Journal de la Société Française de Statistique
Vol. 155 No. 1 (2014)

Multi-state analysis of kidney transplant recipients outcome: a semi-Markov model for studying the role of pre-transplant sensitization against Angiotensin II Type 1 receptor.

Titre: Analyse multi-états des évènements survenant chez les patients transplantés rénaux : un modèle semi-Markovien pour étudier le rôle de l'immunisation pré-greffe contre le récepteur de type I de l'Angiotensine II

Florence Gillaizeau[1,2], Magali Giral[2], Etienne Dantan[1], Duska Dragun[3], Jean-Paul Soulillou[2] and Yohann Foucher[1,2]

Abstract: Chronic diseases are characterized by their long duration and generally their slow progression. To study the time to several stages of progression, traditional survival analyses are not appropriate and the use of multi-state models is required. Of these, the Semi-Markov model (SMM) is convenient because it considers that the probability that a patient goes from a state to another depends on the time already spent in this state.
In this paper, we illustrate the interest of using a SMM by re-analysing the data of an observational study which was designed to investigate the relationship between the pre-graft level of the angiotensin II type 1 receptor antibodies (AT_1R-Abs) and the evolution of kidney transplant recipients (KTR). Previous results were obtained by a multivariate Cox proportional hazards model and showed that patients with high pre-graft level of AT_1R-Abs seemed to have more risk of early acute rejection episodes (ARE) and return to dialysis after 3 years post-transplantation. Nevertheless, it was not possible to distinguish whether AT_1R-Abs had a direct correlation with the graft failure or if this correlation went through an increased incidence of ARE. Thus, a four-state model is proposed to study the graft without any ARE, the graft with at least one ARE, the return in dialysis and the patient death. 599 KTR transplanted in Nantes University Hospital between 1998 and 2007 were included. The baseline hazard functions of the sojourn time distributions were modelled using the generalized Weibull distribution.
At the time of the study, 403 (67%) patients had a functional graft without ARE whereas 105 (15%) patients returned to dialysis, 64 (11%) patients had an ARE and 50 (8%) patients died with a functional graft. Taking into account of traditional factors associated to the recipient's evolution, a high pre-graft level of AT_1R-Abs ($\geq$10U) was associated to an increased risk of ARE. For patients without ARE, there was no evidence of association between the pre-graft level of AT_1R-Abs and the risk of graft failure within the first 3 years following the transplantation. In contrast, a high pre-graft level of AT_1R-Abs seemed to increase this risk beyond 3 years post-transplantation. Finally, the association between the pre-graft level of AT_1R-Abs and the time to death was not significant. The goodness-of-fit of the SMM to our data seemed correct.
This study shows the SMMs are particularly adapted to investigate the relationship between a biomarker and the evolution of disease. These models offer additional information to physicians/scientists about the mechanistic associated to a biomarker. The biostatistical community underutilizes these models, which is counter-productive regarding the

[1] Université de Nantes, EA 4275 Biostatistiques, Pharmacoépidémiologie et Mesures Subjectives en Santé,1 rue Gaston Veil BP 53508, 44035 Nantes Cedex 1, France.
E-mail: florence.gillaizeau@univ-nantes.fr
[2] INSERM CR1064 Centre pour la Recherche en Transplantation et Immunointervention (CRTI), Institut Transplantation-Urologie-Néphrologie (ITUN), 30 Bd Jean Monnet, 44093 Nantes Cedex 01, France.
[3] Department of Nephrology, Charité University Hospital, Berlin, Germany.

 ISSN: 2102-6238

original results they offer in translational research. Further efforts are needed to promote such models to biostatisticians to expand their daily use.

