPV categorized to high and low according to 1st and 3rd terciles of distribution with exclusion 2nd tercile) (71). Three main attitudes towards immunosuppressive regimen were determined in 90 participants: *confident and accurate* (n=40), *concerned and vigilant* (n=38) and *appearance oriented and assertive* (n=12). According to BAASIS interview, 17 % of patients were non-adherent and no significant association of self-reported non-adherence with medication attitudes or within-patient variability in TAC blood concentration was found, but a significant association was reported between higher WPV (i.e. the patients from the 3rd tercile) and "*concerned and vigilant"* attitude (X^2 test, p = 0.036).

Combination of more than two methods of adherence assessment was also reported in kidney transplantation mainly to evaluate diagnostic accuracy of different adherence assessment methods according to reference standard (64,87). In this context, we already discussed the study of Schafer-Keller *et al.* earlier in this chapter (64).

In effort to evaluate the traditional and everyday measures of cognitive ability as potential predictors of non-adherence to TAC and CsA and employment status in kidney transplant recipients, Gelb and colleagues crossed three different methods of adherence assessment (90). These included self-reporting based on Transplant Effects Questionnaire (TxEQ), review of prescription refills expressed as medication possession ratio and CNI serum concentrations. Three most recent TAC and CsA serum concentrations prior to neuropsychological testing as measured by drug concentrations at two hours post dose (C2) were used to classify patients as "achieving target" if at least two of them reached the target range and "not achieving target" elsewhere. Serum CNI concentrations were correlated with prescription refill data expressed as MPR (Kendall's tau-b r=0.24, p<0.05) but not with self-reported non-adherence measured with TxEQ.

As seen above, combining different methods of adherence assessment is not unusual in clinical practice, and it is strongly recommended due to absence of gold standard (49,50,61). In studies with focus on non-adherence after kidney transplantation, the authors mainly opted

for the combination of blood IS levels and one self-reporting method. However, due to poor between-method correlation which was reported, further studies are needed to (i) confirm the results obtained with previously used combinations and (ii) explore other combinations of methods of adherence assessment.

II.2. Association between non-adherence and clinical outcomes in kidney transplant recipients

The association between adherence to IST and different outcomes has been extensively described in literature (24,25,27,51,53,54,62,67,71,73–75,83,89,94,95,99,100), with vast majority of previous studies focusing on the association between non-adherence to IST and clinical outcomes (i.e. acute rejection, development of *de-novo* DSA) or graft survival.

While some studies reported significant association between adherence to IST and onset of graft rejection (27,51,53,54,99,100), other studies failed to prove this association (62,71,73–75,83). Recently, in a cross-sectional retrospective study of renal transplant patients, Scheel *et al*. reported that late acute rejection (i.e. occurring after 6 months post transplantation) was associated with higher percentage of sub-therapeutic IS trough levels in a logistic regression model (OR: 6.136, 95% CI: 1.524 – 24.708), but not with percentage of IS trough level variability and self-reported non-adherence measured with BAASIS questionnaire (51). No association was observed between any of three methods of non-adherence assessment used in current study.

In a prospective cohort of kidney transplant recipients, non-adherence (assessed with ITAS score as a time-dependent covariate over 48 months post-transplantation) was the single most important predictor of biopsy proven acute rejection (BPAR) in a Cox stepwise regression ($p<0.001$, HR not reported) (99). The authors also reported that the percentage of death-censored graft failure was higher in the group of non-adherent patients when compared to the group of adherent patients (78.4% vs 7.8%, $p<0.001$). These results are in partial concordance with other studies where poor adherence was associated with increased mortality but not with BPAR or number of AR episodes (62,73).

Meta-analysis performed by Butler *et al.* on ten cohort studies revealed that a median of 36% of kidney graft losses (IQ range 14%-65%) were associated with prior non-adherence to IST (57). In the same study, the pooled random effects odds ratio for graft failure was seven-fold greater in non-adherent patients compared to adherent patients (random effects combined OR: 7.1; 95% CI: 4.4%-11.7%).

Prihodova *et al.* explored the association between adherence to IS measured as combination of patients self-report and clinicians estimate in the first post-transplant year and occurrence of graft loss/mortality in the period from 3 to 11 years following the measurement of adherence (mean follow-up 7.1 years) in 325 kidney transplant recipients (62). Poor adherence was significantly associated with higher risk for graft loss (HR: 6.03, $p<0.05$) and patient mortality (HR: 3.07, $p<0.05$) in the Cox regression model.

In a large population study of kidney transplant recipients, Pinsky *et al*. investigated economic consequences and transplant outcomes associated with poor adherence to IST (27). Adherence was assessed with prescription refills as the percentage of medication possession ratio (MPR) at the end of the first, the second and the third post-transplant year and was categorized according to MPR 75th percentile, median and 25th percentile as poor, fair, good and excellent. Acute rejection was associated with a decreased likelihood of persistent excellent compliance in the first year after transplantation (adjusted OR: 0.81, $p=0.04$) in a logistic regression model. Compared to patients with excellent adherence, fair adherence was associated with increased risk of dying and increased risk of graft failure (HR for death: 1.54; 95% CI [1.19-2.00]; HR for graft failure: 1.63, 95% CI: [1.37-1.93]) while poor adherence was associated with increased risk of graft failure only (HR: 1.80, 95% CI [1.52-2.13]). The authors also reported that low adherence was associated with $12 840 increase in individual 3-year medical costs.

II.3. Limitations of previous studies – personal point of view

Herein, some principal limitations of previously discussed studies are revealed and, if available, a solution for their overcoming are proposed:

- Adherence assessment was usually based on a single time point, and patients were sometimes at very different time at inclusion even within the same study (i.e. ranging from several months to tens of years post-transplantation); longitudinal assessment of adherence over time should receive more attention in future studies;

- Categorisation of adherence data was performed according to different cut-off values that are not clinically justified; this can result in increased bias and reduced statistical power, and thus, continuous measure of adherence should be preferred (101);

- There was a big discrepancy in goals that were investigated in previous studies; while the predictors of non-adherence to IST and the relationship adherence-clinical outcomes were explored in overwhelming majority of studies, adherence time-evolution received only marginal attention;

- Due to predominant cross-sectional and/or retrospective design of previous studies, it was not possible to establish the causality between adherence to IST and different outcomes; further prospective studies should help in overcoming this drawback;

- Most importantly, the poor methodological concept of studies is evident in terms of statistical analysis and modelling approach and need to be improved; in contrast to basic statistical tests or simple regression techniques (i.e. linear, logistic or Cox regression) used in numerous studies to explore predictors and outcomes of non-adherence , there is now a need for a shift towards models which would provide deeper insight into the dynamic nature and the evolution of adherence to IST in kidney transplant recipients.

In summary, providing a satisfactory description of adherence data, with respect to IS use in kidney transplant recipients, and determining the relation of adherence with exposure and outcomes is more complex than it may initially appear. Herein, it should be kept in mind that the adherence to IS can be considered both as a cause and as a consequence (outcome) of drug response. Accordingly, only by studying the inter-association between adherence,

exposure and outcomes together, we might be able to fully understanding their impact on kidney transplant recipients.

II.4. Objectives of the thesis

In current work, we aimed to investigate the factors associated with poor long-term graft survival in kidney transplant recipients. Two main studies were conducted in this context.

In the first part of this work, by using the repeated measurements of SCr as a surrogate marker of kidney function, we investigated the existence of homogenous groups of patients characterized with specific evolution with time of SCr and group-specific risk for graft failure by jointly analysing longitudinal and time-to-event data. Herein, we hypostatized that on average higher SCr time-profiles over the first 18 months post-transplantation would be associated to higher risk of long-term graft failure. We further investigated the impact of individual factors on evolution with time of SCr and risk for graft failure. Since many previous studies evoked the important impact of development of *de novo* DSA on graft survival, we aimed to develop individual predictions of graft failure for patients who did as well as in patients who did not develop *dn*DSA over their follow-up.

In the second part of this work, we evaluated the inter-association between patient-reported non-adherence, TAC concentration-time profiles and treatment efficacy. Herein, our main assumption was that the non-adherent patients would, on average, have higher variability in TAC concentration-time profiles and/or lower concentration-time profiles compared to adherent patients. Thus, the objective of this part of work was threefold. First, we aimed to describe the evolution with tile of TAC through level concentrations (C_0) and analyse the variability in TAC C_0 over the two years post-transplantation period in kidney transplant recipients. Second, we investigated the association between variability in TAC C_0-time profiles of patient-reported non-adherence. Last, we studied the association of TAC C_0-time profiles and/or variability in TAC C_0-time profiles with the efficacy outcome defined as the first acute rejection onset during the 2 years post-transplantation.

EXPERIMENTAL WORK

CHAPTER III: Joint modelling of longitudinal evolution of SCr and 10-years kidney graft survival

Abstract:

Chronic kidney disease (CKD) is a world-wide recognized health problem, leading progressively to the end stage renal disease (ESRD) and kidney failure. Kidney transplantation remains the best treatment option for patients with ESRD, resulting in improving patient's quality of life and reduced morbidity and mortality together with reduced treatment expenses in comparison to other available options (1,2). Unlike the recent improvement in short-term kidney graft survival due to introduction of potent immunosuppressant drugs, the long-term kidney graft survival improved only marginally within the last few decades (3–5). Two types of data are typically collected in kidney transplant recipients over their follow-up: measurements of longitudinal marker such as serum creatinine (SCr) collected repeatedly over time and data collected at a given time or once during the follow-up. From a methodological standpoint, these two types of data are frequenlty analyzed separately, ignoring the fact that the kidney graft function is often deteriorated long before graft failure and consequentially, that the evolution with time of SCr as the surrogate marker of graft function is possibly associated with this failure. In addition, studying the association of graft failure and its potential predictors, the majority of previous studies focused only on factors collected at baseline (i.e. known at time of transplantation or at 1st transplant anniversary) without considering longitudinal markers of kidney function or factors that can occur after 1 year post-transplantation, such as development of *de novo* donor-specific anti-human leukocyte antigen (HLA) antibodies (*dn*DSA) and onset of late acute rejection (6–10).

In the retrospective cohort of kidney graft recipients transplanted in University Hospital of Limoges (CHU Limoges), joint latent class mixed model approach was used to describe the evolution with time of SCr and identify groups of patients (i.e. latent classes) with homogenous SCr time-profiles over the first 18 months post-transplantation on one hand, and to evaluate impact of SCr time-profiles and other individual factors on 10-years graft failure, on the other. The developed model identified 3 latent classes of patients with respect to time-trajectories of SCr: two latent classes with stable SCr time-profiles over the 18 months post-transplantation and higher (class 2, 63.6 % of patients) or lower baseline SCr value (class 1, 30.7% of patients) and a latent class characterized by a steep increase in SCr over the first 18 months post transplantation (class 3, 5.7% of patients) and associated with significantly higher risk of 10-years graft failure compared to class 1 and class 2 ($p<0.0001$). The ability of model to discriminate between these three latent classes was satisfactory as indicated by high mean posterior probabilities of belonging to each class, ranging from 82.6% to 89.2%, Donor age contributed significantly to explain latent class membership. In addition to latent classes, other

factors associated with higher risk of graft failure included proteinuria at M12 and pre-transplant anti-HLA antibodies as baseline covariates and the interaction term between developed *dn*DSA and onset of AR over the patient's follow-up. The model suggested that deleterious effect of AR on graft survival is much more important in patients who previously developed *dn*DSA. Finally, the developed model was used to provide individualized predictions of the probability of graft failure at different time horizons and a good accuracy was obtained in group of patients who did not develop *dn*DSA.

Individualized prediction of kidney graft failure: development and application of a joint model for longitudinal evolution of serum creatinine and 10-years kidney graft survival

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INTRODUCTION

In kidney transplantation, a new challenge in modelling is individualized prediction of graft failure risk over time. Up to now, no study has reported such a model to access the individual risk and its evolution with time. Numerous risk factors of kidney graft failure are known: factors linked to donor (e.g. age, cause of death, serum creatinine, living or deceased donor, cause of death) (4,9,11–15), to transplantation (e.g. cold ischemia time, transplantation rank) (13,16,17) and to recipients (demographic, clinical, immunological and biological factors) (8,10,13,15,18–23). Composite criteria (e.g. Expanded Criteria Donor – ECD) were proposed to define the suitability of organ donor (17,24). Several recent studies have identified donor-specific anti-HLA antibodies (DSA) and chronic antibody-mediated rejection as a primary causes of late allograft failures (10,19,20,25). Consequently, a particular interest has been brought on the development of new drugs focused on modifying B-cells and alloantibody responses. Ways to improve graft survival in patients who do not develop DSA are less studied although as many graft failures are observed in patients without DSA (26).

Prediction of graft failure is a topic of interest in patients with, as well as without DSA. Currently, there are very few new immunosuppressant (IS) drugs for prophylaxis of graft rejection in kidney transplant recipients. As there are few clinical trials for novel IS drugs they should include patients at high risk of graft failure. Ideally, it would be necessary to know early after transplantation the long-term graft failure risk for each patient. This requires predictive models of graft failure using data collected before and after transplantation.

Graft failure was found associated with SCr in studies taking into account SCr levels measured at specific time-points and/or SCr linear evolution with time after transplantation (i.e. usually SCr slopes between two measurements only) (14). It seems more appropriate to consider the whole dynamic history of SCr (i.e. SCr evolution with time) as a predictor of graft failure risk for individualized predictions.

Latent class models and joint models are innovative statistical tools which allow studying the heterogeneity in the individual time-trajectories of SCr and its impact on the graft failure risk. The ability of latent class models to identify patient subgroups who share common characteristics of renal function time-trajectories was demonstrated in chronic kidney disease by Boucquemont *et al.* (27). Interestingly, these authors showed that their model was appropriate to adequately describe the population heterogeneity without introducing any covariate.

The joint models allow to study the association between evolution of markers over time (i.e. time-trajectories of continuous variable), fixed covariates (i.e. individual factors collected at a given time) and onset of an event (14,28)**.** Statistical developments in the joint modelling area rely either on the shared random-effects models that include characteristics of the longitudinal marker as predictors in the model for the time-to-event (29,30) or on the joint latent class

models which assume that the population is heterogeneous and can be parted into several homogeneous subgroups (corresponding to several latent classes), with a class-specific time-evolution of the marker and a class-specific risk of the event (31,32). Interestingly, Fournier *et al.* reported a shared random effect model to analyze the relationship between time-varying renal serum creatinine beyond the first year post-transplantation and graft failure (14). Whatever the post-transplantation time, they found that current value and current slope of SCr were associated with the risk of graft failure up to 13 years after transplantation. Of note, the same linear model and the same survival model were used for all patients. A limitation of the Fournier et al's study is that it did not consider the impact of onset of *de novo* DSA (*dn*DSA) on graft outcome. No joint latent class model has been developed previously to predict graft failure.

As several papers reported models predictive of graft failure using data collected up to one year after transplantation, it seemed relevant (i) to jointly model the change of serum creatinine over at least the first year post-transplantation and the graft failure risk in this period and (ii) to investigate in such a model the impact of individual factors reported as associated with this change of SCr and/or graft failure risk (of which donor age, cause of death of the donor, presence of anti-HLA antibodies before transplantation and development of *dn*DSA post-transplantation) (7,8,10,33). Therefore, the objectives of the present study were (i) to develop a joint latent class model investigating the impact of serum creatinine time-trajectories and onset of *dn*DSA on graft survival and (ii) to study the possibility of individualized risk prediction of kidney graft failure in patients with and without *dn*DSA.

MATERIAL AND METHODS

Study population

Data was extracted from the retrospective cohort of kidney transplant recipients grafted at the University Hospital of Limoges (France) between 1984 and the end of 2011 (n=819). Among these patients, 616 who had sufficiently data with a clinical and immunological follow-up of at least one year were included in the study. A flowchart showing patient selection is presented in Figure 1.

All patients received an induction therapy. The maintenance immunosuppressive regimen consisted of triple therapy in general, including one calcineurine inhibitor (i.e. cyclosporine or, since 2001, tacrolimus), one anti-metabolite (i.e. azathioprine or, since 1996 mycophenolate mofetil) and corticosteroids mainly stopped after 3 to 6 months post-transplantation.

The study database was approved by the French Informatics and Liberty National Commission (CNIL, registration number 1795293). All the grafts came from heart-beating deceased donor. More details about the patients included in current study can be found in a previous work of our group (15).

Outcomes and study endpoint

Graft failure, defined as return to dialysis or pre-emptive retransplantation was used as the outcome variable and was studied as the event of interest during the first 10 years post transplantation period. Death was considered as a censored event when the recipient died with a functioning graft. When graft function was unknown on the exact date of death, the patient's follow-up was censored on the date of his/her last biological assessment before death. For patients who died because of graft loss, death was considered as a graft failure.

Available variables

Donor-specific variables were age and cause of death (categorized to vascular, traumatic vehicle accident, traumatic non-vehicle accident and other). Transplantation-related variables included cold ischemia time, transplantation rank and transplantation period (from 1984 to the end of 1993, from 1994 to the end of 2002 and from 2003 to the end of 2011). Recipient-specific variables included: recipient age at transplantation, gender, onset of non-donor specific anti-human leucocyte antigen antibodies before transplantation [anti-HLA], initial immunosuppressive regimen and proteinuria levels between month 12 and month 18 after transplantation (in case of missing data for proteinuria at month 12 after transplantation, the first value collected between month 12 and month 18 was used). Additionally, variables collected over the patient follow-up were taken into account: repeated measures of serum creatinine [SCr] within the first 18 months after transplantation (for the majority of patients at M1, M3, M6, M12 and M18, median number of SCr measurements: 5, range: 2-8), diagnosis of the first acute rejection episode [AR] and onset of *de novo* donor specific anti-HLA antibodies [*dn*DSA].

Anti-HLA antibodies were screened and identified using Luminex® solid-phase assay (One Lambda LABScreen assays) in samples collected before transplantation, at three, six and twelve months post-transplantation and annually thereafter or whenever clinically indicated. All sera collected and tested using the Complement Dependent Cytotoxicity method prior to availability of Luminex® technology in our center (2007) were reanalyzed using Luminex®. Since DQ, DP and C HLA typing was not previously systematically performed in our center, a molecular DNA typing of donor and recipient was performed in case of detection by Luminex® of an anti-HLA-C, -DQ or –DP antibodies during the survey in order to determine antibody's specificity (i.e. DSA vs. non DSA). DSA diagnosis prior to kidney transplantation was an exclusion criterion for transplantation in our center. Patients in whom the Luminex® reanalysis identified presence of DSA before transplantation were excluded from the database studied

Donor, recipient and transplant characteristics are presented in Table 1.

Statistical analysis

Joint Latent class model

A joint latent class model for a longitudinal outcome and a right-censored (left-truncated) time-to-event outcome was developed in the 'lcmm' R-Package entitled the Extended Mixed Models Using Latent Classes and Latent Processes, version 17.8 (available at https://cran.r-project.org/web/packages/lcmm/lcmm.pdf). This model considers the population of subjects as heterogeneous, and assumes that the population consists of a finite number of homogeneous subgroups (so called latent classes because they are not directly observed). Each latent class is characterized by a class-specific marker trajectory (e.g. a class-specific time-trajectory of SCr) and a class-specific risk of the event (e.g. the graft failure) (32,34). Thus, this type of joint model is constituted of three submodels: (1) a multinomial logistic submodel aiming to calculate each patients probability of belonging to each latent class, (2) mixed effect model to describe the marker time-trajectories specific of each class (3) survival submodel to describe the risk of event specific of each class. The individual probability of belonging to a given class can be partly explained by covariate(s) included in the multinomial logistic regression submodel. A general mathematical representation of these sub-models, as well as R codes can be found elsewhere (27,35).

The model was constructed in a step-by-step procedure. The first step of model building aimed to define (i) a mixed-effects model for the SCr trajectories, (ii) the baseline risk function and (iii) the number of latent classes (1 to 5). Thus in first, a latent class mixed effects model with only one latent class (i.e. which reduces in this case to a classical mixed effects model) was developed to describe the SCr time-trajectories. Linear and quadratic functions of time post-transplantation with correlated random effects were tested. Different link functions were compared to transform the observed SCr values into a Gaussian latent variable (i.e. herein, the unobserved kidney function): (i) a linear transformation, (ii) a rescaled cumulative distribution function of a beta distribution (iii) quadratic I-splines with equidistant nodes and (iv) quadratic I-splines with nodes located at the quantiles of marker distribution. The most appropriate link function was selected on the basis of goodness-of-fit as measured by the discretized Akaike criterion (dAIC) (36). Then, in a joint model (with one latent class), the risk of graft failure was modelled using a parametric proportional-hazards model. Weibull, piecewise constant and M-splines baseline risk functions were tested and compared using the AIC criterion. The joint latent class model was estimated for a number of latent classes varying from 1 to 5 and the Bayesian information criterion (BIC) was used to compare them (35). As previously recommended, the analyses were repeated using different combinations of starting values to minimize the chance of the models converging to a local maximum. The conditional independence assumption of the SCr repeated measures and time to event given the latent class structure was studied using a Chi-square statistics.

In the second step, the impact of covariates as well as the impact of their interaction on (i) the specific class-membership, (ii) both the SCr trajectories and the graft failure risk for each class,

was studied through fixed effects common within all classes and/or class-specific effects. If the onset of *dn*DSA was retained as covariate, its impact would be studied by taking into account several post-*dn*DSA follow-up periods because associated adverse effects are known to be delayed from their onset. After identification of the best-fitting model, the posterior probability for each patient of belonging to each class was calculated, and each patient was *a posteriori* classified in the class to which he/she had the highest probability of belonging (35). The criteria for final model selection were the BIC and the highest mean posterior class membership probabilities which assess the ability of the model to discriminate between the different latent classes. Finally, the predicted class-specific survivals were compared with the observed survivals within the same classes using Kaplan-Meier analysis.

Because certain research teams studied the factors predictive of short-term graft survival (10), we also analyzed the factors predictive of 5-years graft survival. Numerous studies having investigated the predictive factors of graft failure among the individual factors known up to one year post-transplantation, the final joint model was compared to a model including follow-up data collected up to 1 year post-transplantation only.