Résumé : Les maladies chroniques sont caractérisées par leur longue durée et généralement leur lente progression. Pour étudier le délai de progression vers différents stades, les analyses de survie traditionnelles ne sont pas adaptées et l'utilisation de modèles multi-états est nécessaire. Parmi ceux-ci, le modèle semi-markovien (MSM) est intéressant car il considère que la probabilité qu'un patient passe d'un état à l'autre dépend du temps déjà passé dans cet état.
Dans cet article, nous illustrons l'intérêt d'utiliser un MSM en ré-analysant les données d'une étude observationnelle mise en place pour étudier la relation entre le niveau pré-greffe des anticorps anti-récepteurs de l'angiotensine II de type 1 (AT_1R-Ac) et l'évolution des patients transplantés rénaux (PTR). Les résultats précédents obtenus avec un modèle de Cox à risques proportionnels multivarié montraient que les patients avec un niveau pré-greffe élevé d'AT_1R-Ac semblaient avoir un risque plus élevé d'épisodes de rejets aigus (ERA) précoces et de retour en dialyse après 3 ans post-transplantation. Cependant, ces analyses ne permettaient pas de distinguer si AT_1R-Ac avait une corrélation directe avec l'échec de la greffe ou si cette corrélation était due à l'augmentation de l'incidence d'ERA. Par conséquent, nous proposons un modèle à 4 états pour étudier la greffe sans ERA, la greffe avec au moins un ERA, le retour en dialyse et le décès du patient. 599 PTR transplantés au CHU de Nantes entre 1998 et 2007 ont été inclus. Les fonctions de risque de base des distributions des temps d'attente dans les états ont été modélisées à partir d'une distribution de Weibull Généralisée.
Au moment de l'étude, 403 (67%) patients avaient un greffon fonctionnel sans ERA tandis que 105 (15%) patients étaient retournés en dialyse, 64 (11%) patients avaient eu un ERA et 50 (8%) patients étaient décédés avec un greffon fonctionnel. En tenant compte des facteurs usuels liés à l'évolution du receveur, un niveau pré-greffe élevé d'AT_1R-Ac ($\geq$10U) était associé à un risque accru de faire un ERA. Chez les patients n'ayant pas eu d'ERA il n'était pas mis en évidence d'association entre le niveau pré-greffe d'AT_1R-Ac et le risque d'échec de la greffe dans les 3 premières années suivant la transplantation, alors qu'un niveau pré-greffe élevé d'AT_1R-Ac semblait augmenter ce risque au delà de 3 ans post-transplantation. Enfin, l'association entre le niveau pré-greffe d'AT_1R-Ac et le temps de décès n'était pas significative. La qualité d'ajustement du MSM à nos données semblait correcte.
Cette étude montre que les MSM sont particulièrement adaptés pour étudier la relation entre un biomarqueur et l'évolution d'une maladie. Ces modèles offrent des informations supplémentaires aux médecins/scientifiques sur la mécanistique associée au biomarqueur. La communauté biostatistique sous-utilise ces modèles, ce qui est contre-productif vis-à-vis des résultats originaux qu'ils offrent en recherche translationnelle. Des efforts supplémentaires sont nécessaires pour promouvoir ces modèles aux biostatisticiens afin d'étendre leur utilisation au quotidien.

Keywords: Survival Analysis, Multistate models, Semi-Markov Process, Disease Progression, Kidney Transplantation
Mots-clés : Analyse de survie, Modèles multi-états, Processus semi-markovien, Progression de la maladie, Transplantation rénale
AMS 2000 subject classifications: 62N01, 62P10, 90C40

1. Introduction

Medical researchers are often interested to investigate the relationship between covariates and the time until clinical events such as disease progression or patient death. The most widely used method in survival analysis is the Cox proportional hazards model (Christensen, 1987). However, this regression is only appropriate to study the time to a single event while a patient can experience multiple events. This is especially true in chronic diseases where the diseases are long with several stages of progression.

The disease progression in a patient is characterized by a succession of events or stages and may be analysed using multi-states models (Andersen and Keiding, 2002; Commenges, 1999; Lau et al., 2009; Meira-Machado et al., 2009). The transition intensities define the probability that a patient goes from a state to another. Time homogeneous Markov models make the strong assumption that transition intensities are constant across time and only depend on the current state. This assumption is not realistic in most of medical applications. In contrast, time non-homogeneous

 ISSN: 2102-6238

Markov models consider that the transition intensities depend on the chronologic time (time since the baseline of the study) but this assumption may be not relevant again. Semi-Markov models (SMM) can be viewed as alternative because they consider that the transition intensities depend on the time already spent in the current state (Huzurbazar, 2004; Lagakos et al., 1978), assumption medically relevant. In renal transplantation, we have illustrated the goodness-of-fit of such SMM in previous studies (Foucher et al., 2007, 2010).

In this paper, we illustrate the interest of using a SMM by re-analysing the data of an observational study which was designed to investigate the relationship between the pre-graft level of the angiotensin II type 1 receptor antibodies and the evolution of kidney transplant recipients (KTR). The two main failures observed in KTR are the return in dialysis and the death. The first occurence of an acute rejection episode also represents an important serious event which increases the risk of return in dialysis.

2. Methods

2.1. The semi-Markovian process: definitions and notations

The disease progression in a patient is characterized by a succession of transitions between distinct clinical states that can occured at various times. The semi-Markovian process considers that the times of transitions from one state to another depend on the time already spent in the current state (Foucher et al., 2005; Perez Ocon et al., 1999). We adopt the notation T for the chronologic time (time since baseline of the study) and D for the duration in a state. Two parts can be modelled: the sequence of the observed states and the sojourn time distributions given sequences. Let $\mathscr{X} = \{1,2,...,S\}$ the finite state space of the possible clinical states. The stochastic process $\{X_m, T_m, m \in \mathbb{N}\}$ records the state X_m of the patient after the m-th transition occuring at time T_m after the beginning of the study with $T_1 < T_2 < ... < T_m$, and $T_0 = 0$ by convention. Let ε the set of possible transitions ij with $(i,j) \in (\mathscr{X}, \mathscr{X})$, where i is a transient state with j distinct from i.