Individual predictions in an independent patient group

Data from independent group of 80 patients (the second database) grafted and followed-up in another French transplant center (CHU Tours, Astre database approved by the CNIL, Authorization number DR-2012-518) was used to calculate individual predicted probabilities of graft failure for different horizon times using the developed joint model. (37). Individual predictions were performed in 60 patients who did not develop *dn*DSA (of which 36 with graft failure) and 20 patients who developed *dn*DSA. As in the main cohort which was used for model development, all patients used for computation of individual predictions were also without pre-transplant DSA.

Sensitivity analysis

In the studied cohort, the cumulative incidence of *dn*DSA was of 9.7%. This was a low value although in accordance with previous studies showing a 5-year post-transplantation cumulative incidence of *dn*DSA from 5.5 to 20% (38,39). Because previous studies have reported cumulative incidence of dnDSA up to 20% and in order to increase the accuracy and the statistical power to study patients with *dn*DSA, we repeated the analysis (development of joint model as previously described) after adding to the initial database data collected in 85 independent patients who developed *dn*DSA and had been grafted in another French transplant center (CHU Tours).

RESULTS

Among the 616 patients studied, graft failure was observed in 68 (11%) patients over the 10 years of follow-up (incidence per 1,000 person-years, 16.8; 95% CI, 13.1 to 21.3). The median

follow-up time in patients up to graft failure was 4.97 years (range: 1-10). Among 548 event-free patients, median follow-up time was 7.13 years (range: 1-10). In the cohort, there were 56 deaths with a functional graft. There were 60 incident cases of *dn*DSA (incidence per 1 000 person-years, 14.8; 95% CI, 11.3 to 19.1; median time of onset 3.93 years; range: 0.02-9.8). In the 556 patients who did not developed *dn*DSA, graft failure was observed in 56 patients (11.2%) over the 10 years of follow-up. The median follow-up time to graft-failure in these 556 patients was 4.39 years (range: 0.94-10). One hundred and thirty five patients were treated for a first acute rejection episode over the whole study period, 121 (90%) of which were biopsy proven. T-cell mediated rejection (TCMR) was evidenced in 104 patients, antibody-mediated rejection (ABMR) in 14 patients, and mixed rejection (TCMR+ABMR) in 3 patients. Ninety four first rejections occurred within the first year post-transplantation.

The SCr values were fitted after transformation with a I-spline link function with 5 equidistant nodes since it provided the lowest dAIC (dAIC=35277, i.e. corresponding to at least 114 points less than with the other tested transformation functions). The time-trajectories of SCr after transformation were best described using quadratic function of time to allow non-linear mean trajectories over time. The baseline risk function was modelled parametrically using a two-parameter Weibull baseline risk function since piecewise constant and M-spline functions did not converge. The joint latent class models including 2 and 3 latent classes provided the same best BIC (BIC=36239 and BIC=36238 for 2- and 3- class model, respectively). The conditional independence assumption was rejected for the model with 2 latent classes ($p<0.05$) and the convergence was not reached with the 4-class model. Thus, the joint latent class model including three latent classes for which the conditional assumption was not rejected ($p=0.2762$) was retained. None of aforementioned covariates showed a significant effect on time evolution of SCr in the final joint model (i.e. neither class-specific effect nor common effect over all classes). The class-specific risks of graft failure were described using as covariates, presence of anti-HLA antibodies before transplantation, proteinuria between M12 and M18 greater than 0.275 g/L (yes/no), interaction between onset of acute rejection and development of *dn*DSA (yes/no). The donor age (categorized as greater or not than 60 years) contributed to explain latent class membership with the recipients of kidneys from donors older than 60 years having significantly more probability to be allocated to class 2 and class 3 compared to class 1. The mean posterior probability of belonging to each class ranged from 82.6% in patients allocated to class 1 to 89.2% in class 3, indicating a clear discrimination between the latent classes. Of note, this model including rejection and *dn*DSA data collected over the follow-up outperformed a joint model taking into account data collected up to the end of the first year post-transplantation only ($p=0.001$). This comparison illustrated the added value of the *dn*DSA data collected after one year post-transplantation. The mathematical representation of the final joint latent class model is given in the Equations 1, 2, 3 and 4, respectively.

$$P(c_i = g|X_{ci}) = \frac{e^{\xi_{0g}+DONOR\_AGE60_i\xi_{1g}}}{\sum_{l=1}^{G} e^{\xi_{0l}+DONOR\_AGE60_i\xi_{1l}}}\ , \quad (1)$$

$$\Lambda_i(\mathrm{t})|_{c_i=g} = \beta_{0g} + \beta_{1g} * TIME + \beta_2 * TIME^2 + u_{0ig} + u_{0ig} * TIME + \epsilon_{ij}\ , \quad (2)$$

$$H(Y_{ij};\eta) = \Lambda_i(t_{ij}) + \varepsilon_{ij}\ , \quad (3)$$

$$\lambda_i(t)|_{c_i=g} = \lambda_{0g}(t)e^{HLA*v_1+PROT*v_2+AR*v_3+DSA*v_4+AR*DSA*v_5}\ , \quad (4)$$

where $\xi_{0g}$is the intercept for class *g* and $\xi_{1g}$is the effect of donor age on the class membership probability $P(c_i = g|X_{ci})$ in a multinomial logistic sub-model (Eq. 1); β_{0g}, β_{1g} are the intercept and the linear term for class *g*, β_2 the class-common quadratic term of time, u_{0ig} and u_{1ig} the class-specific random effects for the intercept and the linear term, and ϵ_{ij} the measurements errors which determine class-specific trajectories of latent variable $\Lambda_i(\mathrm{t})|_{c_i=g}$ in the latent class mixed-effects sub-model for (Eq. 2); $H(Y_{ij};\eta)$ corresponds to the I-splines with 5 equidistant knots link-function used to transform the observed SCr values into a latent variable (Eq. 3); $\lambda_i(t)|_{c_i=g}$ is a class-specific risk of graft failure, λ_{0g} is a baseline risk of event in class *g* (a 2-parameter Weibull risk function used in our case) while v_1, v_2, v_3, v_4, and v_5 are the mean regression coefficients corresponding to the explanatory variables in the survival sub-model (Eq. 4). The final joint model estimates and their corresponding 95% CI are reported in Table 2.

Figure 2 shows the estimated trajectories re-translated into SCr and the associated predicted event-free survival for each class. Class 1 with 189 patients (30.7%) was characterized by a mean SCr value close to 100 µmol/L at inclusion, a slow decrease in SCr within the first 18 months post-transplantation and a mean risk of graft failure at 10 years post-transplantation close to 5%. Class 2 corresponding to the majority of the patients (n=392, 63.6%) was characterized by a mean SCr value close to 150 µmol/L at inclusion and a stable mean trajectory over the first 18 months post-transplantation while the mean risk of graft failure at 10 years post-transplantation achieved 10%. In comparison with class 1, it was associated with a significant increase in the observed incidence of graft failure at 10 years post-transplantation (log-rank test, p=0.0346). Finally, class 3 with 35 patients (5.7%) was characterized by a mean SCr value close to that of class 2 at baseline followed by a rapid rise of SCr within the first 18 months post-transplantation. In comparison with class 1 and class 2, it was associated with a significant increase in the observed incidence of graft failure (p<0.0001). The mean risk of graft failure at 10 years post-transplantation in this class was 100%, and no subject in this class had a graft survival greater than seven years.

Since two dominant latent classes (i.e. class 1 and class 2) from the final model englobed almost 94% of patients and showed similar 10-years graft-survival, we also performed a

separate Kaplan-Meier survival analysis for patients in these two classes excluding the patients from class 3. In this scenario, there was still a significant difference in the 10-years cumulative incidence of graft-failure between these two major classes (score test: 4.5, p=0.034). Of note, when the two latent class joint model with the same model-specifications was inspected, the repartition of patients within these two classes was 79% and 21% with their corresponding 10-years cumulative incidence of graft-failure of 5% and 43%, respectively (results not shown).

The risk of 5-years graft failure was also studied using the developed joint latent class model. The 5-years risk of graft failure in a three latent class model was significantly associated with serum creatinine latent classes (p<0.0001), proteinuria (p=0.003) and pre-transplant anti-HLA antibodies (p=0.034). Contrary to the 10-years model, the effect of interaction between *dn*DSA and acute rejection was not significant any more.

Individual predictions in an independent patient group

Individual predictions of graft failure up to the end of follow-up were computed for 60 patients from the validation dataset who had not developed *dn*DSA, according to their observed history of SCr and the covariates retained in the final joint model. Using data collected up to 12 months after transplantation, graft failure was adequately predicted in 28 out of the 36 tested patients in whom the graft loss was observed within ten years post transplantation as the 95% confidence interval of the predicted probability of graft failure included probabilities greater than 0.5. In the 24 patients who did not experience graft failure, no event was predicted by our model: the predicted probability of graft failure remained lower than 30% (with an upper limit of the 95% confidence interval<0.5) until the end of the follow-up. Thus, using data collected up 12 months post-transplantation in this patient subpopulation, sensitivity, specificity and overall accuracy of the graft failure prediction at ten years were 77.7%, 100% and 86.6 % respectively. Figure 3 depicts the predicted probability of graft failure in 21 patients randomly selected from this subgroup.

In the 20 tested patients who had developed *dn*DSA, the model predicted an increased risk of graft failure, but the individual risk of graft failure was not adequately predicted for most of these patients. The best and worth predicted curves of graft failure obtained in this patient subgroup are shown in Figure 4.

Sensitivity analysis

When 85 patients with *dn*DSA followed in a second center were added to the database initially used for model development, the best joint latent class model identified again three latent classes with similar SCr time-trajectories and similar graft survival to those obtained in our main analysis (Figure 5)

The retained covariates were presence of anti-HLA antibodies before transplantation (p=0.031), proteinuria between M12 and M18 (p=0.002), interaction between onset of *dn*DSA

and acute rejection (p= 0.038), i.e. the same covariates as in the joint model developed in the main analysis. Interestingly, adding patients with *dn*DSA led to finding a significant effect of onset of *dn*DSA (p=0.029) on the increased risk of graft failure. Donor age (greater or not than 60 years) contributed significantly to explain latent class membership. The mean posterior probability of belonging to each class ranged from 81.9% in class 1 to 89.8% in class 3. Impact of *dn*DSA on the graft failure was statistically significant from 2 years after their diagnosis (p= 0.006).

DISCUSSION

This study proposes a new approach to predict the evolution over time of individual risk of graft failure based on a joint latent class model. Individualized risk predictions of graft failure were obtained with a good accuracy in patients who did not developed *dn*DSA. The probability of graft failure was increased after development of *dn*DSA, and even more when *dn*DSA were associated with acute rejection in the same patient. However, for patients with *dn*DSA, individual prediction of graft failure risk over time was not obtained with a so good accuracy.

The variables retained in the final model are patient variables routinely collected to manage the optimal renal function in kidney transplant recipients and are classically reported to be associated with graft failure (measurements of SCr and proteinuria, presence of pre-transplant non-donor specific anti-HLA antibodies, *dn*DSA, acute rejection and donor age) (7,8,10,15,21,40,41). The model included an interaction term between *dn*DSA and acute rejection showing, as previously reported, that *dn*DSA are more deleterious for graft survival when the patient has also experienced acute rejection (19). Our study confirms the association between donor age above sixty years and both worse renal function (e.g. high SCr levels) and shorter graft survival (17). In the model developed herein, the proteinuria level observed at one year after transplantation also contributed to explain the graft failure risk. Proteinuria at M12 was previously retained in association with several SCr values determined within the first year post transplantation in the KTFS score aiming to predict the graft survival at 8 years (7).

The main differences between the present model and the previously published tools for graft failure prediction are in (i) use of a latent class modelling approach, (ii) adding *dn*DSA among the variables tested and retained in the survival model for graft failure and (iii) individualized predictions of probabilities of graft loss over time in patients who had not developed *dn*DSA (10,14). The approach of latent class modelling identified 3 homogeneous subgroups (i.e. 3 latent classes) of SCr time-trajectories within the first 18 months post-transplantation with class-specific risks of graft failure. Recently Fournier *et al.* used another type of joint model, so-called shared-random joint model to study the impact of SCr evolution on graft failure (14). Beyond one year post transplantation, these authors found that graft failure risk depended on both the last SCr value and its evolution since the previous measurement (i.e. current slope of

SCr per year). In this shared-random joint model, the inter-individual variability of parameters of the SCr linear model and of the survival model was considered through common random effects. As this type of model does not use the statistical approach of mixture models, the same equations were used for all the patients. On the contrary, in the joint (latent class) mixed model used here, the time-course of SCr and graft survival processes were described with different shapes of SCr trajectories and different mean parameters in each class. The ability of the developed joint latent class model to discriminate between the three identified classes of SCr time-trajectories with specific risk of graft failure was confirmed by high mean posterior probability of belonging to each class.

Overall 10-years survival in current work which included only the patients without presence of circulating DSA on the day of transplantation was 0.82, which is in accordance with the findings from Aubert *et al.*'s study for the similar group of patients (i.e. 7-year survival in the expanded criteria donor and the standard criteria donor groups of patients without presence of circulating DSA on the day of transplantation were 0.85 and 0.90, respectively) (17). Although numerous works highlighted the potential impact of certain DSA classes on graft failure (10,38,42), nearly all reported survival models and scoring systems developed to predict kidney graft survival did not take into account the onset of *dn*DSA (7,8,14). Ignoring the impact of *dn*DSA on the prediction of graft failure risk could lead to underestimating this risk in patients with *dn*DSA and overestimating the risk in patients without *dn*DSA in a long term. In present study, taking into account *dn*DSA improved on average the long-term survival prediction but not the short-term (e.g. 5 years graft survival). Consistently, in a risk model based on the Birmingham score (which incorporates recipient factors at 1 year, including age, sex, ethnicity, renal function, proteinuria, and acute rejection) (8), Gonzales *et al.* (10) found that adding *dn*DSA diagnosed up to 1 year post-transplantation did not improve predictive ability of graft loss by 5 years. This result could be due to a too short time horizon because (i) *dn*DSA occur all over the follow-up and are mostly absent in the first year post transplantation (ii) graft loss attributable to *dn*DSA can occur several years after their onset (43). Recently, significant progress has been made to understand the pathophysiology of DSA-mediated injuries and the determinants of graft loss (22,42,44). Taking into account the characteristics of the DSA (e.g. mean fluorescence intensity, C1q-binding capacity, IgG subclass) and their potential evolution over time may improve individual prediction of graft loss. However, such individual prediction remains challenging in this subpopulation.

Preliminary tests were performed from our model for making individualized risk predictions in distinguishing patients with and without *dn*DSA.

The graft failure risk is less studied in patients who had not developed *dn*DSA. However, most of the kidney graft failures are observed in this group of patients. The frequency of graft failure observed herein (in database used for model development) was similar to the frequency

reported by Terasaki's team (11% allograft loss) with a similar follow-up (median of 94 months) (26). In this population predictive performance of our model seemed high. Using the validation dataset, graft loss was actually observed in all the patients without *dn*DSA for whom the graft failure was predicted by our model (i.e. no false positives). As comparison, a sensitivity of 0.72 and a specificity of 0.71 were reported for the Kidney Transplant Failure Score (7). Although our model might not be appropriate for predictions of graft loss in the global kidney transplant population, it can still be used to generate more than satisfying individual predictions in the majority of this population (i.e. the patients who do not develop *dn*DSA). To our knowledge, the evolution of individual risk-profiles of graft failure is a topic which has not previously received much attention in kidney transplant recipients and it may be of crucial interest for the clinical management of these patients. While we are in an era with very few new therapeutic strategies and new immunosuppressive drugs, individual prognostic tools are necessary for the optimal selection of patients in clinical trials. To demonstrate significant effects of candidate molecules, future trials should focus on patients with poor renal prognoses, and we believe that our model may be a valuable tool for identification of these patients.

Last, our findings should be interpreted by taking into account the limitations of current study. For instance, some factors whose impact on graft survival is well known (such as delayed graft function, type of calcineurine inhibitor, extended criteria donor, recurrent nephropathies, and BKV/CMV infections) were missing in our study. However, we believe that the effect on graft survival of some these factors can be at least partially excluded in current work. As an example, we were unable to directly test the impact of immunosuppressive regimen (i.e. calcineurine inhibitors, corticosteroids, antimetabolites) and their blood levels on graft failure because of dose adjustments and switches from one regimen to another which occurred frequently in patients over such a long study period (from 1984 until 2011). However, we would expect that the different immunosuppressive regimens are at least in part related to different transplantation period, and the period of transplantation was not among the covariates retained in our final joint model. Similarly, 2 of 4 criteria for expended donation (i.e. donor SCr and history of hypertension) were missing in present study but by combining the two remaining criteria in a single dichotomous variable (i.e. donor age ≥60 years or between 50 and 59 years with cardiovascular accident vs. other), we did not observe a better performance of the model than by using donor age alone. Finally, graft failure in present study was defined as the earliest of three events (i.e. return to dialysis, pre-emptive retransplantation or death with non-functional graft) and that the developed joint model was unable to consider the competing risks from different causes of graft failure and/or death. We admit that the proportional sub-distribution hazards model described by Fine and Gray (45) might be a more appropriate approach to use in presence of such competing risks. However, it should be kept in mind that this model relies on the proportional-hazards assumption, which is usually violated for

endogenous time-dependent covariates. To our best knowledge, no previous study in the domain of kidney transplantation reported the use of this type of model with time-dependent variables. Thus, the use of competing risks methodology in the concept of joint latent class mixed models should receive more attention in future works.

CONCLUSION

In conclusion, this study identified 3 groups of kidney transplant recipients with homogenous SCr time-profiles over the first 18 months after transplantation and group-specific risk of 10-year graft failure. The individualized predictions of graft failure risk over time developed from the joint latent class model for patients who did without *dn*DSA were satisfying. Further studies should focus on development of dynamic prognostic tools which would be able to update individual predictions of graft failure risk over patients' follow up whenever new information becomes available, taking into account the measurements of SCr collected after M18 as well.

REFERENCES

1. Laupacis A, Keown P, Pus N, Krueger H, Ferguson B, Wong C, et al. A study of the quality of life and cost-utility of renal transplantation. Kidney Int. 1996 Jul;50(1):235–42.

2. Wolfe RA, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LYC, et al. Comparison of Mortality in All Patients on Dialysis, Patients on Dialysis Awaiting Transplantation, and Recipients of a First Cadaveric Transplant. N Engl J Med. 1999 Dec 2;341(23):1725–30.

3. Meier-Kriesche H-U, Schold JD, Kaplan B. Long-term renal allograft survival: have we made significant progress or is it time to rethink our analytic and therapeutic strategies? Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg. 2004 Aug;4(8):1289–95.

4. Lamb KE, Lodhi S, Meier-Kriesche H-U. Long-term renal allograft survival in the United States: a critical reappraisal. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg. 2011 Mar;11(3):450–62.

5. Gondos A, Döhler B, Brenner H, Opelz G. Kidney graft survival in Europe and the United States: strikingly different long-term outcomes. Transplantation. 2013 Jan 27;95(2):267–74.

6. Nyberg SL, Matas AJ, Kremers WK, Thostenson JD, Larson TS, Prieto M, et al. Improved scoring system to assess adult donors for cadaver renal transplantation. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg. 2003 Jun;3(6):715–21.

7. Foucher Y, Daguin P, Akl A, Kessler M, Ladrière M, Legendre C, et al. A clinical scoring system highly predictive of long-term kidney graft survival. Kidney Int. 2010 Dec;78(12):1288–94.

8. Shabir S, Halimi J-M, Cherukuri A, Ball S, Ferro C, Lipkin G, et al. Predicting 5-year risk of kidney transplant failure: a prediction instrument using data available at 1 year posttransplantation. Am J Kidney Dis Off J Natl Kidney Found. 2014 Apr;63(4):643–51.

9. Brown TS, Elster EA, Stevens K, Graybill JC, Gillern S, Phinney S, et al. Bayesian modeling of pretransplant variables accurately predicts kidney graft survival. Am J Nephrol. 2012;36(6):561–9.

10. Gonzales MM, Bentall A, Kremers WK, Stegall MD, Borrows R. Predicting Individual Renal Allograft Outcomes Using Risk Models with 1-Year Surveillance Biopsy and Alloantibody Data. J Am Soc Nephrol JASN. 2016 Oct;27(10):3165–74.

11. Goldfarb-Rumyantzev AS, Scandling JD, Pappas L, Smout RJ, Horn S. Prediction of 3-yr cadaveric graft survival based on pre-transplant variables in a large national dataset. Clin Transplant. 2003 Dec;17(6):485–97.

12. de Bruijne MHJ, Sijpkens YWJ, Paul LC, Westendorp RGJ, van Houwelingen HC, Zwinderman AH. Predicting kidney graft failure using time-dependent renal function covariates. J Clin Epidemiol. 2003 May;56(5):448–55.

13. Schnitzler MA, Lentine KL, Axelrod D, Gheorghian A, You M, Kalsekar A, et al. Use of 12-month renal function and baseline clinical factors to predict long-term graft survival: application to BENEFIT and BENEFIT-EXT trials. Transplantation. 2012 Jan 27;93(2):172–81.

14. Fournier M-C, Foucher Y, Blanche P, Buron F, Giral M, Dantan E. A joint model for longitudinal and time-to-event data to better assess the specific role of donor and recipient factors on long-term kidney transplantation outcomes. Eur J Epidemiol. 2016 Feb 1;1–11.

15. Prémaud A, Filloux M, Gatault P, Thierry A, Büchler M, Munteanu E, et al. An adjustable predictive score of graft survival in kidney transplant patients and the levels of risk linked to de novo donor-specific anti-HLA antibodies. PLOS ONE. 2017 Jul 3;12(7):e0180236.

16. Debout A, Foucher Y, Trébern-Launay K, Legendre C, Kreis H, Mourad G, et al. Each additional hour of cold ischemia time significantly increases the risk of graft failure and mortality following renal transplantation. Kidney Int. 2015 Feb;87(2):343–9.

17. Aubert O, Kamar N, Vernerey D, Viglietti D, Martinez F, Duong-Van-Huyen J-P, et al. Long term outcomes of transplantation using kidneys from expanded criteria donors: prospective, population based cohort study. BMJ. 2015 Jul 31;351:h3557.

18. Cherukuri A, Welberry-Smith MP, Tattersall JE, Ahmad N, Newstead CG, Lewington AJP, et al. The clinical significance of early proteinuria after renal transplantation. Transplantation. 2010 Jan 27;89(2):200–7.

19. Cooper JE, Gralla J, Cagle L, Goldberg R, Chan L, Wiseman AC. Inferior kidney allograft outcomes in patients with de novo donor-specific antibodies are due to acute rejection episodes. Transplantation. 2011 May 27;91(10):1103–9.

20. Lentine KL, Gheorghian A, Axelrod D, Kalsekar A, L'italien G, Schnitzler MA. The implications of acute rejection for allograft survival in contemporary U.S. kidney transplantation. Transplantation. 2012 Aug 27;94(4):369–76.

21. Everly MJ, Rebellato LM, Haisch CE, Ozawa M, Parker K, Briley KP, et al. Incidence and impact of de novo donor-specific alloantibody in primary renal allografts. Transplantation. 2013 Feb 15;95(3):410–7.

22. Lefaucheur C, Viglietti D, Bentlejewski C, Duong van Huyen J-P, Vernerey D, Aubert O, et al. IgG Donor-Specific Anti-Human HLA Antibody Subclasses and Kidney Allograft Antibody-Mediated Injury. J Am Soc Nephrol JASN. 2016 Jan;27(1):293–304.

23. Lepeytre F, Dahhou M, Zhang X, Boucquemont J, Sapir-Pichhadze R, Cardinal H, et al. Association of Sex with Risk of Kidney Graft Failure Differs by Age. J Am Soc Nephrol JASN. 2017 Jun 7;

24. Rao PS, Schaubel DE, Guidinger MK, Andreoni KA, Wolfe RA, Merion RM, et al. A comprehensive risk quantification score for deceased donor kidneys: the kidney donor risk index. Transplantation. 2009 Jul 27;88(2):231–6.

25. Hourmant M, Cesbron-Gautier A, Terasaki PI, Mizutani K, Moreau A, Meurette A, et al. Frequency and clinical implications of development of donor-specific and non-donor-specific HLA antibodies after kidney transplantation. J Am Soc Nephrol JASN. 2005 Sep;16(9):2804–12.

26. Wu P, Everly MJ, Rebellato LM, Haisch CE, Briley KP, Bolin P, et al. Trends and characteristics in early glomerular filtration rate decline after posttransplantation alloantibody appearance. Transplantation. 2013 Nov 27;96(10):919–25.

27. Boucquemont J, Loubère L, Metzger M, Combe C, Stengel B, Leffondre K, et al. Identifying subgroups of renal function trajectories. Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc. 2016 Dec 28;

28. Daher Abdi Z, Essig M, Rizopoulos D, Le Meur Y, Prémaud A, Woillard JB, et al. Impact of longitudinal exposure to mycophenolic acid on acute rejection in renal-transplant recipients using a joint modeling approach. Pharmacol Res. 2013 Jun;72:52–60.

29. McCrink LM, Marshall AH, Cairns KJ. Advances in Joint Modelling: A Review of Recent Developments with Application to the Survival of End Stage Renal Disease Patients. Int Stat Rev. 2013 Aug 1;81(2):249–69.

30. Asar Ö, Ritchie J, Kalra PA, Diggle PJ. Joint modelling of repeated measurement and time-to-event data: an introductory tutorial. Int J Epidemiol. 2015 Feb;44(1):334–44.

31. Proust-Lima C, Joly P, Dartigues J-F, Jacqmin-Gadda H. Joint Modelling of Multivariate Longitudinal Outcomes and a Time-to-event: A Nonlinear Latent Class Approach. Comput Stat Data Anal. 2009 Feb;53(4):1142–1154.

32. Proust-Lima C, Séne M, Taylor JM, Jacqmin-Gadda H. Joint latent class models for longitudinal and time-to-event data: A review. Stat Methods Med Res. 2014 Feb 1;23(1):74–90.

33. Kaplan B, Schold J, Meier-Kriesche H-U. Poor predictive value of serum creatinine for renal allograft loss. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg. 2003 Dec;3(12):1560–5.

34. Jacqmin-Gadda H, Proust-Lima C, Taylor JMG, Commenges D. Score test for conditional independence between longitudinal outcome and time to event given the classes in the joint latent class model. Biometrics. 2010 Mar;66(1):11–9.

35. Proust-Lima C, Dartigues J-F, Jacqmin-Gadda H. Joint modeling of repeated multivariate cognitive measures and competing risks of dementia and death: a latent process and latent class approach. Stat Med. 2016 Feb 10;35(3):382–98.

36. Proust-Lima C, Amieva H, Jacqmin-Gadda H. Analysis of multivariate mixed longitudinal data: A flexible latent process approach. Br J Math Stat Psychol. 2013 Nov 1;66(3):470–87.

37. Proust-Lima C, Taylor JMG. Development and validation of a dynamic prognostic tool for prostate cancer recurrence using repeated measures of posttreatment PSA: a joint modeling approach. Biostat Oxf Engl. 2009 Jul;10(3):535–49.

38. Hourmant M, Cesbron-Gautier A, Terasaki PI, Mizutani K, Moreau A, Meurette A, et al. Frequency and clinical implications of development of donor-specific and non-donor-specific HLA antibodies after kidney transplantation. J Am Soc Nephrol JASN. 2005 Sep;16(9):2804–12.

39. Wiebe C, Gibson IW, Blydt-Hansen TD, Karpinski M, Ho J, Storsley LJ, et al. Evolution and clinical pathologic correlations of de novo donor-specific HLA antibody post kidney transplant. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg. 2012 May;12(5):1157–67.

40. Kasiske BL, Andany MA, Danielson B. A thirty percent chronic decline in inverse serum creatinine is an excellent predictor of late renal allograft failure. Am J Kidney Dis Off J Natl Kidney Found. 2002 Apr;39(4):762–8.

41. Loupy A, Lefaucheur C, Vernerey D, Chang J, Hidalgo LG, Beuscart T, et al. Molecular microscope strategy to improve risk stratification in early antibody-mediated kidney allograft rejection. J Am Soc Nephrol JASN. 2014 Oct;25(10):2267–77.

42. Viglietti D, Loupy A, Aubert O, Bestard O, Van Huyen J-PD, Taupin J-L, et al. Dynamic Prognostic Score to Predict Kidney Allograft Survival in Patients with Antibody-Mediated Rejection. J Am Soc Nephrol JASN. 2017 Dec 18;

43. Moore J, He X, Shabir S, Hanvesakul R, Benavente D, Cockwell P, et al. Development and evaluation of a composite risk score to predict kidney transplant failure. Am J Kidney Dis Off J Natl Kidney Found. 2011 May;57(5):744–51.

44. Lefaucheur C, Viglietti D, Mangiola M, Loupy A, Zeevi A. From Humoral Theory to Performant Risk Stratification in Kidney Transplantation. J Immunol Res. 2017;2017:5201098.

45. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. J Am Stat Assoc. 1999;94(446):496–509.

Table 1: Immunological parameters, donor, recipient and transplant characteristics (n=616)

Donor characteristics	
Mean age (SD) [years]	43.5 (16.4)
Age ≥60 years (n)	110 (17.8%)
Cause of death (n)	
Vascular	268 (43.5%)
Traumatic vehicle accident	106 (17.2%)
Traumatic non-vehicle accident	116 (18.8%)
Other	36 (5.8%)
Unknown	90 (14.6%)
Recipient characteristics	
Age (years, mean (SD))	49.5 (13.8)
Male/Female (n)	375/241
Biological parameters	
Mean proteinuria measured between M12 and M18 [g/L] (SD)	0.166 (0.451)
Mean serum creatinine at month 12 [µmol/L] (SD)	139 (67)
Clinical characteristics	
Death with functioning graft (n)	56 (9.1%)
Acute rejection (n)	135 (21.9%)
Graft failure (n)	68 (11.0%)
Initial immunosuppressant	
AZA/MMF	134/473
Unknown	9
Immunological parameters	
De novo donor specific anti-HLA antibodies (n)	60 (9.7%)
Non donor specific anti-HLA before transplantation (n)	96 (15.6%)
Transplant characteristics	
Graft rank >1 (n)	52 (8.5%)
Mean cold ischemia time [minutes] (SD)	1138 (369)
Period of transplantation (n)	
from 1984 to the end of 1993	99 (16.0%)
from 1994 to the end of 2002	194 (31.5%)
from 2003 to the end of 2011	323 (52.5 %)

AZA = azathioprine, MMF = mycophenolate mofetil

Table 2: Fixed-effects parameter estimates (95%CI) of the final joint latent class model (n=616)

Parameter	Common effects	Effects specific to each latent class			p-value
		1	2	3	
Multinomial logistic regression sub-model for class membership probability					
ξ_0 (intercept)		reference class	0.49 (0.09;0.89)	-2.4 (-3.01;-1.79)	
ξ_1 (donor age ≥ 60 years)		reference class	1.74 (0.78;2.71)	3.24 (2.12;4.38)	
Mixed-effects model for SCr time-trajectories[*]					
β_0 (intercept)		-2.26 (-2.69;-1.83)	not estimated	0.84 (-3.02;-1.79)	
β_1 (time [years])		-0.12 (-0.46;0.22)	0.44 (0.16;0.74)	2.17 (1.59;2.76)	
β_2 (time^2 [years^2])	-0.15 (-0.31-0.01)				
Survival sub-model for risk of graft failure					
$\sqrt{\zeta_1}^{**}$	0.20 (0.17;0.23)				
$\sqrt{\zeta_2}^{**}$	1.75 (1.56;1.95)				
g (latent class)		reference class	0.73 (-0.33;1.71)	5.66 (4.29;7.03)	
v_1 (anti-HLA Ab before transplantation [yes vs. no])	1.18 (0.56;1.81)				<0.01
v_2 (proteinuria at M12 [>0.275 g/L vs. ≤0.275 g/L])	0.88 (0.20;1.56)				0.01
v_3 (*dn*DSA [yes vs. no])	-0.72 (-2.93;1.49)				0.52
v_4 (acute rejection [yes vs. no])	-0.24 (-0.93;0;44)				0.48
v_5 (*dn*DSA*AR interaction)	2.73 (0.43;5.02)				0.02

*in the latent variable scale, **parameters of the Weibull baseline hazard function, HLA=human leukocyte antigen, Ab= antibodies, *dn*DSA=de novo donor-specific anti-HLA antibodies;

$$P(c_i = g|X_{ci}) = \frac{e^{\xi_{0g}+DONOR\_AGE60_i\xi_{1g}}}{\sum_{l=1}^{G} e^{\xi_{0l}+DONOR\_AGE60_i\xi_{1l}}};$$

$$\Lambda_i(\mathrm{t})|_{c_i=g} = \beta_{0g} + \beta_{1g} * TIME + \beta_2 * TIME^2 + u_{0ig} + u_{0ig} * TIME + \epsilon_{ij};$$

$$H(Y_{ij};\eta) = \Lambda_i(t_{ij}) + \varepsilon_{ij};$$

$$\lambda_i(t)|_{c_i=g} = \lambda_{0g}(t)e^{HLA*v_1+PROT*v_2+AR*v_3+DSA*v_4+AR*DSA*v_5}$$

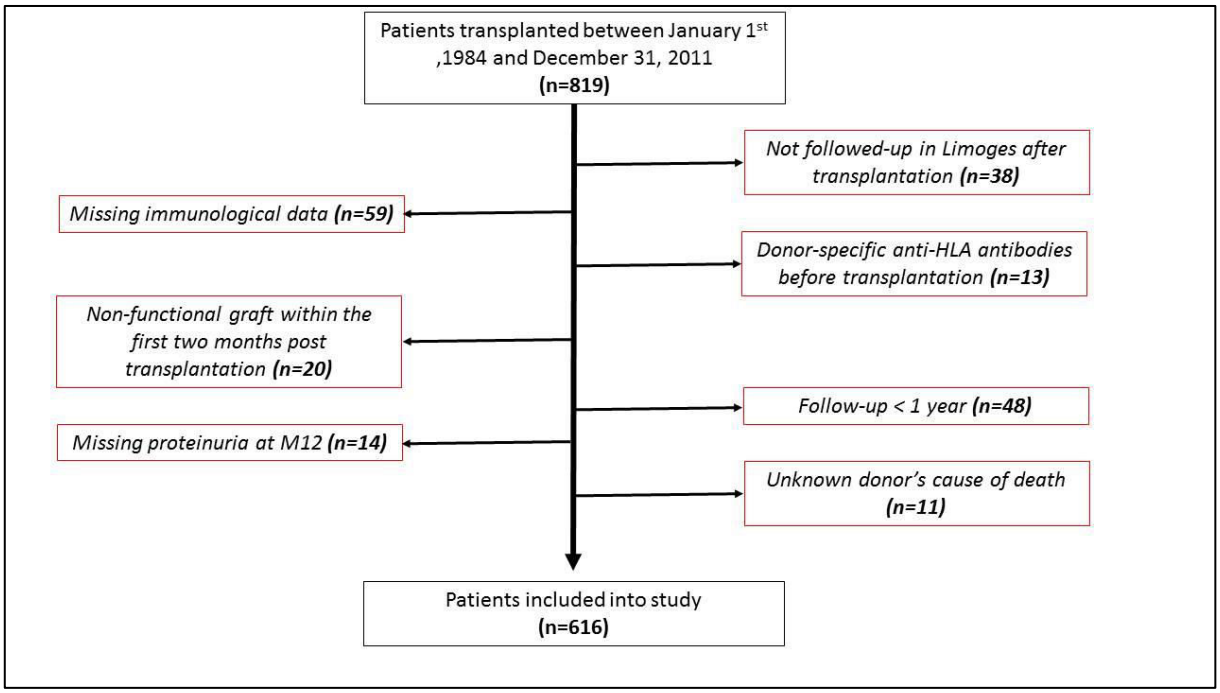

Figure 1: Flowchart showing selection of renal transplant recipients included in the study

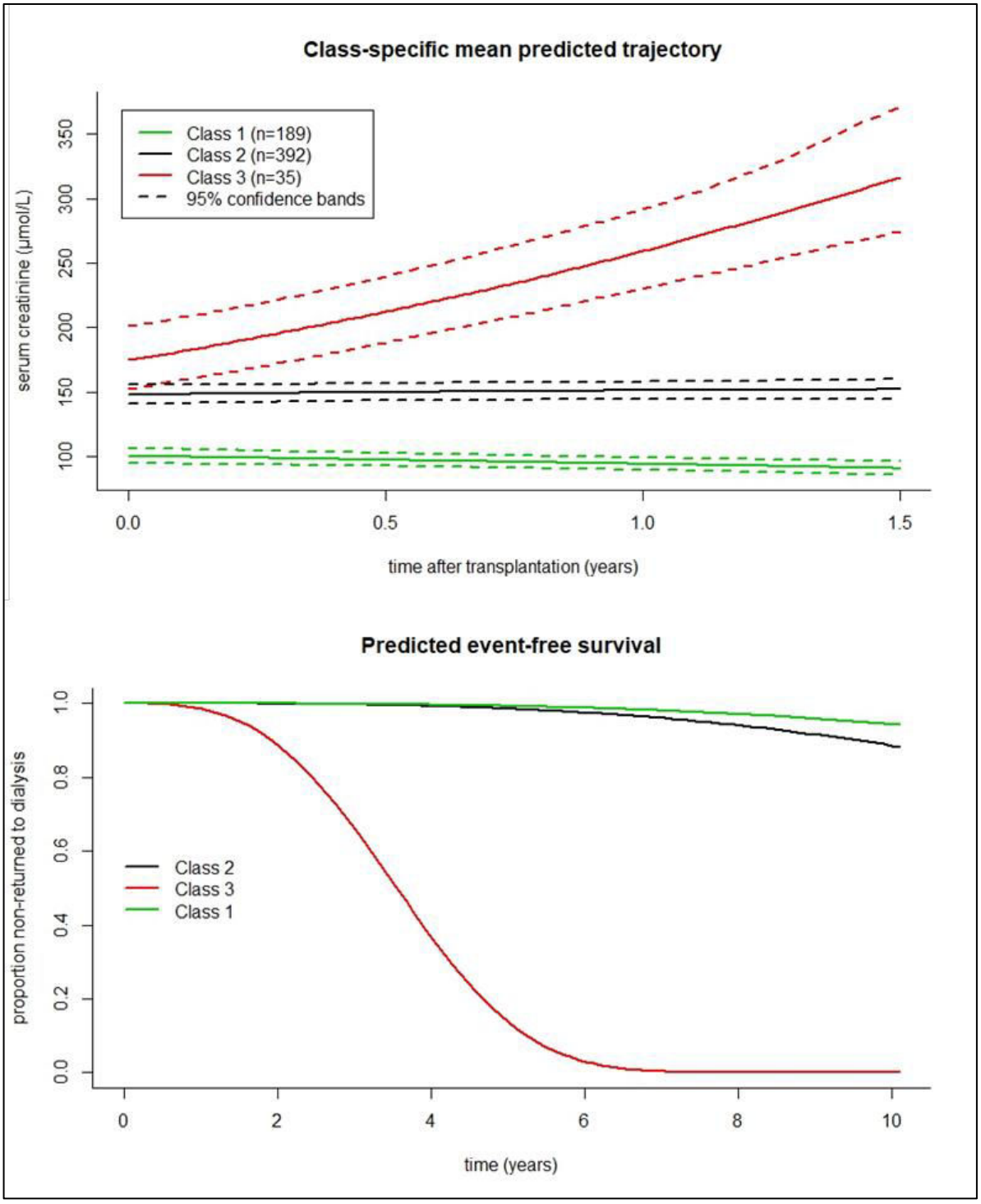

Figure 2: Class-specific predicted mean trajectories (top panel) and class-specific predicted event-free probabilities (bottom panel) from the final joint latent-class mixed mode; class 1(n=189) is in green, class 2 (n=392) in black and class 3 (n=35) in red. Dashed lines are the computed 95 % confidence intervals

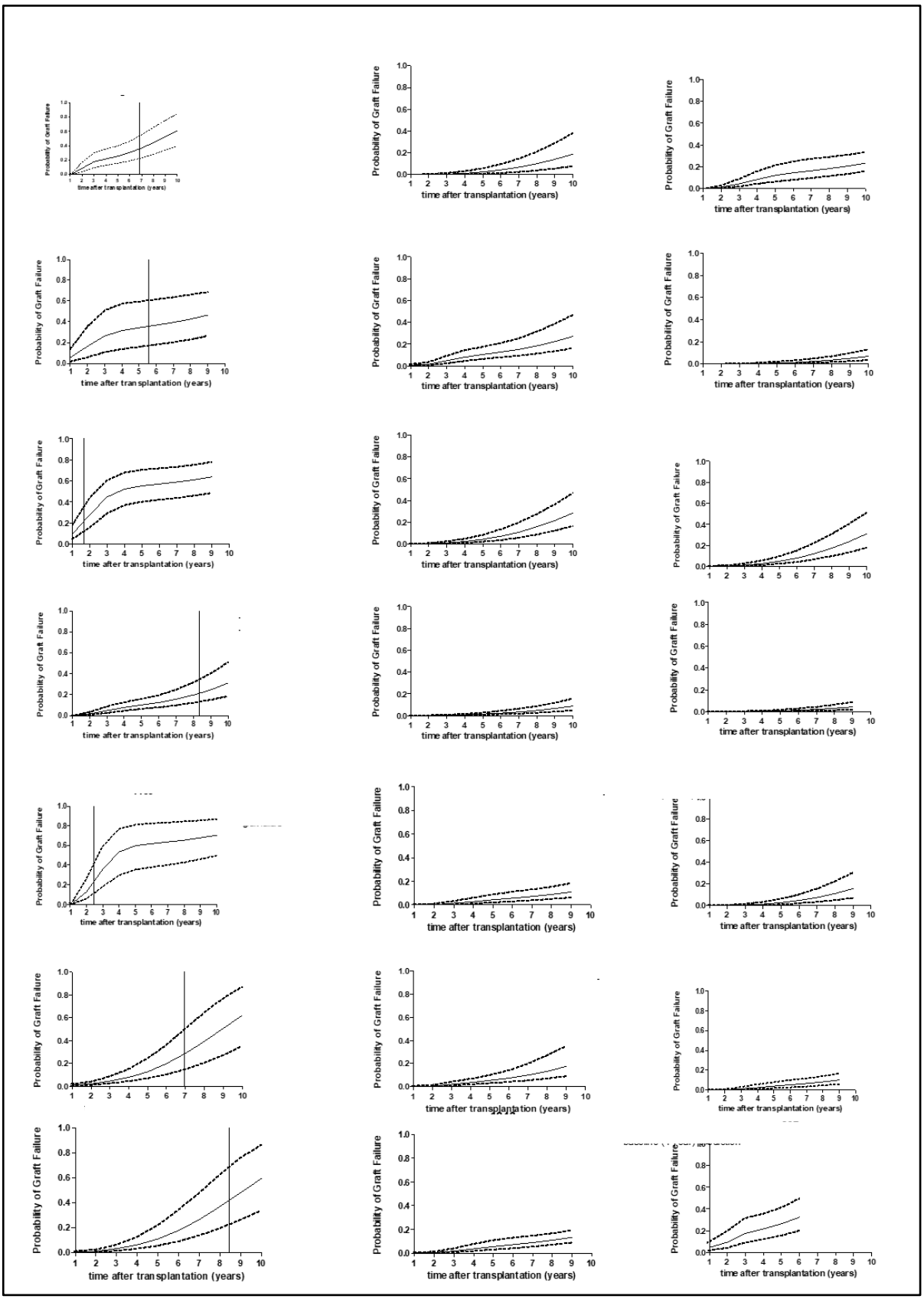

Figure 3: Individual predictions of risk of 10-years graft failure with 95% confidence intervals from the final joint latent-class mixed model for 21 patients who did not develop *dn*DSA based on covariates known at 1 year post-transplantation; the vertical black line for the patients on the left indicates the time of graft failure