The probabilities of next transition correspond to the probabilities that a patient in a transient state i enters in a state j on its next transition, for $ij \in \varepsilon$. They are defined by:

$$p_{ij} = P(X_{m+1} = j | X_m = i) \text{ , respecting the constraint } \sum_{j:ij\in\varepsilon} p_{ij} = 1 \tag{1}$$

The sojourn time distributions can be characterized by the instantaneous hazard function $\lambda_{ij}(d)$ of the duration in the state i given that j is the next state. This instantaneous hazard function is defined by:

$$\lambda_{ij}(d) = \lim_{\Delta d \to 0^+} P(d \leq T_{m+1} - T_m < d + \Delta d | T_{m+1} - T_m > d, X_{m+1} = j, X_m = i)/\Delta d \tag{2}$$

Let $S_{ij}(d)$ be the corresponding survival function with:

$$S_{ij}(d) = P(T_{m+1} - T_m > d | X_{m+1} = j, X_m = i) = \exp\left(- \int_0^d \lambda_{ij}(u)\, \mathrm{d}u \right) \tag{3}$$

The corresponding probability density function $f_{ij}(d)$ can be directly obtained from (2) and (3) since $f_{ij}(d) = \lambda_{ij}(d) S_{ij}(d)$.

 ISSN: 2102-6238

Regardless the possible covariates, the SMM implies that the instantaneous joint probability of jumping towards the state j from state i at duration d depends only on the current state i and the sojourn time d in this state.

2.2. *Modelling of the sequence of the states*

We note W_{ij} the matrix of the covariates (in which the first column is composed of ones) associated with the probability p_{ij} and γ_{ij} the corresponding vector of regression coefficients. This probability can be modelled using multinomial logistic functions (Foucher et al., 2007):

$$p_{ij}(W_{ij}) = \exp(\gamma'_{ij}W_{ij}) / \sum_{j:ij\in\varepsilon} \exp(\gamma'_{ij}W_{ij}) \tag{4}$$

In order to satisfy the constraint (1), a reference transition ij_{ref} has to be defined with $\gamma_{ij_{ref}} = 0$.

Similarly to a multinomial logistic regression model, the exponential of regression coefficients can be interpreted as Odds Ratios (OR). It is therefore straightforward to interpret these reults in terms of risk factors associated to a certain sequence of states.

2.3. *Modelling of the sojourn times*

We note Z_{ij} the matrix of the covariates associated with the duration in the state i before the transition to the state j, and β_{ij} the corresponding vector of regression coefficients. By assuming the proportionality of hazards (PH), the instantaneous hazard function λ_{ij} can be decomposed in the following way:

$$\lambda_{ij}(d|Z_{ij}) = \lambda_{ij,0}(d)\exp(\beta'_{ij}Z_{ij}) \tag{5}$$

where $\lambda_{ij,0}(d)$ is the baseline hazard function. For our application, we chose to use the generalized Weibull distribution (Nikulin and Haghighi, 2009):

$$\lambda_{ij,0}(d) = \frac{1}{\theta_{ij}}\left(1+\left(\frac{d}{\sigma_{ij}}\right)^{\nu_{ij}}\right)^{\frac{1}{\theta_{ij}}-1}\nu_{ij}\left(\frac{1}{\sigma_{ij}}\right)^{\nu_{ij}} d^{\nu_{ij}-1} \tag{6}$$

with $\sigma_{ij} > 0$, $\nu_{ij} > 0$ and $\theta_{ij} > 0$.

The interesting feature of the hazard function of the generalized Weibull family is that it assumes different shapes: constant, monotone, $\cap$ or $\cup$-shaped.

PH assumptions can be inspected on log-minus-log plot of the survival probability as function of the time spent in each state. When covariates have time-varying effects on sojourn time distributions, the hazard function (5) can be piecewise-defined by assuming a common baseline hazard function but different regression coefficients for each time intervals. In this case, the SMM remains homogeneous regarding the chronological since the regression coefficients still depend on the duration in the current state.

Similarly to usual PH models, the exponential of the regression coefficients can be interpreted as Hazards Ratio (HR). Among patients experiencing the transition ij, HR greater than one (respectively lower) illustrate risk factors for more rapid transitions (respectively less rapid).