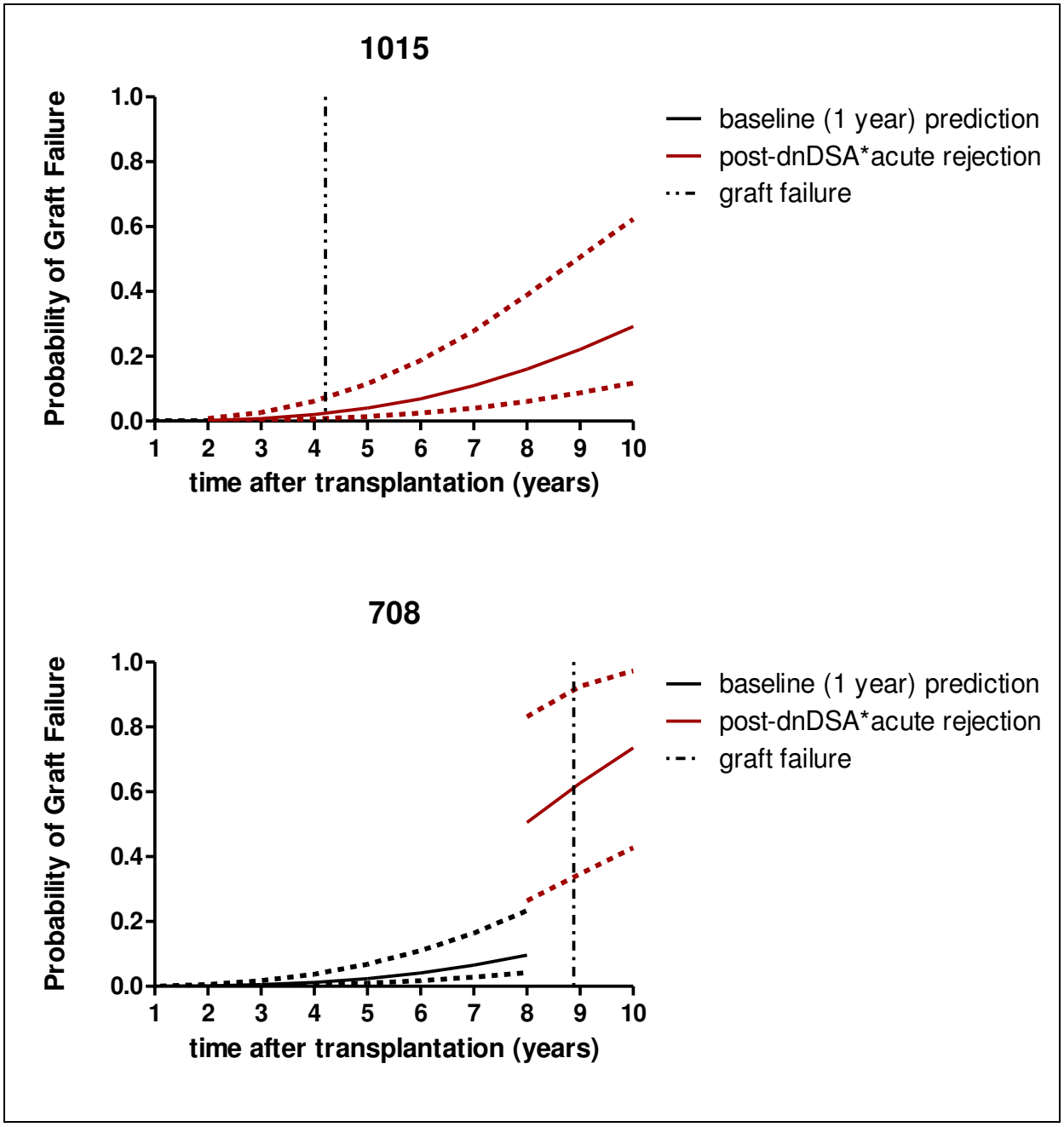

Figure 4: The best (patient ID = 708) and the worst (patient ID = 1015) prediction of probability of 10-years graft failure with 95% confidence intervals from the final joint latent-class mixed model among the group of patients who developed *dn*DSA; the black part of the curve corresponds to predictions based on covariates known up to 1 year post-transplantation while the red curve corresponds to prediction recalculated after onset of both *dn*DSA and acute rejection; the vertical dashed black line indicates the time of graft failure

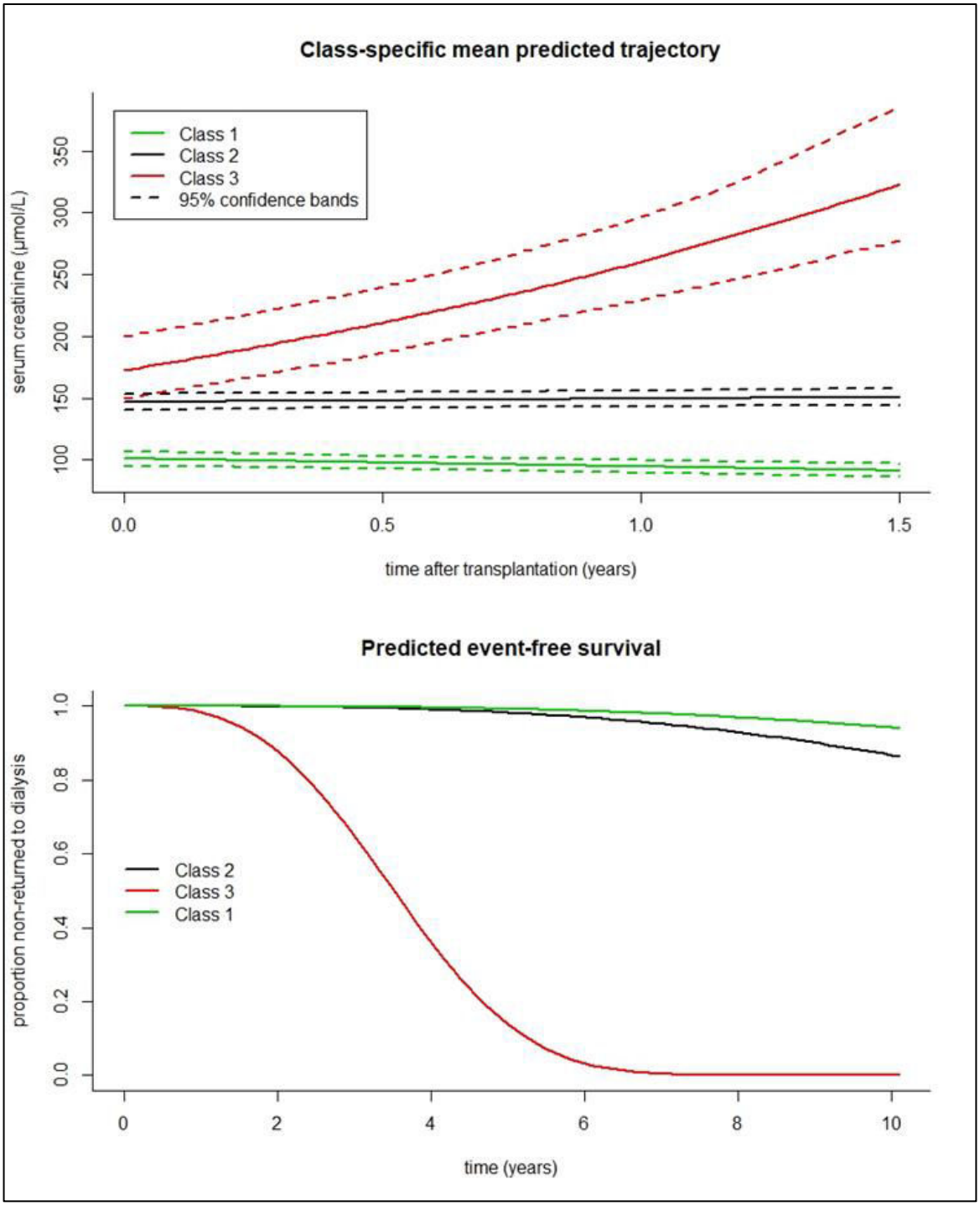

Figure 5: Sensitivity analysis: class-specific predicted mean trajectories (top panel) and class-specific predicted event-free probabilities (bottom panel) from the final joint latent-class mixed model recalculated after adding to the initial data set (n=616), data collected in 85 independent patients who had developed *dn*DSA and were followed-up in a second transplant center

CHAPTER IV: Study of relationships between tacrolimus through concentration, adherence to tacrolimus and treatment efficacy in kidney transplant recipients

Abstract:

Despite the significant improvement in short-term graft survival in kidney transplant recipients over the past decades, acute rejection (AR) still remains one of the most important predictors of kidney graft failure (1,2). Immunosuppressant drugs are used nowadays to prevent the onset of AR in these patients, with tacrolimus (TAC) being the backbone of immunosuppressant treatment since its introduction in the early 90s'. However, this is a critical dose drug which requires the therapeutic drug monitoring (TDM) due to its narrow therapeutic range and large variability in its time-exposure. Non-adherence to prescribed immunosuppressive treatment is one of most important risk factor of AR and graft failure in kidney transplant recipients (3–13) and can potentially lead to higher fluctuations in TAC blood concentrations and increased variability in its through concentrations (C0). Previous studies addressed the within-patient variability (WPV) in TAC blood levels mainly though studying the standard deviation the coefficient of variation of trough concentrations over a small interval of time after kidney transplantation (4,9,10,13,14). No previous study has attempted to study the association between evolution with time of TAC C0 and AR (i.e. treatment efficacy) by identifying subpopulations of patients with distinct profiles of TAC C0 trajectories over time and/or with different variability levels of TAC C0 within these individual time-profiles.

We developed a non-linear mixed-effects model with mixture based on residual (within-patient) variability to describe the time-course of TAC C0 over the first two post-transplant years and to identify subpopulations of patients with respect to variability in TAC C0-time profiles. The model identified two subpopulations of patients: 116 patients (42.2%) were classified in the *subpopulation* 1 characterized by the lower residual variability of 21% whereas 159 patients (57.8%) were classified in the subpopulation 2 with the higher residual variability of 35%. All AR observed after M3 occurred in the subpopulation of higher residual variability (n=9) and the rejection-free survival between M3 and 2 years was significantly lower in this subpopulation compared to the subpopulation of lower residual variability (log rank test: 7.7, p=0.01). No significant association was observed between subpopulations of TAC C0-time variability and time-evolution of self-reported non-adherence over the first 2 years after transplantation previously described using latent class mixed model and no significant association of self-reported non-adherence with AR was found. These results are strongly supportive of TAC TDM in order to prevent the onset of AR as the major predictor of graft failure in kidney transplant recipients.

Study of association between within-patient variability over time in tacrolimus through concentrations, treatment efficacy and patient-reported adherence

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INTRODUCTION

Since its introduction in the early 1990s, tacrolimus (TAC) has become the cornerstone of maintenance immunosuppressant regimen after organ transplantation. This is a critical dose drug characterized by a narrow therapeutic range and large variability in its dose-exposure relationships. Consequently, therapeutic drug monitoring (TDM) is mandatory for TAC in order to optimize its efficacy and prevent adverse effects. Although the AUC (area under concentration-time curve) is considered as the best marker of TAC exposure (15,16), the whole blood trough concentrations (TAC C_0) are usually used in practice for both financial and practical reasons and thanks to correlation between AUC and C_0 (17–19).

Immunosuppressive medication non-adherence was reported as a risk factor of graft rejection and graft failure (3–13). To date, there is no standardized and reliable method of adherence assessment which could be used to predict kidney graft survival in everyday clinical practice. Different approaches which are proposed for medication non-adherence monitoring in kidney transplant recipients include self-reporting, pill counts, electronic monitoring, prescription refill recording, physician-reported estimate and the measurement of individual drug concentrations and/or their variability.

Several works have studied the association between TAC within-patient variability (WPV) and clinical outcomes of kidney transplantation in adults (20–22) and in paediatrics patients (4,23,24). In most of these studies, the standard deviation (SD) of TAC levels or the coefficient of variation (CV%) of TAC trough concentrations were used to quantify the WPV in exposure to TAC (4,9,10,13,14). *Sapir-Pichhadze et al.* investigated association between variability of TAC though levels beyond one year post-transplant and a composite end point including late allograft rejection, transplant glomerulopathy, graft loss and death using a time-dependent cox model (22). They found a significant increase in the adjusted hazard of the composite end point for every 1-unit increase in SD of TAC levels. In pediatric population, *Hsiau et al.* found that median CV% of TAC blood trough levels was significantly higher in patients with biopsy-proven rejection than in those without rejection (4). However, caution should be made when SD and CV% are used to quantify WPV in TAC levels, and especially when the purpose is to use WPV in TAC C_0 as surrogate marker for non-adherence assessment. As it was previously pointed-out by some authors, patients with higher mean TAC levels have higher SD of TAC C_0 and there is a need to exclude outliers from the calculation of SD of TAC levels (4,13). On the other hand, the CV% of TAC through blood levels combines in a single measure two components (i.e. the mean of TAC through concentrations and the standard deviation of TAC though levels) and each of them be independently correlated with non-adherence and/or acute rejection episodes (22,25). Coefficient of variation is a relative measure of variability which compares heterogeneity in TAC C_0 blood levels for each individual relative to his/her own mean of TAC though concentrations. That is to say, two patients with the same CV% in TAC C_0 levels can

have very different means of TAC C_0 levels and/or show very different absolute variability in TAC through concentrations (i.e. SD). Modeling evolution with time of TAC through blood levels and taking into account each patient's variability around mean TAC C_0-time profile can be an interesting alternative to use of SD and CV% of TAC levels in the assessment of adherence to TAC. Tacrolimus through levels usually decrease with time post-transplantation as a consequence of reduction in TAC target levels. We hypothesized herein that the lower mean TAC C_0-time profiles and the higher WPV in these profiles could help to *a priori* identify non-adherent patients and can be associated with acute rejection episodes. No previous study has attempted to identify subpopulations of patients with distinct TAC concentration-time profiles and/or with different variability within these individual time-profiles.

Non-Linear Mixed Effects Models (NLMEM) are appropriate for the analysis of longitudinal (i.e. collected over time) data and allow to describe the evolution over time of drug concentrations, i.e. herein the individual TAC C_0 over time. NLMEM estimates population mean parameters (i.e. fixed-effect part of the model), and variability of these parameters through random-effects part taking into account the correlation between repeated measures of TAC C_0 taken on the same individual over period of time. Identification of subpopulations with specific fixed and/or random effect parameters is possible by combining NLMEM and mixture models (26,27). Using mixture models, the fraction of individuals belonging to each subpopulation is estimated and each patient is allocated to one and only one subpopulation. In longitudinal studies, mixture model on the fixed effects can estimate different mean parameters for each of these subpopulations, i.e. potentially different mean time-profiles; additionally a mixture on the random effects distribution can allow classification of subjects according to their different WPV. For these reasons, NLMEM with mixture seems a method of choice to study the heterogeneity in individual TAC trajectories.

To our knowledge, no previous study of longitudinal TAC exposure reported mixture models to identify subpopulations with different TAC C_0-time profiles (i.e. different shape of profiles, different concentration levels or different variability magnitude of concentrations). Thus, the aims of this study were (i) to describe the evolution with time of TAC C_0 over the first two years post-transplantation and identify subpopulations of TAC C_0-time profiles using NLMEM and the mixture model approach (ii) to investigate whether subpopulations of TAC C_0-time profiles were associated with self-reported non-adherence and (iii) to explore the association between the subpopulations with different TAC C_0-time profiles and acute rejection.

MATERIALS AND METHODS

Patients and treatment

Out of 361 adult kidney transplant recipients from the prospective multicentre French cohort named "EPIGREN" (28,29), we considered in current analysis patients who met the following

criteria: (i) being followed in one of the three centres (i.e. University Hospitals of Bordeaux, Limoges and Toulouse); (ii) understanding study protocol; (iii) having at least one measurement of TAC whole blood trough level (TAC C_0) during the first two years after transplantation (iv) having functioning graft during at least first three weeks post transplantation; (v) being able to read and understand French. Thus, after excluding from the initial group patients who did not met the aforementioned criteria, a total of 275 patients were included in current study. More details about patient selection steps are given in figure 1. All patients gave written consent and were followed-up during the first two years post transplantation. The study was completed with the legal requirements of the Declaration of Helsinki and received approval and authorization from the regional Ethics committee (no 06-040 on 19/05/2006), the French Medicine Agency (no 060566 on 08/08/2006) and the National Committee for Informatics and Liberties (907275 ACT) in 2006.

All patients received TAC as a part of dual or triple maintenance therapy with mycophenolate mofetil (MMF) and corticosteroids. Patients also received induction therapy (basiliximab, daclizumab or thymoglobuline) in conjunction with their maintenance therapy. Therapeutic drug monitoring of TAC was performed for patients from all three centres by using whole blood trough (C_0) levels. Target concentrations for TAC C_0 were predetermined in line with recommendations from the European Consensus Conference (15,16). More detailed description of patients is given in Table 1.

Modelling the evolution over time of tacrolimus through concentrations

Measurements of TAC C_0 were performed at different time points over the period from three weeks to two years after transplantation using high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS). A total of 4618 TAC C_0 values were available. Median number of recordings per patient was 13 (IQ 9-21). The modelling framework for evolution with time of TAC C_0 consisted of two steps.

In the first modelling step, non-linear mixed effects model was used to describe evolution of TAC C_0 over the first two years after transplantation. NLMEM allows the response variable (i.e. TAC C_0) to be expressed as a non-linear function of both fixed (population mean parameters) and random effects (between patient variability and residual variability). Herein, time course of TAC C_0 was described with an exponential equation validated in a previous study of our group (30):

$$C_0(t) = \theta_1 + \theta_2 * e^{-\theta_3 * t}$$

where C_0 is TAC trough concentration and θ_1, θ_2, θ_3 are fixed-effects model parameters. An exponential random effects model was used to account for between patient variability in the model parameters. A proportional error model was used to describe residual variability (also called within-patient variability). In order to assess the evolution with time of TAC C_0 at different

periods post-transplantation, the separate analysis was repeated with TAC C_0 measured before 3 months post-transplantation only.

In the second modelling step, the presence of subpopulations of patients according to TAC C_0-time profiles was investigated using the mixture model approach (26,31). From the previously described model for longitudinal TAC C_0 evolution (step 1), mixture was tested on (i) fixed-effect parameters exponential equation (on each as well as on all parameters, i.e. θ_1, θ_2 and θ_3) to identify subpopulations with different shapes of TAC C_0 time-profiles and (ii) residual error to identify subpopulations with different levels of within-patient variability in TAC C_0 over time (32).

The judgemental criteria for choosing the final model between the models tested were: (i) the NONMEM objective function value (OFV, corresponding to value of statistical function equal to -2 * ln(likelihood)); (ii) the standard error of estimated parameters; (iii) the residual variability.

The evaluation of the selected final model was performed using (i) Visual Predictive Check plot (VPC) (33,34) and (ii) non-parametric bootstrap analysis (35). Briefly, VPC plots were obtained by simulating 1000 data sets using the final model. The distribution of the simulated TAC exposure profiles (median, 5^{th} and 95^{th} percentiles) were plotted and compared with the observed profiles. Five hundred bootstrap sets were simulated by resampling from the original dataset. The 95% confidence intervals were estimated from the model parameters obtained by fitting the final model on these 500 simulated bootstrap sets and were then compared with the corresponding mean population estimates obtained with the original dataset.

The first order conditional estimation method with interaction was used for estimation of model parameters and VPC simulations. The whole modelling framework of these two steps was implemented in NONMEM software version 7.3.0 (36) along with the module PSN (Pearl Speaks NONMEM, version 4.6.0) (37,38).

Non-adherence assessment using self-reported questionnaires

Details about assessment and modelling steps of self-reported adherence can be found in the recent study by our group (29).

Briefly, drug adherence was assessed at seven different time points over the first two years post-transplantation (39) in 345 patients of the EPIGREN cohort using self-reported Morisky 4-Item Medication Adherence Scale (MMAS-4) (39). Patients are considered good adherent for MMAS-4= 0, medium adherent for MMAS-4= 1 or 2 and non-adherent for MMAS-4= 3 or 4.

The total number of questionnaires collected at each visit and the corresponding MMAS-4 scores are shown in Table 2. The majority of patients (90%) filled in the questionnaires at least at 4 visits during their follow-up. At one month post-transplantation, 93 % of patients were considered as adherent (MMAS-4 equal to 0) while the remaining 7 % were considered medium adherent (MMAS-4 equal to 1 or 2). Non-adherence increased with time, to reach 24%

of medium adherent patients at M18. Only one MMAS-4 score of 3 (non-adherent) was reported for a patient at M6.

Time evolution of MMAS-4 scores over the first two years after transplantation was studied using latent class mixed model implemented in "lcmm" package of R software (40). The developed model identified 2 latent classes or homogeneous subgroups of patients with time-trajectories of MMAS-4 score characteristic of (i) adherent and (ii) medium adherent patients.

Among the 275 patients for whom measures of TAC C_0 were available, 222 were classified *a posteriori* in the latent class of adherent patients, 37 were classified in the non-adherent latent class and 16 were not classified due to too many missing data on self-reported non-adherence (see Figure 1 for more details).

Study endpoints

First episode of acute rejection (AR) occurring in the first two years after transplantation was considered as efficacy end-point and outcome of interest. In the 275 patients included in the study, 16 events were recorded between three weeks and two years post transplantation. In patients who experienced their first AR while being on immunosuppressive regimen which did not include TAC or whose immunosuppressive regimen prior to their first AR episode was unknown, the first AR under TAC was considered (n=2). In each patient, the first biopsy proven AR was considered if available (n=12), or the first episode of AR not proven by biopsy elsewhere (n=4). Thus, 12 of 16 AR (0.75) were proven on a biopsy. (41,42).

In line with the results published by *Israni et al.* (43), separate analyses were conducted to investigate the association between TAC exposure and first acute rejection occurring (i) in the 3 weeks - 2 years period, (ii) before 3 months and (iii) after 3 months post-transplantation (figure 1). For the purpose of this analysis, only the measurements of TAC C_0 available prior to the first episode of AR were considered in the concerned patients. (i.e. the final model was refitted using TAC C_0 available prior to acute rejections only).

Statistical analyses

Pearson's χ^2 test was used to study the associations between (i) adherence and TAC C_0- time profiles and (ii) adherence and onset of acute rejection. Therefore classification of patients as adherent or non-adherent using latent class model for longitudinal data and the subpopulations for TAC C_0 time course were used.

Non-parametric Kaplan-Meier estimator and corresponding log-rank test were used to assess and compare rejection-free survival in the subpopulations with different TAC C_0-time profiles defined with NLMEM. The survival analysis was performed with "survival" R package (44).

The statistical analyses were implemented in R version 3.4.1 (available from www.r-project.org)

RESULTS

Modelling the evolution with time of tacrolimus through blood concentrations

A mixture model based on residual error best described the data; the model was without covariates and separated the database into two subpopulations on the basis of the variability of TAC concentrations over time. One hundred and sixteen patients (42.2%) were classified in the *subpopulation* 1 characterized by a lower residual variability of 21% whereas 159 patients (57.8%) were classified in the subpopulation 2 with a higher residual variability of 35%. The other mixture models tested did not resulted in a significant decrease of the OFV and did not give a good precision of parameters estimation as shown by the very high standard deviations on the estimated parameters in each subpopulation (at least 150% of the parameter values). Parameter estimates of the final mixture model with their corresponding 95% confidence intervals obtained from 500 bootstrap samples are shown in table 3. A full NONMEM code used for development of the final model can be found in appendix.

Visual predictive check (VPC) showed that the mixture model based on residual error provided a good fit of observed data on the whole, with approximately 95% of the observations lying within the simulated 2.5^{th} and 97.5^{th} percentiles and being symmetrically distributed around the median (Figure 2). The VPCs obtained for each subpopulation are presented in Figure 3. They revealed (i) more homogeneity in TAC C_0 over time in subpopulation of patients with lower residual variability (ii) better fit of observed TAC C_0 data for patients classified in the subpopulation with the higher residual variability compared to the patients in subpopulation with lower variability.

Association between tacrolimus concentration-time profiles and self-reported non-adherence

No statistically significant association was found between self-reported non-adherence latent class and subpopulations identified by NONMEM on the basis of TAC C_0 variability over time. Among 37 patients who were previously identified as non-adherent according to latent class model for MMAS-4 trajectories over the first two years post transplantation, 21 patient belonged to *the subpopulation 1 (lower WPV of TAC C_0)* and 16 to *the subpopulation 2 (higher WPV of TAC C_0)*. Table 4 summarizes the results obtained.

Association between tacrolimus concentration-time profiles and onset of acute rejection

Four and twelve patients experienced acute rejections in *the lower* and *the higher WPV subpopulations* of TAC C_0-time profiles, respectively. Over the first 2 years post transplantation, the rejection-free survival was not significantly different between the two subpopulations of TAC C_0-time profiles (0.96 in *the lower WPV subpopulation* versus 0.92 in *the higher WPV subpopulation*; Log-rank test: 1.8, p=0.18).

The first episode of acute rejection occurred in 7 patients over the first three months post-transplantation, and the rejection-free survival was not significantly different between the two subpopulations of TAC C_0-time profiles over this period (4 AR in *the lower WPV subpopulation versus* 3 AR in *the higher WPV subpopulation*; Log-rank test: 2.8, p=0.95). On the contrary, in the period from 3 months to 2 years the rejection-free survival was significantly higher in the subpopulation with *the lower WPV of TAC C_0 over time* (Figure 4, Log-rank test: 7.7, p=0.01). The 9 AR observed after M3 occurred in patients assigned to *the higher WPV subpopulation.*

Association between self-reported non-adherence and onset of acute rejection

Three and 13 AR were recorded among the patients classified to the non-adherent and the adherent latent class according to MMAS-4 trajectories over the first two years post transplantation, respectively. Thus, there was no significant difference in the percentage of patients with acute rejection between these two classes of adherence (8.1% vs. 5.8%, p=0.80)

DISCUSSION

This study identified two subpopulations of kidney transplant patients based on the WPV in TAC C_0 – time profiles and revealed that the subpopulation with the higher WPV in the evolution with time of TAC C_0 was associated with significantly higher incidence of AR beyond three months post-transplantation. This association was not observed between the subpopulation with higher WPV in TAC C_0 - time profiles and AR occurring within the first three months post-transplantation, when the self-reported adherence seemed to be the highest.

A positive association between higher variability in TAC through levels and onset of acute rejection was previously reported in literature (4,10,13,21,22), although some authors did not found this association significant (14,43). However, these results should be taken with caution. In the study of Ro *et al.*, the fluctuations in TAC through concentrations were calculated in the period between 6 and 12 months after transplantation, whereas the majority of AR episodes were observed in the period prior to 6 months (21). The findings of other groups were based on participants with very large period of inclusion (i.e. from 6 months to 34 years after the transplantation) (13), small samples size of paediatric patients only (4,10) or simultaneous kidney-liver transplant recipients (43). To our knowledge, only one study analysed the association between time-dependent variability in tacrolimus blood though levels and efficacy but this study used a composite efficacy end-point of late acute rejection, transplant glomerulopathy, graft failure or death with a functional graft in time-dependent Cox model (22). The authors used time-varying SD in TAC through concentrations even though this measure can be strongly influenced by outliers, as suggested by *Hsiau et al.* (4).

Israni et al. reported no significant difference in tacrolimus trough level variability measured as CV% in the group of subjects with AR before 3 months after transplantation compared to the group with rejection between 3 and 6 months after transplantation and to the group with no

rejection within the first 6 months after transplantation (43). In contrast, beyond M3 post-transplantation, we found an association between AR and WPV in time-evolution of TAC through levels. Our findings are in accordance with results of other studies that reported the existence of association between high variability in tacrolimus through levels and acute rejection (4,10,13,21). Of note, all rejections observed in the period after 3 months in present study (i.e. the period over which patient-reported non-adherence increased with time) were assigned to *the higher WPV subpopulation*. This suggests that decreased WPV in TAC concentration-time profiles could help manage acute rejection risk and adherence monitoring contribute to this objective. Our findings are also consistent with the work recently published by *Scheel et al.* who also reported absence of association between patient-reported non-adherence (measured with BAASIS) and immunosuppressive drugs through level variability/percentage of sub-therapeutic IS trough levels in a retrospective study of 267 adult kidney recipients (13).

Most of the previous studies used summary measures of immunosuppressive drug fluctuations such as CV% or SD over specific periods or at specific time points after transplantation (e.g. the three most recent measures prior to adherence assessment) as measure of WPV in TAC through levels (4,9,10,13,14,20,22,25). The SD of TAC C_0 is biased in patients with higher means and there is often a need to exclude outliers from its calculation, while the CV% of TAC through blood levels is a combination of two components which can be independently correlated with graft rejection and non-adherence (4,13,22,25). Added to this, CV% compares the heterogeneity in TAC C_0 blood levels relative to the mean of TAC though concentrations in each patient and two patients with the same CV% in TAC C_0 levels can have very different means of TAC C_0 levels and/or show very different absolute variability in TAC through concentrations. It should be also mentioned that the CV% is not an ideal index of certainty of a measurement when the total number of TAC C_0 measurements varies across individuals because it does not depend on the total number of TAC measurements. On the other side, very little attention was given to exploring the variability in the trajectories of TAC C_0 evolution with time. In our study, we used exponential model to describe the time course of TAC C_0 during the first two years after transplantation. Two subpopulations characterized by different levels of variability in TAC C_0 – time profiles were identified according to the mixture model based on residual error. The majority of patients (57.8%) were assigned to *the higher WPV subpopulation* with residual variability of 34.9%. Model predicted TAC C_0-time profiles were in good agreement with observed data as shown by VPC. To our knowledge, mixture model was previously applied with tacrolimus to investigate differences in absorption rate between its immediate-release and prolonged-release formulations (i.e. Prograf® and Advagraf®) (45), and this is the first time this approach is used to explore within-patient variability in the evolution with time of TAC blood levels.

The developed mixture model failed to *a priori* detect patients who were non-adherent according to the two latent-classes obtained for MMAS-4 score. Several factors could contribute to explain this failure. First, all patients in our study were either adherent or medium non-adherent, which means a MMAS-4 score range of 0 to 2, and except for one patient at M6, no patient was considered non-adherent (MMAS-4 = 3 or 4). Thus, the question remains whether "*the quantity of non-adherence"* was sufficient in order to be able to cause remarkable changes in the evolution with time of TAC through levels and/or increased WPV in TAC C_0 – time profiles. Moreover, when the self-reporting questionnaire are used for adherence assessment, patients who are adherent tend to be honest when answering the questions which is not always the case for non-adherent patients who might prefer not to reveal their aberrant behaviour (46). Second, within-patient variability in TAC C_0 levels over time can be as well due to other factors such as prescribed dose adjustments. Herein, much like in previously reported studies, the non-adherence increased over time and in particular beyond M3 (32,41–43) while the number of dose-adjustment decreased (39). Interestingly, the present study found a higher incidence of acute rejection beyond M3 in the patients classified in the subpopulation with higher within-patient variability in evolution of TAC C_0 over time. Third, some patients were switched between different maintenance immunosuppressive regimens during the study, and not all of these regimens included TAC. We should keep in mind that MMAS-4 questionnaire in current study was not specific to use of TAC only and as a consequence, we cannot exclude the possibility that patient-reported non-adherence was in part related to use of other immunosuppressive agents as well. Applying a self-reported questionnaire specific to use of TAC only or taking into account whether TAC was a part of patient's immunosuppressive therapy at each occasion the self-reported non-adherence was estimated could be a useful strategy to consider in future works.

The main limitation of the present study is that the developed model did not account for TAC dose-adjustments on the TAC through levels variability. In addition, the interval in which the TAC C_0 measures were taken was unknown in current study. Evaluating the expected variability in TAC C_0 due to dose adjustments and comparing it with the observed variability could be an interesting perspective in this context. Indeed, self-reported non-adherence was low early after transplantation and increased over two years while 86.4% of data on TAC C_0 were collected before year 1. Thus, model-based classification of patients according to their WPV in TAC C_0-time profiles might be biased towards these early TAC C_0 measurements and incapable to detect the increase in patient-reported non-adherence which was the highest later-on in our study. Although one previous study argued in favour of association between MMAS-4 score and fluctuations in TAC serum concentrations, these fluctuations were calculated as the difference between the maximum and the minimum of 3 last measures only and the authors omitted to report the tacrolimus sampling strategy (i.e. C_0 or some other) (47).

Therefore, this interpretation seems questionable. The impact of increased variability in TAC C_0-time profiles on the development of *de novo* donor-specific anti-HLA antibodies development was not among the objectives of current study, but the incidence of these antibodies is usually low in the studied period. Last, due to missing in data, we were unable to determine whether a particular type of AR (i.e. antibody mediated rejection or T-cell mediated rejection) was associated higher WPV in the evolution TAC C_0 with time.

There are several perspectives to currently developed mixture model which can be envisaged in clinical settings. First, a study could be conducted only on the group of patients with higher WPV in TAC C_0-time profiles to explore if the non-adherence rate is elevated in these patients, using the other methods of adherence assessment than the MMAS-4 questionnaire. The educational programs in combination with patient-tailored interventions can be proposed to promote adherence in patients in whom the high non-adherence is confirmed. The current model can be also useful to identify early after transplantation the patients at higher risk of acute rejection based on the WPV in the TAC C_0 evolution with time. The predictive value of WPV in the C_0-time profiles for onset of AR should be evaluated in future works.

CONCLUSION

We conclude that increased WPV in TAC C_0-time profiles was significantly associated with higher incidence of AR after 3 months post-transplantation in current study, which is supportive of tacrolimus TDM. This finding also suggests that, unless necessary, the frequent changes in TAC C_0 levels should be avoided. No association was found in current work between the variability in TAC C_0 time-profiles and patient-reported non-adherence. Further studies should investigate if WPV in TAC C_0-time profiles is better correlated with other methods of TAC adherence assessment and to evaluate whether its use in combination with other methods would better detect the non-adherence.

REFERENCES

1. Lentine KL, Gheorghian A, Axelrod D, Kalsekar A, L'italien G, Schnitzler MA. The implications of acute rejection for allograft survival in contemporary U.S. kidney transplantation. Transplantation. 2012 Aug 27;94(4):369–76.

2. Cooper JE, Gralla J, Cagle L, Goldberg R, Chan L, Wiseman AC. Inferior kidney allograft outcomes in patients with de novo donor-specific antibodies are due to acute rejection episodes. Transplantation. 2011 May 27;91(10):1103–9.

3. Morrissey PE, Reinert S, Yango A, Gautam A, Monaco A, Gohh R. Factors contributing to acute rejection in renal transplantation: the role of noncompliance. Transplant Proc. 2005 Jun;37(5):2044–7.

4. Hsiau M, Fernandez HE, Gjertson D, Ettenger RB, Tsai EW. Monitoring nonadherence and acute rejection with variation in blood immunosuppressant levels in pediatric renal transplantation. Transplantation. 2011 Oct 27;92(8):918–22.

5. Sellarés J, de Freitas DG, Mengel M, Reeve J, Einecke G, Sis B, et al. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg. 2012 Feb;12(2):388–99.

6. Prihodova L, Nagyova I, Rosenberger J, Majernikova M, Roland R, Groothoff JW, et al. Adherence in patients in the first year after kidney transplantation and its impact on graft loss and mortality: a cross-sectional and prospective study. J Adv Nurs. 2014 Dec;70(12):2871–83.

7. Gaynor JJ, Ciancio G, Guerra G, Sageshima J, Hanson L, Roth D, et al. Graft failure due to noncompliance among 628 kidney transplant recipients with long-term follow-up: a single-center observational study. Transplantation. 2014 May 15;97(9):925–33.

8. Spivey CA, Chisholm-Burns MA, Damadzadeh B, Billheimer D. Determining the effect of immunosuppressant adherence on graft failure risk among renal transplant recipients. Clin Transplant. 2014 Jan;28(1):96–104.

9. Pabst S, Bertram A, Zimmermann T, Schiffer M, de Zwaan M. Physician reported adherence to immunosuppressants in renal transplant patients: Prevalence, agreement, and correlates. J Psychosom Res. 2015 Nov;79(5):364–71.

10. Pizzo HP, Ettenger RB, Gjertson DW, Reed EF, Zhang J, Gritsch HA, et al. Sirolimus and tacrolimus coefficient of variation is associated with rejection, donor-specific antibodies, and nonadherence. Pediatr Nephrol Berl Ger. 2016 Dec;31(12):2345–52.

11. Shuker N, Shuker L, van Rosmalen J, Roodnat JI, Borra LCP, Weimar W, et al. A high intrapatient variability in tacrolimus exposure is associated with poor long-term outcome of kidney transplantation. Transpl Int. 2016 Nov 1;29(11):1158–67.

12. Rozen-Zvi B, Schneider S, Lichtenberg S, Green H, Cohen O, Gafter U, et al. Association of the combination of time-weighted variability of tacrolimus blood level and exposure to low drug levels with graft survival after kidney transplantation. Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc. 2017 01;32(2):393–9.

13. Scheel J, Reber S, Stoessel L, Waldmann E, Jank S, Eckardt K-U, et al. Patient-reported non-adherence and immunosuppressant trough levels are associated with rejection after renal transplantation. BMC Nephrol [Internet]. 2017 Mar 29 [cited 2017 Aug 27];18. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5372303/

14. Rodrigo E, Segundo DS, Fernández-Fresnedo G, López-Hoyos M, Benito A, Ruiz JC, et al. Within-Patient Variability in Tacrolimus Blood Levels Predicts Kidney Graft Loss and Donor-Specific Antibody Development. Transplantation. 2016 Nov;100(11):2479–85.

15. Schiff J, Cole E, Cantarovich M. Therapeutic monitoring of calcineurin inhibitors for the nephrologist. Clin J Am Soc Nephrol CJASN. 2007 Mar;2(2):374–84.

16. Wallemacq P, Armstrong VW, Brunet M, Haufroid V, Holt DW, Johnston A, et al. Opportunities to optimize tacrolimus therapy in solid organ transplantation: report of the European consensus conference. Ther Drug Monit. 2009 Apr;31(2):139–52.

17. Balbontin FG, Kiberd B, Squires J, Singh D, Fraser A, Belitsky P, et al. Tacrolimus monitoring by simplified sparse sampling under the concentration time curve. Transplant Proc. 2003 Nov;35(7):2445–8.

18. Undre NA. Pharmacokinetics of tacrolimus-based combination therapies. Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc. 2003 May;18 Suppl 1:i12-15.

19. Scholten EM, Cremers SCLM, Schoemaker RC, Rowshani AT, van Kan EJ, den Hartigh J, et al. AUC-guided dosing of tacrolimus prevents progressive systemic overexposure in renal transplant recipients. Kidney Int. 2005 Jun;67(6):2440–7.

20. Borra LCP, Roodnat JI, Kal JA, Mathot RAA, Weimar W, van Gelder T. High within-patient variability in the clearance of tacrolimus is a risk factor for poor long-term outcome after kidney transplantation. Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc. 2010 Aug;25(8):2757–63.

21. Ro H, Min S-I, Yang J, Moon KC, Kim YS, Kim SJ, et al. Impact of tacrolimus intraindividual variability and CYP3A5 genetic polymorphism on acute rejection in kidney transplantation. Ther Drug Monit. 2012 Dec;34(6):680–5.

22. Sapir-Pichhadze R, Wang Y, Famure O, Li Y, Kim SJ. Time-dependent variability in tacrolimus trough blood levels is a risk factor for late kidney transplant failure. Kidney Int. 2014 Jun 1;85(6):1404–11.

23. Pollock-Barziv SM, Finkelstein Y, Manlhiot C, Dipchand AI, Hebert D, Ng VL, et al. Variability in tacrolimus blood levels increases the risk of late rejection and graft loss after solid organ transplantation in older children. Pediatr Transplant. 2010 Dec;14(8):968–75.

24. Prytula AA, Bouts AH, Mathot RAA, van Gelder T, Croes LK, Hop W, et al. Intra-patient variability in tacrolimus trough concentrations and renal function decline in pediatric renal transplant recipients. Pediatr Transplant. 2012 Sep;16(6):613–8.

25. Pollock-BarZiv SM, Finkelstein Y, Manlhiot C, Dipchand AI, Hebert D, Ng VL, et al. Variability in tacrolimus blood levels increases the risk of late rejection and graft loss after solid organ transplantation in older children. Pediatr Transplant. 2010 Dec 1;14(8):968–75.

26. Lindsay BG. Mixture Models: Theory, Geometry, and Applications. IMS; 1995. 180 p.

27. Lindsay BG. The Geometry of Mixture Likelihoods: A General Theory. Ann Stat. 1983 Mar;11(1):86–94.

28. Villeneuve C, Laroche M-L, Essig M, Merville P, Kamar N, Coubret A, et al. Evolution and Determinants of Health-Related Quality-of-Life in Kidney Transplant Patients Over the First 3 Years After Transplantation. Transplantation. 2016 Mar;100(3):640–7.

29. Villeneuve C. Déterminants et évolution de profils de qualité de vie et d'adhésion aux traitements: analyse dans une cohorte de patients transplantés rénaux [Internet] [Pharmacologie et science du medicament]. Limoges; 2017. Available from: https://tel.archives-ouvertes.fr/tel-01452926/document

30. Daher Abdi Z, Prémaud A, Essig M, Alain S, Munteanu E, Garnier F, et al. Exposure to mycophenolic acid better predicts immunosuppressive efficacy than exposure to calcineurin inhibitors in renal transplant patients. Clin Pharmacol Ther. 2014 Oct;96(4):508–15.

31. Muthén B, Shedden K. Finite mixture modeling with mixture outcomes using the EM algorithm. Biometrics. 1999 Jun;55(2):463–9.

32. Ette EI, Williams PJ. Pharmacometrics: The Science of Quantitative Pharmacology. John Wiley & Sons; 2013. 1300 p.

33. Holford N. The Visual Predictive Check – Superiority to Standard Diagnostic (Rorschach) Plots [Internet]. [cited 2017 Nov 28]. Available from: https://www.page-meeting.org/?abstract=738

34. Karlsson MO, Holford N. A Tutorial on Visual Predictive Checks [Internet]. [cited 2017 Nov 28]. Available from: https://www.page-meeting.org/?abstract=1434

35. Parke J, Holford NH, Charles BG. A procedure for generating bootstrap samples for the validation of nonlinear mixed-effects population models. Comput Methods Programs Biomed. 1999 Apr;59(1):19–29.

36. Bael S, Sheiner LB, Boeckmann A, Bauer RJ. NONMEM User's Guides. Icon Development Solutions, Ellicott City, Maryland, USA, 2006; 1989.

37. Lindbom L, Ribbing J, Jonsson EN. Perl-speaks-NONMEM (PsN)--a Perl module for NONMEM related programming. Comput Methods Programs Biomed. 2004 Aug;75(2):85–94.

38. Lindbom L, Pihlgren P, Jonsson EN, Jonsson N. PsN-Toolkit--a collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM. Comput Methods Programs Biomed. 2005 Sep;79(3):241–57.

39. Morisky DE, Green LW, Levine DM. Concurrent and predictive validity of a self-reported measure of medication adherence. Med Care. 1986 Jan;24(1):67–74.

40. Estimation of Extended Mixed Models Using Latent Classes and Latent Processes: The R Package lcmm | Proust-Lima | Journal of Statistical Software. [cited 2017 Dec 4]; Available from: https://www.jstatsoft.org/article/view/v078i02

41. Racusen LC, Solez K, Colvin RB, Bonsib SM, Castro MC, Cavallo T, et al. The Banff 97 working classification of renal allograft pathology. Kidney Int. 1999 Feb;55(2):713–23.

42. Solez K, Colvin RB, Racusen LC, Haas M, Sis B, Mengel M, et al. Banff 07 classification of renal allograft pathology: updates and future directions. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg. 2008 Apr;8(4):753–60.