 ISSN: 2102-6238

2.4. The likelihood function

The contribution to likelihood for a subject h who jumps from state i to state j after a sojourn time d in this state i given its characteristics W_{ij}^h and Z_{ij}^h is:

$$\lim_{\Delta d \to 0^+} P(d \leq T_{m+1} - T_m < d + \Delta d, X_{m+1} = j | X_m = i, W_{ij}^h, Z_{ij}^h)/\Delta d = p_{ij}(W_{ij}^h) f_{ij}(d|Z_{ij}^h) \tag{7}$$

The contribution to likelihood for a subject h right censored in the state i after a sojourn time d in this state i given its characteristics W_{ij}^h and Z_{ij}^h is:

$$P(T_{m+1} - T_m > d | X_m = i, W_{ij}^h, Z_{ij}^h) = \sum_{j:ij \in \varepsilon} p_{ij}(W_{ij}^h) S_{ij}(d|Z_{ij}^h) \tag{8}$$

Let ε_h the set of transitions ij observed for the patient h at respective durations d_{ij} before its last time of follow-up. Let ε'_h the set of possible next transitions that may occur for the patient h after duration d_i in the state i at its last time of follow-up. The likelihood for a sample H of subjects can therefore be written:

$$L = \prod_{h \in H} \prod_{ij \in \varepsilon_h} p_{ij}(W_{ij}^h) f_{ij}(d_{ij}|Z_{ij}^h) \left[\sum_{ij \in \varepsilon'_h} p_{ij}(W_{ij}^h) S_{ij}(d_i|Z_{ij}^h) \right]^{\delta'_h} \tag{9}$$

with $\delta'_h = 1$ if the patient h is in a transient state at the last time of follow-up and $\delta'_h = 0$ otherwise.

We used the R statistical software (R Core Team, 2012) with *optim()* function to maximise the likelihood function and to compute the corresponding Hessian matrix (Nelder and Mead (1965) algorithms).

2.5. Goodness-of-fit of the SMM

We used the goodness-of-fit test proposed by Foucher et al. (2010) to check the chronological time homogeneity assumption of the proposed SMM. Briefly, this Pearson-type test compares observed and expected numbers of failures according to chronological intervals l. Using the notation $o_{l,f}$ and $e_{l,f}$ for observed and expected numbers of failures respectively, the Pearson statistic is defined by:

$$G = \sum_{l \in L} \sum_{f \in F} (o_{l,f} - e_{l,f})^2 / e_{l,f} \tag{10}$$

with F the set of final events and L the number of chronological time intervals. A semi-parametric boostrap procedure was implemented with the constitution of 300 bootstrap samples in order to estimate the distribution of this statistic.

In parallel, in order to evaluate the adequation of the parametric assumptions, we also compared for each transition the estimations of the cumulative incidence functions (CIFs) from the univariate SMM including the biomarker of interest and from the stratified non-parametric Aalen-Johansen estimator (Aalen, 1978). The CIF of an event at time t represents the probability that this event occurs before time t. Resampled parameters from the multinormal distribution were used to estimate the mean and 95% confidence intervals (95%CIs) of the CIFs from the SMM (500 simulations) (Aalen et al., 1997).

 ISSN: 2102-6238

2.6. Simulations

We performed simulations to assess the performance of our SMM model according to varying censoring rates, particularly regarding the high censoring rate observed in our application in kidney transplantation. Patient characteristics were simulated using a multinomial distribution with proportions observed within strata in the original sample. For each patient, and according to its characteristics, the sequence of the states was simulated using a multinomial distribution with parameters estimated from the final model (Table 2). Sojourn time distributions were simulated using parameters estimated from the same final model. Censored survival data were simulated with the Weibull distribution to have censoring rates approximately equals to 30%, 50% and 70% (the latter corresponding to the censoring rate observed in our original sample). A censoring is defined when the patient remained in the initial state at the end of its follow-up. We excluded simulated data sets with missing transitions or with less than ten observations on transitions for which the sojourn time distribution was modeled according to covariates: in practice, a multistate model is not considered when the number of transitions is too small. For each censoring rate, we estimated the parameters of the final SMM for 100 data sets with a sample size of 600 subjects. For each scenario, we reported several criteria: the mean absolute bias (mean difference between the estimate and the true value of parameter), the mean relative bias (mean ratio of the absolute bias and the true value of parameter), the root mean square error (RMSE), the estimated asymptotic standard error (obtained from the inverse of the information matrix), the empirical standard error (estimated as the standard deviation of the estimated effect over simulations), and the empirical coverage of the nominal 95% CIs (estimated as the proportion of samples, in which the 95% CIs included the true effect).