43. Israni AK, Riad SM, Leduc R, Oetting WS, Guan W, Schladt D, et al. Tacrolimus Trough Levels After Month 3 as a Predictor of Acute Rejection following Kidney Transplantation; a Lesson Learned from DEKAF Genomics. Transpl Int Off J Eur Soc Organ Transplant. 2013 Oct;26(10):982–9.

44. Therneau T. A Package for Survival Analysis in S. version 2.38 [Internet]. 2015. Available from: https://CRAN.R-project.org/package=survival

45. Woillard J-B, de Winter BCM, Kamar N, Marquet P, Rostaing L, Rousseau A. Population pharmacokinetic model and Bayesian estimator for two tacrolimus formulations – twice daily Prograf® and once daily Advagraf®. Br J Clin Pharmacol. 2011 Mar;71(3):391–402.

46. Cramer JA, Scheyer RD, Mattson RH. Compliance declines between clinic visits. Arch Intern Med. 1990 Jul;150(7):1509–10.

47. Liu J, Liu S, Yan J, Yi Q, Huang H. Adherence to Immunosuppressive Medication in Renal Transplant Recipients From Follow-up Outpatient in China: Association of 2 Different Measurement Methods. Clin Ther. 2015 Nov 1;37(11):2572–80.

Table 1: Description of patients included in the study (n=275)

Donor characteristics	
Mean age at transplantation [years] (SD)	50.7 (13.2)
Male/Female (n)	169/106
Mean height [cm] (SD)	165.4 (18.9)
Pre-transplant parameters	
Median time spent on dialysis [years] (IQ)	2.0 (1.1-3.9)
Clinical parameters	
Death with functioning graft (n)%	6 (2.2)
Graft failure n(%)	6 (2.2)
Acute rejection under tacrolimus between 3 weeks and 2 years (%)	16 (5.8)

Table 2: Total number of self-reported questionnaires collected at each visit and the corresponding MMAS-4 scores

		M1	**M3**	**M6**	**M9**	**M12**	**M18**	**M24**
	Number of questionnaires	324	302	266	238	232	107	112
	MMAS-4 score							
adherent patient	0	302	267	220	199	187	81	89
		93.2%	88.4%	82.7%	83.6%	80.6%	75.7%	79.5%
Medium and non-adherent patient	1	18	27	40	29	36	23	17
	2	4	8	5	10	9	3	6
	3	0	0	1	0	0	0	0
	MMAS-4 score = 1 to 3	6.8%	11.6%	17.3%	16.4%	19.4%	24.3%	20.5%

Table 3: Parameter estimates of the mixture model for evolution with time of TAC C_0 based on residual variability (step 2)

Parameters	Model estimate (SE %)	Bootstrap* 2.5th-97.5th percentiles
θ_1 [µg/L]	6.65 (5.8)	5.9-7.2
θ_2 [µg/L]	4.1 (6.9)	3.8-4.8
θ_3 [day^{-1}]	2.4 (28.5)	1.5-4.1
Proportion of patients in subpopulation 1	0.42 (17.4)	0.31-0.57
BPV θ_1 (%)	18.1 (39.1)	16.0-21.0
BPV θ_2 (%)	41.3 (54.2)	31.5-54.5
Residual variability subpopulation 1 (%)	21.3 (33.2)	19.8-23.8
Residual variability subpopulation 2 (%)	34.9 (26.2)	33.1-37.4

BPV = between-patient variability, Objective Function Value = 13897.6, *Bootstrap analysis was performed with 500 data samples

Table 4: Repartition (n(%)) of patients according to self-reported adherence latent class and subpopulation of variability in TAC C_0-time profiles (n=259)

	Subpopulation 1 (lower WPV)	Subpopulation 2 (higher WPV)
Adherent latent class	91 (81.3)	131 (89.1)
Non-adherent latent class	21 (18.7)	16 (10.9)

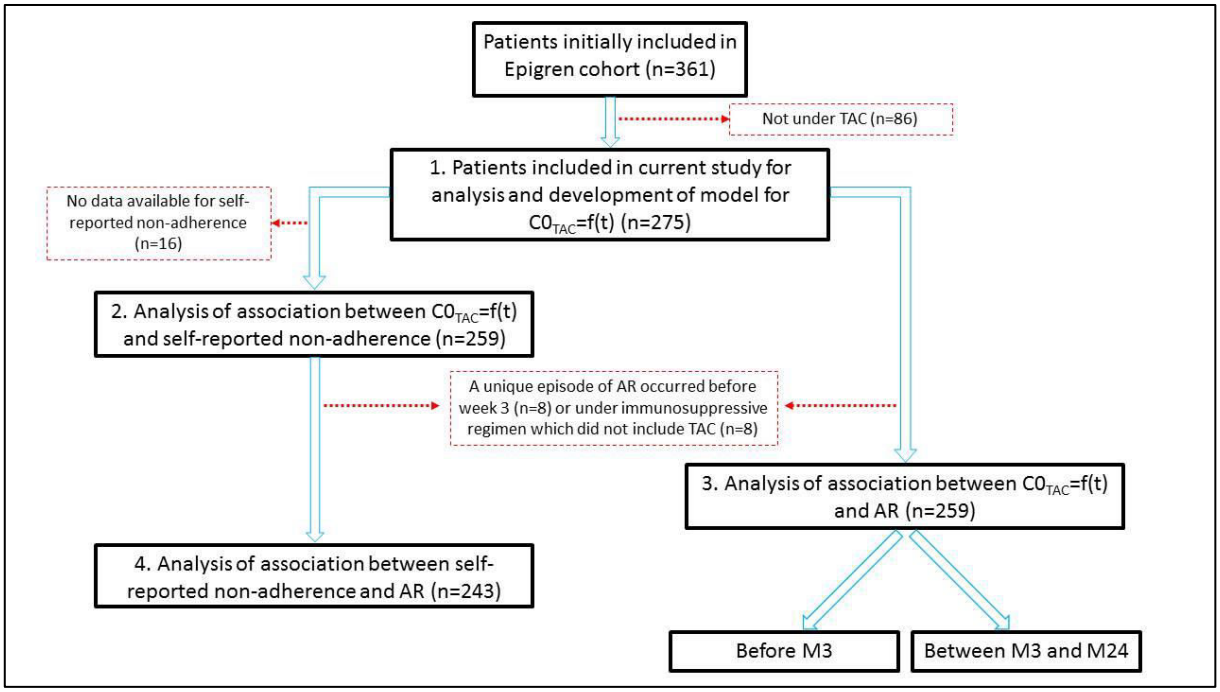

Figure 1: Flowchart showing patient selection and analyses performed in current study

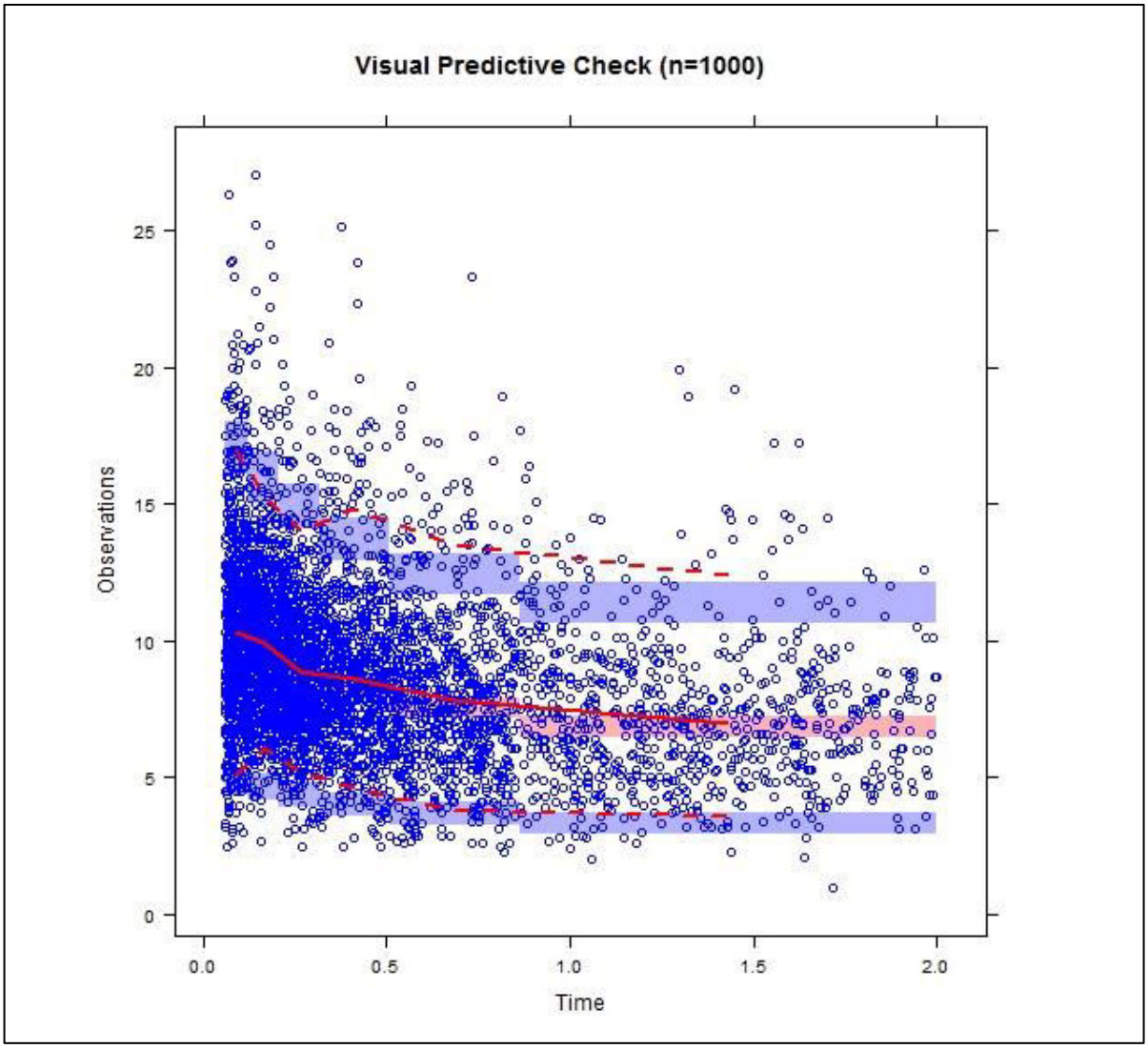

Figure 2: Visual predictive check (VPC) of the final mixture model for TAC C_0-time course. The model accounts for the fixed effects (the population mean evolution with time of TAC C_0) and the random effects (between-patent variability and residual variability in TAC C_0-time profiles); the mixture is based on residual variability in TAC C_0-time profiles The solid red line represents the median TAC C_0 levels, and the area around this line represents a simulation-based 95% confidence interval for the median, based on the developed model. The observed 5th and 95th percentiles are presented with dashed red lines, and the 95% confidence intervals of the corresponding model-predicted percentiles are shown as blue areas around these lines. The observed TAC trough concentration values are represented by points.

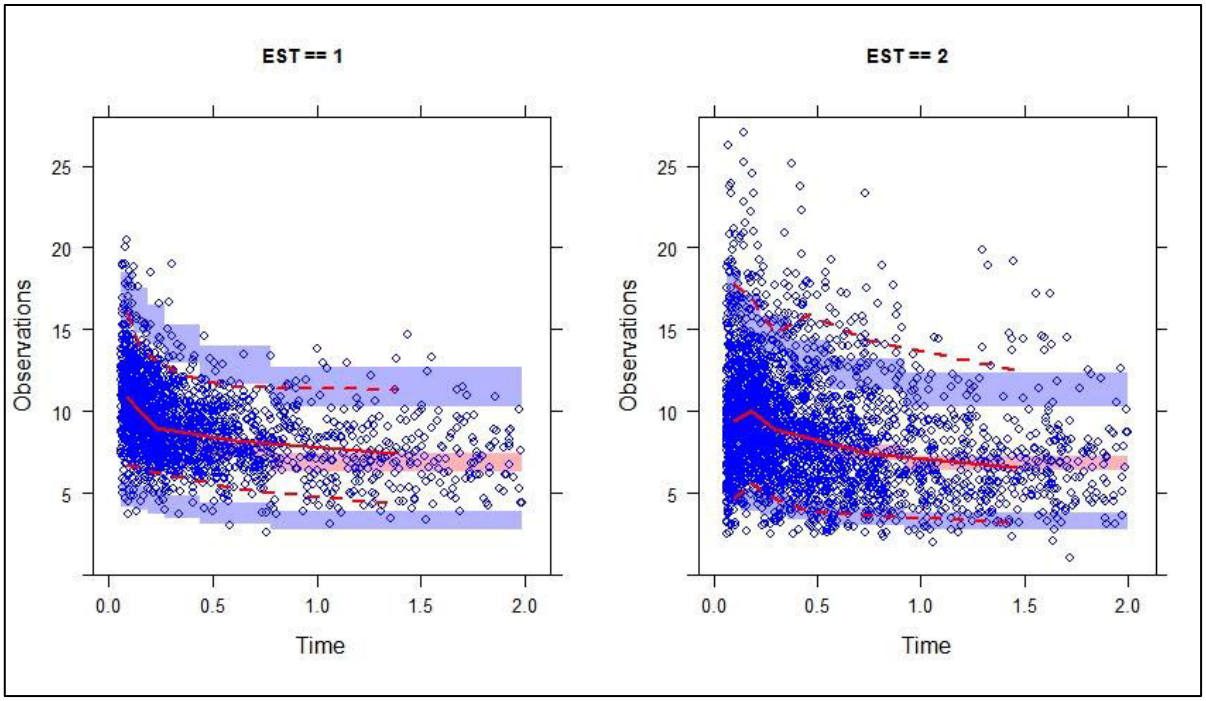

Figure 3: Visual predictive check (VPC) of the final mixture model for the subpopulation with low WPV in TAC C_0-time course (left panel) and the subpopulation with high WPV in TAC C_0-time course (right panel). The model accounts for the fixed effects (the population mean evolution with time of TAC C_0) and the random effects (between-patent variability and residual variability in TAC C_0-time profiles); the mixture is based on residual variability in TAC C_0-time profiles The solid red line represents the median TAC C_0 levels, and the red area around this line represents a simulation-based 95% confidence interval for the median, based on the developed model. The observed 5th and 95th percentiles are presented with dashed red lines, and the 95% confidence intervals of the corresponding model-predicted percentiles are shown as blue areas around these dashed lines. The observed TAC trough concentration values are represented by points.

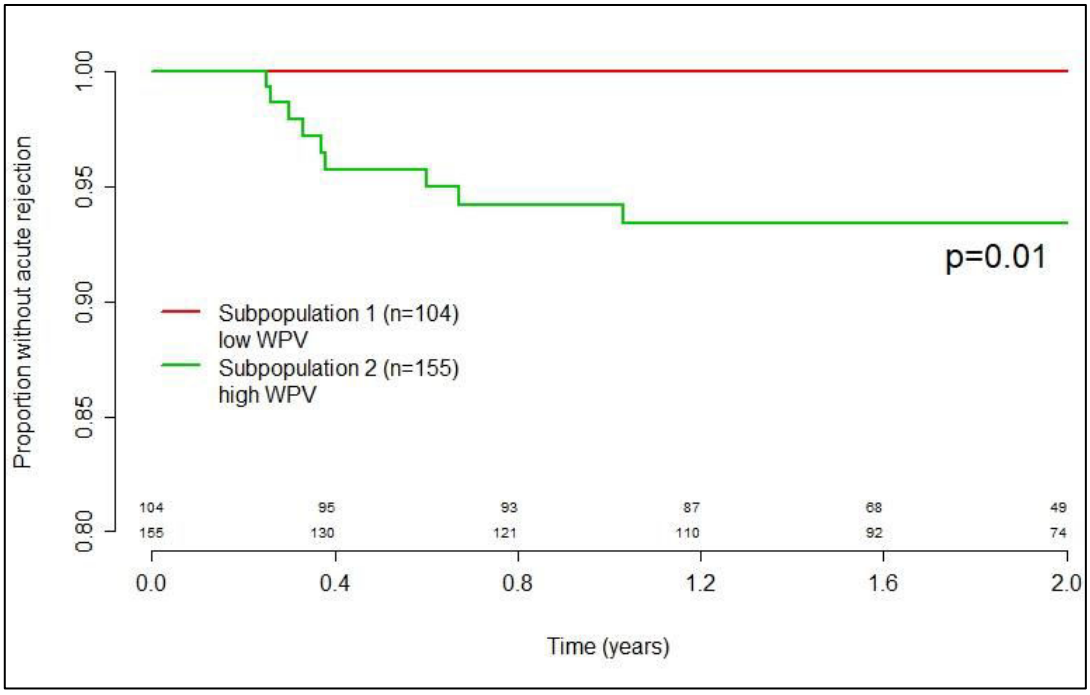

Figure 4: Kaplan-Meier curves for rejection-free survival in subpopulations of WPV in TAC C_0-time profiles for the period between 3 months and 2 years post-transplantation

CHAPTER V: Discussion and perspectives

Whether for a transplant recipient or a clinician, evaluation of the graft failure risk after kidney transplantation is crucial for optimizing clinical management as well as patients' quality of life. Therefore, the main objectives of this PhD thesis were to explore the factors associated with long-term graft failure in kidney transplant recipients. Two main studies were conducted in this context. First, we investigated the risk factors of 10-year kidney graft failure in taking into account the evolution of SCr over the first 18 months after transplantation and covariates collected pre- and post-transplantation. Second, we studied the association between longitudinal exposure to IS, non-adherence to prescribed IS treatment, and onset of acute rejection (AR). Acute rejection is, in clinical trials, frequently used as immunosuppressive treatment efficacy end-point.

Numerous factors have been identified as associated or predictive of graft failure. These factors, which were presented in details in section II of this work, include:

- donor specific factors: age, cause of death, type of donation (i.e. living vs. deceased donor), expanded criteria donor (13,14,17,22,34,46)
- recipients specific factors: age, gender, cause of ESRD, history of comorbidities, type of pre-transplant RRT (15,17,22,46)
- factors related to transplantation: warm and cold ischemia time, retransplantation, pre-emptive transplantation, period of transplantation, delayed graft function(12,34,47,102,103)
- biological and clinical factors collected or measured over patients' follow-up: SCr, proteinuria, GFR, acute rejection (18,19,23,30,47,104,105)
- immunological factors: number of HLA mismatches, pre-formed or onset of *de novo* DSA (20,21,23,47)
- treatment-related factors: maintenance immunosuppressive regimen, variability in IS exposure, non-adherence (28,99)

In vast majority of studies investigating risk factors of graft failure the attention was drawn to factors specific of only one of aforementioned groups, ignoring the global vision of kidney transplant recipient in these settings. For instance, while certain groups of authors frequently focused on immunological risk-factors such as development of *dn*DSA and antibody-mediated rejection (20,21,23,32), the others analyzed risk factors linked to donor-specific characteristics (13,14,17) or to clinical and biological parameters collected over the first post-transplant year (15,22,46). Several prognostic scores of short- and long-term graft failure (5 to 10 years post-transplantation) were recently reported in kidney transplantation, taking into account donor-specific characteristics, recipient pre-transplant characteristics or

data obtained over the clinical and biological follow-up in the period after transplantation (22,33,46,106,107). Generally, these scores only considered factors collected at specific fixed time-points after transplantation, usually at M6 or 1 year, and did not include evolution of these factors with time. Moreover, to our knowledge, apart from a scoring system recently proposed by our group (47), none of previously developed scores took into account immunological factors, such as existence of anti-human leukocyte antigen (HLA) antibodies or development of *de novo* DSA, beyond 1 year post-transplantation, even though these factors were shown to be significantly associated with higher risk of graft failure (108). In order to assess prediction of individual risk of graft failure over time not only for a majority of patients but for all kidney transplant patients, we developed a time-to-event model which puts together the evolution of kidney function with time (instead of a measure of biomarker collected at a specific time), and the onset over time of *dn*DSA.

Many previous studies have explored the association between graft failure and renal function assessed through single-time measures of SCr; few studies used current value of SCr or the last slope of SCr levels (12,18,34,47,105). Herein, we hypothesized that modelling profiles of SCr evolution with time better depicts patient's kidney function than single-time measurements of SCr. However, the time-trajectory of kidney function remains incompletely understood to date and while some studies suggested almost linear change in renal function over time and slow progressive worsening until graft failure (109), others reported episodes of non-linearity and rapid progression in deterioration of kidney function before graft failure (104). In current work, the impact of the evolution of SCr with time after transplantation on graft failure risk was studied using joint latent-class mixed model approach, recently proposed in the area of biostatistics. Concomitantly, this approach allows investigating the impact of individual factors on evolution of the studied longitudinal marker and graft failure risk. Recently, Boucquemont *el al.* used latent class mixed model (i.e. the longitudinal part of joint-latent class mixed model only) to explore the heterogeneity in SCr time-profiles in the large-population of patients with ESRD (45). However, Boucquemont *el al.*'s findings cannot be generalized to patients after kidney transplantation and to our best knowledge, the present work is the first to use joint latent-class mixed models in a population of kidney transplant recipients to identify homogenous groups of patients (i.e. latent classes) with evolution over time of graft function and class-specific risk of graft failure. We found that donor age was the only predictor of class-membership probability in the multinomial logistic-regression sub-model, indicating that receiving an older kidney was significantly associated with higher probability of belonging to latent classes with lower baseline or decline with time in SCr (i.e. class 2 and class 3). These findings are in line with results of previous studies who reported the association of higher donor age with shorter graft survival (13,14,22,46). Accordingly, our results are supportive of the strategy where it might be more appropriate to allocate older kidney to older rather than

younger recipients. For the group of patients with rapid decline in renal function and the corresponding high risk of long-term graft failure, therapeutic management can be adapted by clinician. Indeed, clinicians should decide for each of these patients individually whether the more frequent follow-up might be necessary at a specific moment after kidney transplantation or whether a change in current treatment or its readjustment should be considered. Since we live the era where the constant raise in need for kidney transplantation is not accompanied with proportional increase in number of kidneys available for transplantation, this can represent an important strategy to fight against donor shortage. Our findings suggest that the majority of patients at high risk of graft failure can be identified early after transplantation (before the 1st transplant anniversary).

For an overwhelming majority of patients with ESRD, receiving a new kidney represents a gift that makes them free of everyday-life constraints imposed by dialysis. From the perspective of patient who underwent kidney transplantation, graft survival now becomes all that matters. According to a recent study by Howell and colleagues, graft failure is perceived by a patient as the least desirable of 9 different adverse outcomes associated with kidney transplantation, followed by death and development of cancer (110); in this context, kidney transplant recipients seem to be more willing to experience even *hypothetical* death before graft failure than the graft failure itself over the first post-transplant year (110). Thus, questions such as *"what are my chances to experience graft failure within the next xx years"* or *"how long can I expect my new kidney to function properly"*, are not unusual; some patients prefer to seek an answer on their own, others address these questions directly to their nephrologist. The interpretation of risk factor of graft failure is not the same not only at two different time-points after transplantation but also for a specific time-point depending on whether the instant or cumulative risk of graft failure is considered. Indeed, the cumulative risk of graft failure constantly increase with time post-transplantation whereas the instant risk of graft failure or death is relatively high in the period immediately and early after transplantation (i.e. due to surgical complications, graft injury, delayed graft function, inadequate immunosuppression, the reaction of recipient cellular and humoral immune system against the new organ leading to acute rejections), decreases over the 1st post-transplant year and continues to increase progressively thereafter. In addition, not only the risk of graft failure differ between patients with respect to their pre-transplant and transplant characteristics (i.e. static, time-fixed risk-factors), but it can also change at any moment after transplantation according to biological and clinical parameters that become available over patients follow-up (i.e. time-varying factors). Unfortunately, this dynamic nature of risk of graft failure is often ignored in clinical settings. We identified the evolution with time of SCr, presence of anti-HLA antibodies before transplantation, proteinuria at M12 greater than 0.275 g/L and the interaction between onset of AR and development of *dn*DSA as independent predictors of increased risk of 10-years graft

failure in present work. In addition, we found that the risk of graft failure was only moderately increased in patients who developed *dn*DSA but who did not experience acute rejection episode. Consequentially, in patients with moderate to high risk of graft failure, in presence of at risk clinical context (e.g. comorbidities such as cancer), a more intensive surveillance of rejections without the need for specific individual adjustment of immunosuppressive regimen (i.e. increase of the doses) for DSA may be recommended. Nevertheless, surveillance should include a close monitoring of immunosuppressive drug exposure to avoid suboptimal exposure. On the other hand, in patients with a short-term high to very high risk of graft failure, specific medical strategy linked with onset of *dn*DSA might be personalized regarding the comorbidities of the patient and balancing between the probability of maintaining a functioning graft and side effects associated between these treatments. Accordingly, a higher level of immunosuppression might be proposed for these patients over the certain time periods in order to avoid the development of *dn*DSA and onset of antibody mediated graft rejection. The prognostic tool developed from joint latent class model can guide clinicians in the management of individual risk assessment for kidney transplant recipients. As it was shown, this tool provided reliable and satisfying individual predictions of graft-failure in group of patients who do not develop *dn*DSA, and unlike the majority of previous prognostic tools, it included the evolution with time of renal function. However, there is a need now for shift towards a dynamic and personalized risk-based concept in kidney transplantation to help clinicians guide their decisions: the one approach to fit all patients at risk of graft failure is no longer applicable. Such dynamic predictive tool which would englobe pre-transplant and transplant characteristics of each patient with ability to be updated over patients follow-up whenever the new information becomes available, could be considered a final goal of individualized risk-assessment of graft failure. The prognostic tool issued from the joint latent class model used in present work can be considered as a step towards development of such dynamic individual predications in kidney transplant recipients.

For a patient who underwent kidney transplantation, the use of immunosuppressant medication becomes a lifelong constraint in the sense that a regular intake of immunosuppressive treatment (IST) on every-day basis is mandatory to assure the optimal graft function. With introduction of cyclosporine A (CsA) and more recently tacrolimus (TAC) as the part of IST in organ transplantation, there has been an important improvement in short-term kidney graft survival, mainly due to reduction in number of AR episodes which occur within the early post-transplantation period. Nevertheless, AR still remains one of primary causes of kidney graft failure nowadays. One of the key determinants of AR is insufficient immunosuppression which can occur in kidney transplant recipients due to low exposure to IS drugs or high variability in exposure to IST. Another possible explanation for insufficient

immunosuppression which is less evident and complicated to control in practice is the non-adherence to prescribed IST. Unfortunately, the majority of non-adherent patients are willing to admit their aberrant behaviour only once the severe or irreversible consequences (such as AR or graft failure) already occurred and the question remains nowadays how to reliably evaluate non-adherence in absence of gold-standard of adherence assessment. Acknowledging the limitations of all subjective and objective methods that are currently available for this purpose, the World Health Organization recommends the use of multi-method approach (49).

We hypothesized in present work that the patients who were less adherent to their prescribed IST would show on average lower C_0-time profiles compared to adherent patients and/or that the non-adherence would translate into higher variability of IS C_0-time profiles in these patients. In previous works, our research group acquired a solid background in optimization of IST for prevention of AR episodes after kidney and liver transplantation with the focus being mainly on the therapeutic drug monitoring (TDM) of immunosuppressant drugs. Only recently, in EPIGREN and EPHEGEN cohorts, our interest was oriented towards the adherence to IST as a potentially modifiable factor for optimization of graft survival in kidney transplant recipients. The variability in TAC C_0 and its association with non-adherence and/or clinical outcomes in kidney transplantation was previously analyzed using summary measures of variability in TAC through concentrations such as SD, CV percentage or the difference between maximum and minimum of TAC blood levels (24,51,53,54,92,95). Due to cross-sectional design of some studies, the non-adherence to TAC for each participant was assessed at a single and unspecific time-point after kidney transplantation (51,92). Recently, Sapir-Pichhadze and colleagues reported that increased variability in time-varying exposure to TAC after the first post-transplantation year was significantly associated with higher risk of composite end point (i.e. allograft rejection, transplant glomerulophaty or total graft loss) in the time-dependent Cox model (24). Standard deviation of TAC blood concentrations was estimated for each period over which TAC dose was unchanged and was used as a measure of variability in TAC time-exposure. However, no previous study in analyzed in kidney transplant recipients analyzed TAC C_0-time profiles the variability in TAC C_0, on one hand, and its association with longitudinal profiles of repeatedly measured patient reported adherence and treatment efficacy, on the other. In EPIGREN and EPHEGREN cohorts, the participants were simultaneously followed-up for their IS blood concentrations and asked to fill in the self-reporting MMAS-4 questionnaire repeatedly over the first 2-years after kidney transplantation. Herein, mixture models were used to study TAC C_0-time profiles and the final model identified two subpopulations of kidney transplant recipients with respect to their within-patient variability (WPV) in TAC C_0. No association was found between within patient variability in TAC C_0 and

self-reported adherence. On the contrary, all acute rejections that took place after 3 months post-transplantation were in the group of patients with high variability in TAC C_0. The disparity between our initial hypothesis and the absence of association between non-adherence and variability in TAC C_0 in current study can be (i) for reasons related to assessment of non-adherence, (ii) due to other factors that can be in origin of variability In TAC C_0 or (iii) for both reasons. For instance, the present study did not account for TAC individual dose adjustments as potentially one of the main sources of variability in TAC C_0. This could be the main reason for which relatively high proportion of patients was assigned to subpopulation with high variability in TAC C_0, although this proportion was comparable with the prevalence of non-adherence reported in some previous studies (66,74). White-coat adherence is also one of possible explanations for the lack of association between variability in TAC C_0 and self-reported non-adherence in current work – in order to avoid being blamed for their aberrant behavior, non-adherent patients sometimes tend to take their medication correctly in the period preceding the clinical visit. However, due to relatively high number of measurements of TAC through concentrations (C_0) per patient in our study, it is reasonable to consider that the impact of white-coat adherence was minor. Last, according to self-reporting and the definition of non-adherence used with MMAS-4 questionnaire in present work, all patients were either adherent or moderately non-adherent. Obviously, this render more difficult the establishment of association between non-adherence (or moderate non-adherence in our case) and variability in TAC C_0. However, the most important finding of this study is the association between increased variability in TAC C_0 and onset of late acute rejection. It was shown that in kidney transplant recipients who experience AR episode, the risk of graft failure increase significantly with increase in time elapsed between transplantation and AR occurrence (19). That is to say, patients with AR episodes occurring later during their follow-up are at much higher risk of graft failure to compared to patients with early AR. Herein, increased WPV in TAC C_0 should can considered as an important clue to alarm clinicians for potential AR episodes and the developed model can be useful to identify patients with increased variability in TAC C_0 over the first three months post-transplantation. Furthermore, a more frequent clinical follow-up could be proposed for these patients after M3 in order to lower the variability in TAC C_0 and to determine if non-adherence or some other factor is in its origin. An interesting perspective to mixture model proposed in current study would be to compare the model-predicted variability due to TAC dose adjustments with the observed variability. If the better accordance is attained between variability in TAC C_0 and patients' self-reporting after accounting for individual dose adjustments, the measurement of WPV in TAC C_0 could be implemented as a tool for *a priori* assessment of non-adherence to IST. Specific strategies to improve adherence could be proposed in patients with high WPV in TAC C_0. Currently, the WHO states that the most effective method include the use of educational programs in combination with patient-tailored

intervention to promote adherence (49). Another extension to results obtained in current work could be to describe the evolution with time of multiple longitudinal markers such as exposure to IST as the surrogate marker of non-adherence and SCr as the surrogate marker of graft function and to analyze the impact of change in these markers on risk of graft failure. Given that the risk of death is not the same in patients who do and who do not develop graft failure, the competing risks approach might be considered for analysis of these two outcomes simultaneously. A motivating example on how to study the effect of multiple longitudinal markers as predictors of competing-risks outcomes was recently proposed by Proust-Lima *et al.*, who included in a joint latent class model two tests of semantic memory measured repeatedly over time and the competing risks between death and onset of dementia in elderly population (44).

After all, kidney transplantation remains a story of remarkable achievements and ongoing challenges. Although its success is undeniable, there has not been any major improvement in this field lately that would assure better graft and patient survival. In such circumstances, reevaluating the risk-factors of kidney graft failure in an individualized risk-assessment context can be an important strategy to assure the brighter future for kidney transplant recipients.

CHAPTER VI: Conclusion

The research conducted in the present PhD thesis work was dedicated to exploring in kidney transplant recipients the risk factors of long-term graft failure along with the evolution with time of kidney function. It was divided in two main axes.

In the first part of this thesis, the developed joint latent class mixed model identified three distinct groups of kidney transplant recipients (latent classes) with respect to time-trajectories of SCr over the first 18 months post-transplantation with class-specific risks for 10-years graft failure. Six percent of patients who were characterized by rapid and steep increase in SCr and extremely high risk of 10-years graft failure. Besides, presence of anti-HLA antibodies before transplantation, higher proteinuria at M12 and interaction between acute rejection onset and *dn*DSA development were associated with higher risk of graft failure whereas the donor age contributed to explain latent-class membership. Predictive performance of model for 10-years graft survival was more than satisfying in the group of patients who did not develop *dn*DSA as indicated by high values of sensitivity and specificity. These predictions can further be used as the basis for development of dynamic predictions of graft failure in kidney transplant recipients which are considered the state of the art of today's individualized risk management concept.

The second part of this thesis dealt with inter-association between longitudinal TAC C_0 levels, adherence to TAC and treatment efficacy, with our initial hypothesis being the higher incidence of TAC under-exposure and increased variability in TAC C_0 in non-adherent patients. The developed mixture model identified two subpopulations of patients based on residual variability in TAC C_0. No association was found between sub-populations of TAC C_0 variability and patient-reported adherence. The adequacy of using the mixture modelling approach in this context was further demonstrated by studying the relationship between the evolution of TAC C_0 with tile and the first onset of acute rejection (AR) which in our case was the criteria of treatment efficacy. All AR that occurred beyond 3 months after transplantation were assigned to the subpopulation of patients with higher variability in TAC C_0. These results are strongly supportive of TAC therapeutic drug monitoring. Further studies should determine the potential of monitoring of variability in TAC C_0 for *a priori* assessment of non-adherence after accounting for individual dose-adjustments.

References

1. Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, et al. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Ann Intern Med. 2003 Jul 15;139(2):137–47.

2. Laupacis A, Keown P, Pus N, Krueger H, Ferguson B, Wong C, et al. A study of the quality of life and cost-utility of renal transplantation. Kidney Int. 1996 Jul;50(1):235–42.

3. Wolfe RA, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LYC, et al. Comparison of Mortality in All Patients on Dialysis, Patients on Dialysis Awaiting Transplantation, and Recipients of a First Cadaveric Transplant. N Engl J Med. 1999 Dec 2;341(23):1725–30.

4. Journalist Workshops - Organ donation and transplantation - Public Health - European Commission [Internet]. Public Health. [cited 2018 Feb 6]. Available from: /health/blood_tissues_organs/events/journalist_workshops_organ_en

5. Agence de la biomédecine - Le rapport annuel médical et scientifique 2015 [Internet]. [cited 2018 Feb 6]. Available from: https://www.agence-biomedecine.fr/annexes/bilan2015/donnees/organes/02-organes/synthese.htm

6. Ekberg H, Tedesco-Silva H, Demirbas A, Vítko S, Nashan B, Gürkan A, et al. Reduced exposure to calcineurin inhibitors in renal transplantation. N Engl J Med. 2007 Dec 20;357(25):2562–75.

7. Halloran PF. Immunosuppressive drugs for kidney transplantation. N Engl J Med. 2004 Dec 23;351(26):2715–29.

8. Watson CJE, Dark JH. Organ transplantation: historical perspective and current practice. Br J Anaesth. 2012 Jan;108 Suppl 1:i29-42.

9. Meier-Kriesche H-U, Schold JD, Kaplan B. Long-term renal allograft survival: have we made significant progress or is it time to rethink our analytic and therapeutic strategies? Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg. 2004 Aug;4(8):1289–95.

10. Lamb KE, Lodhi S, Meier-Kriesche H-U. Long-term renal allograft survival in the United States: a critical reappraisal. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg. 2011 Mar;11(3):450–62.

11. Gondos A, Döhler B, Brenner H, Opelz G. Kidney graft survival in Europe and the United States: strikingly different long-term outcomes. Transplantation. 2013 Jan 27;95(2):267–74.

12. de Bruijne MHJ, Sijpkens YWJ, Paul LC, Westendorp RGJ, van Houwelingen HC, Zwinderman AH. Predicting kidney graft failure using time-dependent renal function covariates. J Clin Epidemiol. 2003 May;56(5):448–55.

13. Querard A-H, Foucher Y, Combescure C, Dantan E, Larmet D, Lorent M, et al. Comparison of survival outcomes between Expanded Criteria Donor and Standard Criteria Donor kidney transplant recipients: a systematic review and meta-analysis. Transpl Int Off J Eur Soc Organ Transplant. 2016 Apr;29(4):403–15.

14. Aubert O, Kamar N, Vernerey D, Viglietti D, Martinez F, Duong-Van-Huyen J-P, et al. Long term outcomes of transplantation using kidneys from expanded criteria donors: prospective, population based cohort study. BMJ. 2015 Jul 31;351:h3557.

15. Schnitzler MA, Lentine KL, Axelrod D, Gheorghian A, You M, Kalsekar A, et al. Use of 12-month renal function and baseline clinical factors to predict long-term graft survival: application to BENEFIT and BENEFIT-EXT trials. Transplantation. 2012 Jan 27;93(2):172–81.

16. Debout A, Foucher Y, Trébern-Launay K, Legendre C, Kreis H, Mourad G, et al. Each additional hour of cold ischemia time significantly increases the risk of graft failure and mortality following renal transplantation. Kidney Int. 2015 Feb;87(2):343–9.

17. Lepeytre F, Dahhou M, Zhang X, Boucquemont J, Sapir-Pichhadze R, Cardinal H, et al. Association of Sex with Risk of Kidney Graft Failure Differs by Age. J Am Soc Nephrol JASN. 2017 Jun 7;

18. Kasiske BL, Andany MA, Danielson B. A thirty percent chronic decline in inverse serum creatinine is an excellent predictor of late renal allograft failure. Am J Kidney Dis Off J Natl Kidney Found. 2002 Apr;39(4):762–8.

19. Lentine KL, Gheorghian A, Axelrod D, Kalsekar A, L'italien G, Schnitzler MA. The implications of acute rejection for allograft survival in contemporary U.S. kidney transplantation. Transplantation. 2012 Aug 27;94(4):369–76.

20. Everly MJ, Rebellato LM, Haisch CE, Ozawa M, Parker K, Briley KP, et al. Incidence and impact of de novo donor-specific alloantibody in primary renal allografts. Transplantation. 2013 Feb 15;95(3):410–7.

21. Lefaucheur C, Viglietti D, Bentlejewski C, Duong van Huyen J-P, Vernerey D, Aubert O, et al. IgG Donor-Specific Anti-Human HLA Antibody Subclasses and Kidney Allograft Antibody-Mediated Injury. J Am Soc Nephrol JASN. 2016 Jan;27(1):293–304.

22. Gonzales MM, Bentall A, Kremers WK, Stegall MD, Borrows R. Predicting Individual Renal Allograft Outcomes Using Risk Models with 1-Year Surveillance Biopsy and Alloantibody Data. J Am Soc Nephrol JASN. 2016 Oct;27(10):3165–74.

23. Cooper JE, Gralla J, Cagle L, Goldberg R, Chan L, Wiseman AC. Inferior kidney allograft outcomes in patients with de novo donor-specific antibodies are due to acute rejection episodes. Transplantation. 2011 May 27;91(10):1103–9.

24. Sapir-Pichhadze R, Wang Y, Famure O, Li Y, Kim SJ. Time-dependent variability in tacrolimus trough blood levels is a risk factor for late kidney transplant failure. Kidney Int. 2014 Jun 1;85(6):1404–11.

25. Israni AK, Riad SM, Leduc R, Oetting WS, Guan W, Schladt D, et al. Tacrolimus Trough Levels After Month 3 as a Predictor of Acute Rejection following Kidney Transplantation; a Lesson Learned from DEKAF Genomics. Transpl Int Off J Eur Soc Organ Transplant. 2013 Oct;26(10):982–9.

26. Daher Abdi Z, Essig M, Rizopoulos D, Le Meur Y, Prémaud A, Woillard JB, et al. Impact of longitudinal exposure to mycophenolic acid on acute rejection in renal-transplant recipients using a joint modeling approach. Pharmacol Res. 2013 Jun;72:52–60.

27. Pinsky BW, Takemoto SK, Lentine KL, Burroughs TE, Schnitzler MA, Salvalaggio PR. Transplant outcomes and economic costs associated with patient noncompliance to

immunosuppression. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg. 2009 Nov;9(11):2597–606.

28. Daher Abdi Z, Prémaud A, Essig M, Alain S, Munteanu E, Garnier F, et al. Exposure to mycophenolic acid better predicts immunosuppressive efficacy than exposure to calcineurin inhibitors in renal transplant patients. Clin Pharmacol Ther. 2014 Oct;96(4):508–15.

29. Rabbat CG, Thorpe KE, Russell JD, Churchill DN. Comparison of mortality risk for dialysis patients and cadaveric first renal transplant recipients in Ontario, Canada. J Am Soc Nephrol JASN. 2000 May;11(5):917–22.

30. Cherukuri A, Welberry-Smith MP, Tattersall JE, Ahmad N, Newstead CG, Lewington AJP, et al. The clinical significance of early proteinuria after renal transplantation. Transplantation. 2010 Jan 27;89(2):200–7.

31. Cox DR. Regression Models and Life-Tables. J R Stat Soc Ser B Methodol. 1972;34(2):187–220.

32. Loupy A, Lefaucheur C, Vernerey D, Chang J, Hidalgo LG, Beuscart T, et al. Molecular microscope strategy to improve risk stratification in early antibody-mediated kidney allograft rejection. J Am Soc Nephrol JASN. 2014 Oct;25(10):2267–77.

33. Foucher Y, Daguin P, Akl A, Kessler M, Ladrière M, Legendre C, et al. A clinical scoring system highly predictive of long-term kidney graft survival. Kidney Int. 2010 Dec;78(12):1288–94.

34. Fournier M-C, Foucher Y, Blanche P, Buron F, Giral M, Dantan E. A joint model for longitudinal and time-to-event data to better assess the specific role of donor and recipient factors on long-term kidney transplantation outcomes. Eur J Epidemiol. 2016 Feb 1;1–11.

35. Garre FG, Zwinderman AH, Geskus RB, Sijpkens YWJ. A joint latent class changepoint model to improve the prediction of time to graft failure. J R Stat Soc Ser A Stat Soc. 2008 Jan 1;171(1):299–308.

36. Asar Ö, Ritchie J, Kalra PA, Diggle PJ. Joint modelling of repeated measurement and time-to-event data: an introductory tutorial. Int J Epidemiol. 2015 Feb;44(1):334–44.

37. Laird NM, Ware JH. Random-effects models for longitudinal data. Biometrics. 1982 Dec;38(4):963–74.

38. Wulfsohn MS, Tsiatis AA. A joint model for survival and longitudinal data measured with error. Biometrics. 1997 Mar;53(1):330–9.

39. Proust-Lima C, Taylor JMG. Development and validation of a dynamic prognostic tool for prostate cancer recurrence using repeated measures of posttreatment PSA: a joint modeling approach. Biostat Oxf Engl. 2009 Jul;10(3):535–49.

40. Proust-Lima C, Séne M, Taylor JM, Jacqmin-Gadda H. Joint latent class models for longitudinal and time-to-event data: A review. Stat Methods Med Res. 2014 Feb 1;23(1):74–90.

41. Proust-Lima C, Joly P, Dartigues J-F, Jacqmin-Gadda H. Joint Modelling of Multivariate Longitudinal Outcomes and a Time-to-event: A Nonlinear Latent Class Approach. Comput Stat Data Anal. 2009 Feb;53(4):1142–1154.