3. Application in renal transplantation: the role of pre-graft AT_1R-Abs in the evolution of recipients

3.1. Study population and data collection

The study population was the adult kidney transplant recipients (KTR) with no other simultaneous organ transplantation. Patients from the study had been transplanted between the March 13, 1998 and the November 26, 2007 at Nantes University hospital and were followed up as a part of the DIVAT cohort (Données Informatisées et VAlidées en Transplantation, www.divat.fr). The data were collected until March 29, 2012. Data were prospectively collected, in particular the date of acute rejection episode (ARE), return to dialysis, death, and last follow-up. The quantitative assay of the pre-formed non-HLA antibodies (before the transplantation) against angiotensin II type 1 receptor (AT_1R-Abs) was performed in Berlin (Germany), using extracts of cell overexpressing the human AT_1R as a solid phase, and blinding from information concerning the clinical data. The other studied parameters included the donor age, recipient age, gender, number of previous transplants, panel reactive antibodies (PRA) on T and B cells and HLA-A-B-DR incompatibilities.

Characteristics of the 599 KTR in the sample and according to the pre-graft level of AT_1R-Abs are reported in Table 1. Median age was 51 years (Range: 14-79) and 73 (13%) KTR had already had a previous graft transplantation. Graft transplant mainly came from cadaveric donor (94%). There were 316 patients (52.8%) with a pre-graft level of AT_1R-Abs lower than 10 Units (U) and

 ISSN: 2102-6238

Table 1: Characteristics of KTR at transplantation according to the pre-graft level of AT_1R-Abs

		All sample (N=599)		AT_1R-Abs≥10U (N=316)[1]		AT_1R-Abs<10U (N=283)[1]		
	NA[2]	Mean	SD	Mean	SD	Mean	SD	*p*-value
Quantitative characteristics								
Recipient age (years)	0	48.87	14.23	49.57	14.06	48.08	14.39	0.1999
Body Mass Index (kg/m2)	9	23.64	4.36	23.69	4.40	23.57	4.32	0.7328
Cold ischemia time (hours)	3	24.26	10.49	24.55	10.20	23.94	10.81	0.4825
Donor age (years)	1	46.59	16.39	47.82	16.58	45.23	16.09	0.0529
	NA[2]	N	%	N	%	N	%	*p*-value
Qualitative characteristics								
Male recipient	0	365	60.9	203	64.2	162	57.2	0.0797
Recipient age > 55 years	0	217	36.2	120	38.0	97	34.3	0.3470
Body Mass Index < 20 kg/m2	9	120	20.3	58	18.6	62	22.2	0.2818
Body Mass Index > 25 kg/m2	9	188	31.9	94	30.2	94	33.7	0.3669
Graft rank > 1	0	78	13.0	43	13.6	35	12.4	0.6525
Cold ischemia time > 24 hours	3	264	44.3	145	46.3	119	42.0	0.2939
Delayed graft function	20	217	37.5	110	36.3	107	38.8	0.5406
HLA-ABDR incompatibilities > 5	3	31	5.2	17	5.4	14	5.0	0.8051
Recurent initial disease	5	206	34.7	100	31.9	106	37.7	0.1399
Panel Reactive Antibody on B> 25%	3	81	13.6	40	12.7	41	14.6	0.5010
Panel Reactive Antibody on T> 25%	3	74	12.4	34	10.8	40	14.2	0.2035
Donor age > 55 years	1	190	31.8	113	35.9	77	27.2	0.0231
Male donor	0	379	63.3	204	64.6	175	61.8	0.4906
Positive CMV serology[3]	0	295	49.2	148	46.8	147	51.9	0.2119
Positive HCV serology[4]	0	31	5.2	16	5.1	15	5.3	0.8960
Cadeveric donor	0	564	94.2	301	95.3	263	92.9	0.2268
Induction therapy with ATG[5]	2	203	34.0	108	34.3	95	33.7	0.8777
Induction therapy with Simulect	2	296	49.6	164	52.1	132	46.8	0.1998

[1] AT_1R-Abs≥10U: pre-graft level of angiotensin II type 1 receptor greater or equal to 10U; AT_1R-Abs<10U: pre-graft level of angiotensin II type 1 receptor lower than 10U
[2] NA=Not Available
[3] CMV=Cytomegalovirus
[4] HCV=Hepatitis C virus
[5] ATG=Anti Thymo-Globulin

283 (47.2%) with a level greater or equal to 10U. There were no significant differences between characteristics of the patients according to the pre-graft level of AT_1R-Abs except for donor's age which tended to be higher for KTR with low pre-graft level of AT_1R-Abs (47.8 versus 45.2 years, Student's test *p*-value=0.053). The KTR had a mean follow-up time (±SD) of 6.9 years (±3.4) and a similar median follow-up time (Min-Max:0.5-13.3).

3.2. Results previously obtained from traditional survival analyses

Data of this observational study were previously analysed to investigate the relationship between the pre-graft level of AT_1R-Abs and graft survival (main outcome) and between the pre-graft level of AT_1R-Abs and time-to-ARE (secondary outcome) (Giral et al., rint). These two events

 ISSN: 2102-6238

were analysed independently using two multivariate Cox PH regression models with time-varying coefficients. Death with a functional graft was considered as right censoring in both analyses. Returns to dialysis without previous ARE was also considered as right censoring for the study of time-to-ARE. These two modellings are commonly used in transplantation litterature. A threshold of AT_1R-Abs levels was previously determined at 10U based on a survival analysis of time-to-graft failure and using the methodology proposed by Hothorn and Zeileis (2008).