42. Rizopoulos D. Joint models for longitudinal and time-to-event data: with applications in R. Boca Raton: CRC Press; 2012. 261 p. (Chapman & Hall/CRC biostatistics series).

43. Proust-Lima C, Philipps V, Liquet B. Estimation of extended mixed models using latent classes and latent processes: the R package lcmm. ArXiv Prepr ArXiv150300890 [Internet]. 2015 [cited 2016 Feb 8]; Available from: http://arxiv.org/abs/1503.00890

44. Proust-Lima C, Amieva H, Jacqmin-Gadda H. Analysis of multivariate mixed longitudinal data: A flexible latent process approach. Br J Math Stat Psychol. 2013 Nov 1;66(3):470–87.

45. Boucquemont J, Loubère L, Metzger M, Combe C, Stengel B, Leffondre K, et al. Identifying subgroups of renal function trajectories. Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc. 2016 Dec 28;

46. Shabir S, Halimi J-M, Cherukuri A, Ball S, Ferro C, Lipkin G, et al. Predicting 5-year risk of kidney transplant failure: a prediction instrument using data available at 1 year posttransplantation. Am J Kidney Dis Off J Natl Kidney Found. 2014 Apr;63(4):643–51.

47. Prémaud A, Filloux M, Gatault P, Thierry A, Büchler M, Munteanu E, et al. An adjustable predictive score of graft survival in kidney transplant patients and the levels of risk linked to de novo donor-specific anti-HLA antibodies. PLOS ONE. 2017 Jul 3;12(7):e0180236.

48. Proust-Lima C, Dartigues J-F, Jacqmin-Gadda H. Joint modeling of repeated multivariate cognitive measures and competing risks of dementia and death: a latent process and latent class approach. Stat Med. 2016 Feb 10;35(3):382–98.

49. WHO | ADHERENCE TO LONG-TERM THERAPIES: EVIDENCE FOR ACTION [Internet]. WHO. [cited 2017 Mar 24]. Available from: http://www.who.int.gate2.inist.fr/chp/knowledge/publications/adherence_report/en/

50. Fine RN, Becker Y, De Geest S, Eisen H, Ettenger R, Evans R, et al. Nonadherence consensus conference summary report. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg. 2009 Jan;9(1):35–41.

51. Scheel J, Reber S, Stoessel L, Waldmann E, Jank S, Eckardt K-U, et al. Patient-reported non-adherence and immunosuppressant trough levels are associated with rejection after renal transplantation. BMC Nephrol [Internet]. 2017 Mar 29 [cited 2017 Aug 27];18. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5372303/

52. Ro H, Min S-I, Yang J, Moon KC, Kim YS, Kim SJ, et al. Impact of tacrolimus intraindividual variability and CYP3A5 genetic polymorphism on acute rejection in kidney transplantation. Ther Drug Monit. 2012 Dec;34(6):680–5.

53. Pizzo HP, Ettenger RB, Gjertson DW, Reed EF, Zhang J, Gritsch HA, et al. Sirolimus and tacrolimus coefficient of variation is associated with rejection, donor-specific antibodies, and nonadherence. Pediatr Nephrol Berl Ger. 2016 Dec;31(12):2345–52.

54. Hsiau M, Fernandez HE, Gjertson D, Ettenger RB, Tsai EW. Monitoring nonadherence and acute rejection with variation in blood immunosuppressant levels in pediatric renal transplantation. Transplantation. 2011 Oct 27;92(8):918–22.

55. Dew MA, DiMartini AF, De Vito Dabbs A, Myaskovsky L, Steel J, Unruh M, et al. Rates and risk factors for nonadherence to the medical regimen after adult solid organ transplantation. Transplantation. 2007 Apr 15;83(7):858–73.

56. Morrissey PE, Flynn ML, Lin S. Medication noncompliance and its implications in transplant recipients. Drugs. 2007;67(10):1463–81.

57. Butler JA, Roderick P, Mullee M, Mason JC, Peveler RC. Frequency and impact of nonadherence to immunosuppressants after renal transplantation: a systematic review. Transplantation. 2004 Mar 15;77(5):769–76.

58. Denhaerynck K, Dobbels F, Cleemput I, Desmyttere A, Schäfer-Keller P, Schaub S, et al. Prevalence, consequences, and determinants of nonadherence in adult renal transplant patients: a literature review. Transpl Int Off J Eur Soc Organ Transplant. 2005 Oct;18(10):1121–33.

59. Belaiche S, Décaudin B, Dharancy S, Noel C, Odou P, Hazzan M. Factors relevant to medication non-adherence in kidney transplant: a systematic review. Int J Clin Pharm. 2017 Jun;39(3):582–93.

60. Massey EK, Tielen M, Laging M, Timman R, Beck DK, Khemai R, et al. Discrepancies between beliefs and behavior: a prospective study into immunosuppressive medication adherence after kidney transplantation. Transplantation. 2015 Feb;99(2):375–80.

61. Farmer KC. Methods for measuring and monitoring medication regimen adherence in clinical trials and clinical practice. Clin Ther. 1999 Jun;21(6):1074–1090; discussion 1073.

62. Prihodova L, Nagyova I, Rosenberger J, Majernikova M, Roland R, Groothoff JW, et al. Adherence in patients in the first year after kidney transplantation and its impact on graft loss and mortality: a cross-sectional and prospective study. J Adv Nurs. 2014 Dec;70(12):2871–83.

63. Pabst S, Bertram A, Zimmermann T, Schiffer M, de Zwaan M. Physician reported adherence to immunosuppressants in renal transplant patients: Prevalence, agreement, and correlates. J Psychosom Res. 2015 Nov;79(5):364–71.

64. Schäfer-Keller P, Steiger J, Bock A, Denhaerynck K, De Geest S. Diagnostic accuracy of measurement methods to assess non-adherence to immunosuppressive drugs in kidney transplant recipients. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg. 2008 Mar;8(3):616–26.

65. Dharancy S, Giral M, Tetaz R, Fatras M, Dubel L, Pageaux G-P. Adherence with immunosuppressive treatment after transplantation: results from the French trial PREDICT. Clin Transplant. 2012 Jun;26(3):E293-299.

66. Massey EK, Meys K, Kerner R, Weimar W, Roodnat J, Cransberg K. Young Adult Kidney Transplant Recipients: Nonadherent and Happy. Transplantation. 2015 Aug;99(8):e89-96.

67. Sellarés J, de Freitas DG, Mengel M, Reeve J, Einecke G, Sis B, et al. Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg. 2012 Feb;12(2):388–99.

68. Cramer JA, Matson R. Monitoring compliance with antiepileptic drug therapy. In: Patient compliance in medical practice and clinical trials. New York: Raven Press; 1991. p. 123–37.

69. Morisky DE, Green LW, Levine DM. Concurrent and predictive validity of a self-reported measure of medication adherence. Med Care. 1986 Jan;24(1):67–74.

70. Morisky DE, Ang A, Krousel-Wood M, Ward HJ. Predictive validity of a medication adherence measure in an outpatient setting. J Clin Hypertens Greenwich Conn. 2008 May;10(5):348–54.

71. Tielen M, van Exel J, Laging M, Beck DK, Khemai R, van Gelder T, et al. Attitudes to medication after kidney transplantation and their association with medication adherence and graft survival: a 2-year follow-up study. J Transplant. 2014;2014:675301.

72. Chisholm-Burns M, Pinsky B, Parker G, Johnson P, Arcona S, Buzinec P, et al. Factors related to immunosuppressant medication adherence in renal transplant recipients. Clin Transplant. 2012 Oct;26(5):706–13.

73. Tsapepas D, Langone A, Chan L, Wiland A, McCague K, Chisholm-Burns M. A longitudinal assessment of adherence with immunosuppressive therapy following kidney transplantation from the Mycophenolic Acid Observational REnal Transplant (MORE) study. Ann Transplant. 2014 Apr 17;19:174–81.

74. Griva K, Davenport A, Harrison M, Newman SP. Non-adherence to immunosuppressive medications in kidney transplantation: intent vs. forgetfulness and clinical markers of medication intake. Ann Behav Med Publ Soc Behav Med. 2012 Aug;44(1):85–93.

75. Couzi L, Moulin B, Morin M-P, Albano L, Godin M, Barrou B, et al. Factors predictive of medication nonadherence after renal transplantation: a French observational study. Transplantation. 2013 Jan 27;95(2):326–32.

76. Villeneuve C. Déterminants et évolution de profils de qualité de vie et d'adhésion aux traitements: analyse dans une cohorte de patients transplantés rénaux [Internet] [Pharmacologie et science du medicament]. Limoges; 2017. Available from: https://tel.archives-ouvertes.fr/tel-01452926/document

77. De Geest S, Burkhalter H, Bogert L, Berben L, Glass TR, Denhaerynck K, et al. Describing the evolution of medication nonadherence from pretransplant until 3 years post-transplant and determining pretransplant medication nonadherence as risk factor for post-transplant nonadherence to immunosuppressives: the Swiss Transplant Cohort Study. Transpl Int Off J Eur Soc Organ Transplant. 2014 Jul;27(7):657–66.

78. Cramer JA, Mattson RH, Prevey ML, Scheyer RD, Ouellette VL. How often is medication taken as prescribed? A novel assessment technique. JAMA. 1989 Jun 9;261(22):3273–7.

79. Lam WY, Fresco P. Medication Adherence Measures: An Overview [Internet]. BioMed Research International. 2015 [cited 2018 Jan 15]. Available from: https://www.hindawi.com/journals/bmri/2015/217047/

80. Joost R, Dörje F, Schwitulla J, Eckardt K-U, Hugo C. Intensified pharmaceutical care is improving immunosuppressive medication adherence in kidney transplant recipients during the first post-transplant year: a quasi-experimental study. Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc. 2014 Aug;29(8):1597–607.

81. Hilbrands LB, Hoitsma AJ, Koene RA. Medication compliance after renal transplantation. Transplantation. 1995 Nov 15;60(9):914–20.

82. Russell CL, Moore S, Hathaway D, Cheng A-L, Chen G, Goggin K. MAGIC Study: Aims, Design and Methods using SystemCHANGE™ to Improve Immunosuppressive Medication Adherence in Adult Kidney Transplant Recipients. BMC Nephrol [Internet]. 2016 Jul 16 [cited 2017 Mar 29];17. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4947243/

83. Russell CL, Ashbaugh C, Peace L, Cetingok M, Hamburger KQ, Owens S, et al. Time-in-a-bottle (TIAB): a longitudinal, correlational study of patterns, potential predictors, and outcomes of immunosuppressive medication adherence in adult kidney transplant recipients. Clin Transplant. 2013 Oct;27(5):E580-590.

84. Russell C, Conn V, Ashbaugh C, Madsen R, Wakefield M, Webb A, et al. Taking immunosuppressive medications effectively (TIMELink): a pilot randomized controlled trial in adult kidney transplant recipients. Clin Transplant. 2011 Dec;25(6):864–70.

85. Nevins TE, Kruse L, Skeans MA, Thomas W. The natural history of azathioprine compliance after renal transplantation. Kidney Int. 2001 Oct;60(4):1565–70.

86. Feldman HI, Hackett M, Bilker W, Strom BL. Potential utility of electronic drug compliance monitoring in measures of adverse outcomes associated with immunosuppressive agents. Pharmacoepidemiol Drug Saf. 1999 Jan;8(1):1–14.

87. Butler JA, Peveler RC, Roderick P, Horne R, Mason JC. Measuring compliance with drug regimens after renal transplantation: comparison of self-report and clinician rating with electronic monitoring. Transplantation. 2004 Mar 15;77(5):786–9.

88. Kuypers DRJ, Peeters PC, Sennesael JJ, Kianda MN, Vrijens B, Kristanto P, et al. Improved adherence to tacrolimus once-daily formulation in renal recipients: a randomized controlled trial using electronic monitoring. Transplantation. 2013 Jan 27;95(2):333–40.

89. Spivey CA, Chisholm-Burns MA, Damadzadeh B, Billheimer D. Determining the effect of immunosuppressant adherence on graft failure risk among renal transplant recipients. Clin Transplant. 2014 Jan;28(1):96–104.

90. Gelb SR, Shapiro RJ, Thornton WJL. Predicting medication adherence and employment status following kidney transplant: The relative utility of traditional and everyday cognitive approaches. Neuropsychology. 2010 Jul;24(4):514–26.

91. Rozen-Zvi B, Schneider S, Lichtenberg S, Green H, Cohen O, Gafter U, et al. Association of the combination of time-weighted variability of tacrolimus blood level and exposure to low drug levels with graft survival after kidney transplantation. Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc. 2017 01;32(2):393–9.

92. Liu J, Liu S, Yan J, Yi Q, Huang H. Adherence to Immunosuppressive Medication in Renal Transplant Recipients From Follow-up Outpatient in China: Association of 2 Different Measurement Methods. Clin Ther. 2015 Nov 1;37(11):2572–80.

93. Shuker N, Shuker L, van Rosmalen J, Roodnat JI, Borra LCP, Weimar W, et al. A high intrapatient variability in tacrolimus exposure is associated with poor long-term outcome of kidney transplantation. Transpl Int. 2016 Nov 1;29(11):1158–67.

94. Borra LCP, Roodnat JI, Kal JA, Mathot RAA, Weimar W, van Gelder T. High within-patient variability in the clearance of tacrolimus is a risk factor for poor long-term

outcome after kidney transplantation. Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc. 2010 Aug;25(8):2757–63.

95. Rodrigo E, Segundo DS, Fernández-Fresnedo G, López-Hoyos M, Benito A, Ruiz JC, et al. Within-Patient Variability in Tacrolimus Blood Levels Predicts Kidney Graft Loss and Donor-Specific Antibody Development. Transplantation. 2016 Nov;100(11):2479–85.

96. Cramer JA, Scheyer RD, Mattson RH. Compliance declines between clinic visits. Arch Intern Med. 1990 Jul;150(7):1509–10.

97. Shuker N, van Gelder T, Hesselink DA. Intra-patient variability in tacrolimus exposure: causes, consequences for clinical management. Transplant Rev Orlando Fla. 2015 Apr;29(2):78–84.

98. Shemesh E, Fine RN. Is calculating the standard deviation of tacrolimus blood levels the new gold standard for evaluating nonadherence to medications in transplant recipients? Pediatr Transplant. 2010 Dec;14(8):940.

99. Gaynor JJ, Ciancio G, Guerra G, Sageshima J, Hanson L, Roth D, et al. Graft failure due to noncompliance among 628 kidney transplant recipients with long-term follow-up: a single-center observational study. Transplantation. 2014 May 15;97(9):925–33.

100. Morrissey PE, Reinert S, Yango A, Gautam A, Monaco A, Gohh R. Factors contributing to acute rejection in renal transplantation: the role of noncompliance. Transplant Proc. 2005 Jun;37(5):2044–7.

101. Tueller SJ, Deboeck PR, Van Dorn RA. Getting less of what you want: reductions in statistical power and increased bias when categorizing medication adherence data. J Behav Med. 2016 Dec;39(6):969–80.

102. Goldfarb-Rumyantzev AS, Scandling JD, Pappas L, Smout RJ, Horn S. Prediction of 3-yr cadaveric graft survival based on pre-transplant variables in a large national dataset. Clin Transplant. 2003 Dec;17(6):485–97.

103. Heylen L, Jochmans I, Samuel U, Tieken I, Naesens M, Pirenne J, et al. The duration of asystolic ischemia determines the risk of graft failure after circulatory-dead donor kidney transplantation: A Eurotransplant cohort study. Am J Transplant. :n/a-n/a.

104. Ferro CJ, Hodson J, Moore J, McClure M, Tomson CRV, Nightingale P, et al. Bayesian analysis of glomerular filtration rate trajectories in kidney transplant recipients: a pilot study. Transplantation. 2015 Mar;99(3):533–9.

105. Kaplan B, Schold J, Meier-Kriesche H-U. Poor predictive value of serum creatinine for renal allograft loss. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg. 2003 Dec;3(12):1560–5.

106. Nyberg SL, Matas AJ, Kremers WK, Thostenson JD, Larson TS, Prieto M, et al. Improved scoring system to assess adult donors for cadaver renal transplantation. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg. 2003 Jun;3(6):715–21.

107. Brown TS, Elster EA, Stevens K, Graybill JC, Gillern S, Phinney S, et al. Bayesian modeling of pretransplant variables accurately predicts kidney graft survival. Am J Nephrol. 2012;36(6):561–9.

108. Hourmant M, Cesbron-Gautier A, Terasaki PI, Mizutani K, Moreau A, Meurette A, et al. Frequency and clinical implications of development of donor-specific and non-donor-specific HLA antibodies after kidney transplantation. J Am Soc Nephrol JASN. 2005 Sep;16(9):2804–12.

109. Kasiske BL, Gaston RS, Gourishankar S, Halloran PF, Matas AJ, Jeffery J, et al. Long-term deterioration of kidney allograft function. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg. 2005 Jun;5(6):1405–14.

110. Howell M, Wong G, Rose J, Tong A, Craig JC, Howard K. Patient Preferences for Outcomes After Kidney Transplantation: A Best-Worst Scaling Survey. Transplantation. 2017 Nov;101(11):2765–73.

Appendix

Appendix I: Nonmem code for the final mixture model for tacrolimus time-exposure based on residual error

```
$PROB MODEL TACRO
$INPUT ID TIME MDV DV
$DATA Expo_Tacro_275.csv
      IGNORE @

$PRED

E0=THETA(1)*EXP(ETA(1))
E1=THETA(2)*EXP(ETA(2))
K=THETA(3)*EXP(ETA(3))

TACRO=E0+E1*EXP(-K*TIME)

EST=MIXEST
IF(MIXNUM.EQ.1) THEN
Y=TACRO+(TACRO*ERR(1))
ELSE
Y=TACRO+(TACRO*ERR(2))

ENDIF
IPRED=Y
$MIX
NSPOP=2
P(1)=THETA(4)
P(2)=1-P(1)

$THETA
(0,10); E0
(0,10); E1
(0,0.1,10; K
(0,0.3,1)

$OMEGA
0.01; ETA1
0.01; ETA2
0 FIXED; ETA3


$SIGMA
0.1; PROP PP1
0.1; PROP PP2

$EST METHOD=1 INTER MAX=9999 SIGDIGIT=4 POSTHOC NOABORT PRINT=5
$COV
$TABLE      ID EST TIME DV PRED IPRED CWRES NOPRINT ONEHEADER
FILE=sdtabC
$TABLE      ID E0 E1 K ETA(1) ETA(2) NOPRINT NOAPPEND
            ONEHEADER FILE=patabC
$TABLE      ID NOPRINT NOAPPEND ONEHEADER FILE=cotabC
```

Modélisation conjointe pour données longitudinales et données de survie: analyse des facteurs prédictifs du devenir de la greffe rénale

La prédiction du devenir du greffon et de sa survie permettrait d'optimiser la prise en charge des patients transplantés. Le suivi des patients transplantés rénaux inclue des mesures répétées de marqueurs longitudinaux tels que la créatinine sérique et l'exposition aux médicaments immunosuppresseurs ainsi que des donnés sur la survenue des évènements cliniques. Dans la première partie de ce travail, trois groupes homogènes des patients caractérisés par une trajectoire spécifique de l'évolution de la créatinine sérique en fonction du temps et un risque d'échec de greffe spécifique ont été identifiés en utilisant le modèle conjoint à classes latentes. Le risque individuel d'échec de greffe en fonction du temps était prédit avec un niveau de performance satisfaisant en termes de spécificité, sensibilité et précision chez les patients qui n'avaient pas développé d'anticorps anti-HLA spécifiques du donneur avec ce modèle. L'utilité clinique de cet outil devra être évaluée avec une approche dynamique. Dans la seconde partie, les modèles non linéaires à effets mixtes combinés avec l'approche des modèles de mélange ont été utilisée pour analyser (i) l'association entre la variabilité des concentrations de tacrolimus au cours du temps et l'adhésion au traitement rapportée par le patient et (ii) l'impact de cette variabilité des concentrations sur le risque de rejet aigu. Ce modèle a montré un effet significatif de la variabilité des concentrations du tacrolimus au cours du temps sur la survenu de rejet aigu au-delà de 3 mois post-transplantation mais aucune association entre l'adhésion et la variabilité des concentrations de tacrolimus d'une part, et le risque de rejet aigu d'autre part n'a été observée dans cette étude qui n'incluait que des patients modérément non-adhérents. Ce résultat pose la question de l'impact d'une non-adhésion modérée sur le devenir du greffon.

Mots-clés : transplantation rénale, modèle conjoint, profils de créatinine sériques, exposition longitudinale, tacrolimus, adhésion, rejet aigu, échec de greffe.

Joint modelling of longitudinal and time-to-event data: analysis of predictive factors of graft outcomes in kidney transplant recipients

Prediction of graft outcome would be useful to optimize patient care. Follow-up of kidney transplant patients include repeated measurements of longitudinal markers, such as serum creatinine and immunosuppressive drug levels as well as data about clinical outcomes. In the first part of present work, the joint latent class model identified three homogenous groups of patients with a class-specific time-evolution of serum creatinine and risk of graft failure. The individual predicted probabilities of graft failure calculated from this joint model were satisfying in terms of sensitivity, specificity and overall accuracy for group of patients who did not develop *de novo* donor specific anti-HLA antibodies. The clinical usefulness of developed predictive tool needs to be evaluated with a dynamic approach. In the second part, non-linear mixed effects models with a mixture of distribution for random effects were used to investigate (i) the association between variability over time in tacrolimus concentration-time (C_0-t) profiles and patient-reported drug adherence and (ii) the impact of this variability on the acute rejection risk. This model found a significant impact of variability in tacrolimus C_0-t profiles on acute rejection onset beyond 3 months post transplantation. On the contrary, no association between non-adherence and (i) variability in tacrolimus C_0-t profiles (ii) acute rejection was observed in our study which included moderate non-adherent patients only. This result questions the impact of moderate non adherence on graft outcome

Keywords : kidney transplantation, joint model, serum creatinine profiles, tacrolimus, longitudinal exposure, drug adherence, acute rejection, graft failure