The results indicated that patients with pre-graft level of AT_1R-Abs$\geq$10U had more risk to return to dialysis after 3 years post-transplantation and more risk to do an ARE in the 4 months post-transplantation. The delayed correlation between pre-graft level of AT_1R-Abs and time-to-dialysis could be due to the significant higher frequency of ARE during the first months of the transplantation. However the hypothesis of a chronological mechanistic of the pre-graft level of AT_1R-Abs on the evolution of recipients could not be assessed from these analyses. We thus proposed to re-analyse the data using a multi-state SMM.

The multi-state model is represented in Figure 1. The set of possible states was $\mathscr{X} = \{1,2,3,4\}$ and the set of possible transitions was $\varepsilon = \{12,13,14,23,24\}$. The ARE (state 2) is a transient state while the return in dialysis (state 3) and death with a functional graft (state 4) constitute two absorbing states. A patient could have been into a maximum of three states with two transitions occuring at the chronological times T_1 and T_2. No return transition is clinically possible.

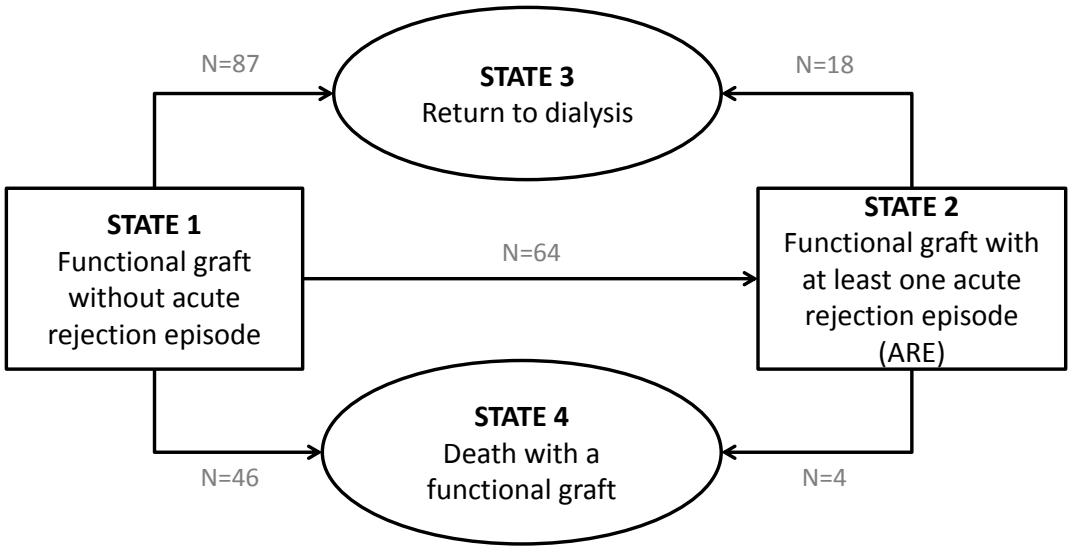

Figure 1: The multi-state structure (states and possible transitions) for the analysis of kidney transplant data.
Squares represent transient states and ellipses absorbing states. Arrows correspond to possible transitions between the states at beginnings of arrows and the states at points. Numbers above arrows indicate the numbers of observed transitions among the 599 patients initially included. Among the 599 patients initially included, 402 were right-censored at the end of the follow-up period (no transition observed).

 ISSN: 2102-6238

3.3. *Strategy of modelling*

To compare the results from the SMM and from the traditional PH models previously described, we used the same threshold of 10U for AT_1R-Abs. Covariates for adjustment were dichotomized according to clinical criteria (reference level indicated): donor age ($\leq$ 55 years), recipient age ($\leq$ 55 years), gender (female sex), number of previous transplants (one), panel reactive antibodies (PRA) on T and B cells ($\leq$ 25%) and HLA-A-B-DR incompatibilities ($\leq$ 5).

In a first time, we analysed the sojourn time distributions $\lambda_{ij}(d)$ without covariates and assumed the generalized Weibull distribution for the baseline hazard function (6). This distribution was simplified into the Weibull or the Exponential distributions when there was no evidence of lack of fit, according to non-parametric CIFs and likelihood ratio statistic (LRS). We then forced the covariate AT_1R-Abs in the SMM on the probabilities associated to the sequence of the states and on the transition intensities with possible time-varying effect.

Finally, the multivariate analysis was performed in a stepwise selection way. Each covariate was successively candidate in the SMM for the probabilities associated to the sequence of the states and for the transition intensities. Significant regression coefficients at 0.20 level were kept in the forward selection. Non significant terms (p-value > 0.05) were removed in a backward selection to obtain the final model. In order to avoid confounding results, the covariates were kept if they caused relative variations greater than 20% on other parameters when they were removed from the model. The LRS was used for variable selection. Due to few deaths after ARE, we did not analyze any association between covariates and death occurring after ARE. Thus, no covariate was included in the probability associated with the sequence of states or in the distribution of time-to-death after ARE.

3.4. *The estimated SMM*

Estimated parameters from the multivariate SMM are presented in Table 2. We will focus the interpretation of results on the AT_1R-Abs effects unless the regression model was multivariate to take account of the potential confounding factors. Log-minus-log plot of the survival probabilities according to AT_1R-Abs are given in Appendix.

We chose ARE as the reference modality to model the probability of second visited state. The coefficients $\gamma_{13}AT_1$R-Abs and $\gamma_{14}AT_1$R-Abs are thus respectively associated to the odds ratio of returning to dialysis or dying directly after transplantation rather than doing an ARE, for patients with high pre-graft level of AT_1R-Abs. The negative coefficients indicated that high pre-graft levels of AT_1R-Abs were associated to an increased risk to do an ARE. Patients with pre-graft AT_1R-Abs$\geq$10U were about 5 times more likely to have an ARE (adjusted OR[95%CI] of return to dialysis directly =0.24[0.08-0.70], and adjusted OR[95%CI] of death directly =0.18[0.07-0.47]).

Distribution of time-to-ARE (transition 12) was modelled using the generalized Weibull function for the baseline hazard function (three parameters σ_{12},ν_{12},θ_{12}), with a time-varying regression coefficient for the pre-graft level of AT_1R-Abs at 4 months post-transplantation (see Appendix). The null hypothesis $\theta_{12} = 0$ was rejected (SMM without covariate: LRS=32.6, df=1, p-value < 0.0001; final SMM: LRS=15.9, df=1, p-value < 0.0001) indicating that the generalised Weibull distribution was more appropriate than the simple Weibull distribution. In addition, this reflects a medical reality for which the rejection process results in clinical signs after a few weeks of

 ISSN: 2102-6238

Table 2: Final Semi Markov Model for the analysis of the relationship between AT_1R-Abs and the time-to-events (N=575)[1].

Coefficient[2]	Estimate	SE	Wald	exp(Est)	95%CI	*p*-value
Generalized Weibull distribution						
$\log(\sigma_{12})$	-4.12	0.19	-22.08	0.02	[0.01-0.02]	0.0000
$\log(\nu_{12})$	1.66	0.45	3.68	5.28	[2.18-12.75]	0.0002
$\log(\theta_{12})$	2.99	0.50	5.95	19.95	[7.49-53.15]	0.0000
$\log(\sigma_{13})$	3.58	0.33	10.74	35.73	[18.71-68.22]	0.0000
$\log(\nu_{13})$	0.26	0.14	1.83	1.29	[0.98-1.70]	0.0673
$\log(\sigma_{14})$	3.68	0.23	15.80	39.49	[25.16-61.98]	0.0000
$\log(\sigma_{23})$	3.43	0.85	4.04	30.85	[5.83-163.23]	0.0001
$\log(\sigma_{24})$	4.03	0.56	7.23	56.03	[18.70-167.93]	0.0000
Probabilities of first transition[3]						
γ_{13} AT_1R-Abs≥10U	-1.41	0.54	-2.62	0.24	[0.08-0.70]	0.0088
γ_{14} AT_1R-Abs≥10U	-1.74	0.50	-3.45	0.18	[0.07-0.47]	0.0006
γ_{13} Recipient Age>55 years	-0.52	0.43	-1.22	0.59	[0.25-1.38]	0.2233
γ_{14} Recipient Age>55 years	1.67	0.43	3.90	5.30	[2.28-12.30]	0.0001
Transition intensities[4]						
From transplantation to ARE						
β_{12} AT_1R-Abs≥10U, t<4 months	-1.16	0.44	-2.65	0.31	[0.13-0.74]	0.0081
β_{12} AT_1R-Abs≥10U, t≥4 months	-2.70	0.65	-4.16	0.07	[0.02-0.24]	0.0000
β_{12} HLA-ABDR incompatibilities>5	1.16	0.48	2.41	3.19	[1.24-8.17]	0.0161
From transplantation to return to dialysis						
β_{13} AT_1R-Abs≥10U, t<3 years	0.10	0.49	0.21	1.11	[0.43-2.90]	0.8298
β_{13} AT_1R-Abs≥10U, t≥3 years	0.86	0.43	2.02	2.37	[1.02-5.50]	0.0430
β_{13} Recipient Age>55 years	1.90	0.48	3.99	6.72	[2.62-17.21]	0.0001
β_{13} PRA on T cells>25%	0.49	0.38	1.30	1.64	[0.78-3.45]	0.1928
From transplantation to death						
β_{14} PRA on B cells>25%	0.94	0.44	2.12	2.56	[1.08-6.08]	0.0341
From ARE to return to dialysis						
β_{23} AT_1R-Abs≥10U	1.38	0.75	1.83	3.98	[0.91-17.29]	0.0672
β_{23} Donor Age>55 years	1.71	0.65	2.62	5.54	[1.55-19.80]	0.0087

[1] Final multivariate SMM model after covariate selection. There were 24 patients excluded from analysis because of missing data.

[2] 12: transition from transplantation to ARE; 13: transition from transplantation to return to dialysis; 14: transition from transplantation to death; 23: transition from ARE to return to dialysis; 24: transition from ARE to death. AT_1R-Abs≥10U: pre-graft level of angiotensin II type 1 receptor greater or equal to 10U. PRA: panel reactive antibodies.

[3] Estimations (SE) of intercepts γ_{13},γ_{14}, and γ_{24} in the multinomial logistic functions were: 1.91 (0.27), 0.90 (0.40), and -0.05 (0.56) respectively (see Equation (4) for details). No covariate included for the probabilities of second transition.

[4] t: time since graft transplantation (years).

transplantation but became rare after 2 years post-transpantation. Regardless its time-dependence, the negative coefficients associated with AT_1R-Abs indicated that, among patients who had an ARE, these acute events seemed to appear earlier for individuals with pre-graft levels of AT_1R-Abs lower than 10U (HR[95%CI]=0.31[0.13-0.74] before 4 months post-transplantation and 0.07[0.02-0.24] after).

Distribution of time-to-dialysis directly after transplantation (transition 13) was modelled using

 ISSN: 2102-6238

the Weibull function for the baseline hazard function (two parameters σ_{13},ν_{13}). There was a time-varying regression coefficient for the pre-graft level of AT_1R-Abs on the time-to-return directly to dialysis at 3 years post-transplantation (see Appendix). The pre-graft level of AT_1R-Abs seemed to not influence the time-to-return directly to dialysis in the 3 years following the transplantation. On the contrary, among patients who returned to dialysis without ARE, an high pre-graft level of AT_1R-Abs seemed to be associated with earlier failure after three years post-transplantation (HR[95%CI]=2.37[1.02-5.50]).

Association between AT_1R-Abs and time-to-death (transition 14) was not significant and was removed. However, there is no clinical justification to expect an association between pre-transplant sensitization against AT_1R and patient survival.

Distribution of time-to-dialysis after ARE (transition 23) was modelled using the Exponential function (one parameter σ_{23}). Patients who returned to dialysis after an ARE tended to have earlier failure when the pre-graft level of AT_1R-Abs was higher than 10U (HR[95%CI]=3.98 [0.91-17.29]).

3.5. Goodness-of-fit

Table 3: Contingency table for the observed and expected counts[1] of final events on the original sample (N=575).[2]

Chronological time (in years)		**Final events**	
		Return to dialysis	**Death with a functional graft**
[0;1.820[	Observed	20	8
	Expected	17.57	11.09
[1.820;3.811[	Observed	18	10
	Expected	22.34	10.41
[3.811;6.013[	Observed	23	6
	Expected	21.33	8.40
[6.013;8.681[	Observed	18	9
	Expected	13.62	5.42
[8.681;13.329[	Observed	16	12
	Expected	7.22	2.96

[1] Expected transitions are derived from the final SMM on the original sample (see Foucher et al. (2010) for details).

[2] The statistic G comparing the number of observed final events on the original sample and the number of expected final events from the SMM was equal to 45.01 (see Equation (10) for details).

The CIFs derived from the univariate SMM including AT_1R-Abs and from the non-parametric Aalen-Johansen estimator are presented in Figure 2. To have a better visualisation, the 95%CI of probabilities are not presented. Since AT_1R-Abs was not included in the SMM for probabilities p_{23},p_{24} and for the transition intensity $\lambda_{24}(d)$ because of the low number of events (N=4), we did not represent the corresponding graph. For all other transitions, both curves from the univariate SMM estimations were close to the stratified non-parametric estimations.

The observed counts of final events and the expected counts derived from the final SMM are presented in Table 3. We chose to compute the goodness-of-fit statistic using five intervals of

 ISSN: 2102-6238

chronological times defined with the quantiles of the times of occurence of return-to-dialysis and death. Value of the goodness-of-fit statistic G_0 of the SMM on the original sample was 45.01. Of the 300 bootstrap samples, a total of 228 goodness-of-fit statistics were greater than or equal to G_0, corresponding to a p-value of 0.76. Thus, the stationary assumption of the SMM can not be rejected regarding the chronological time.

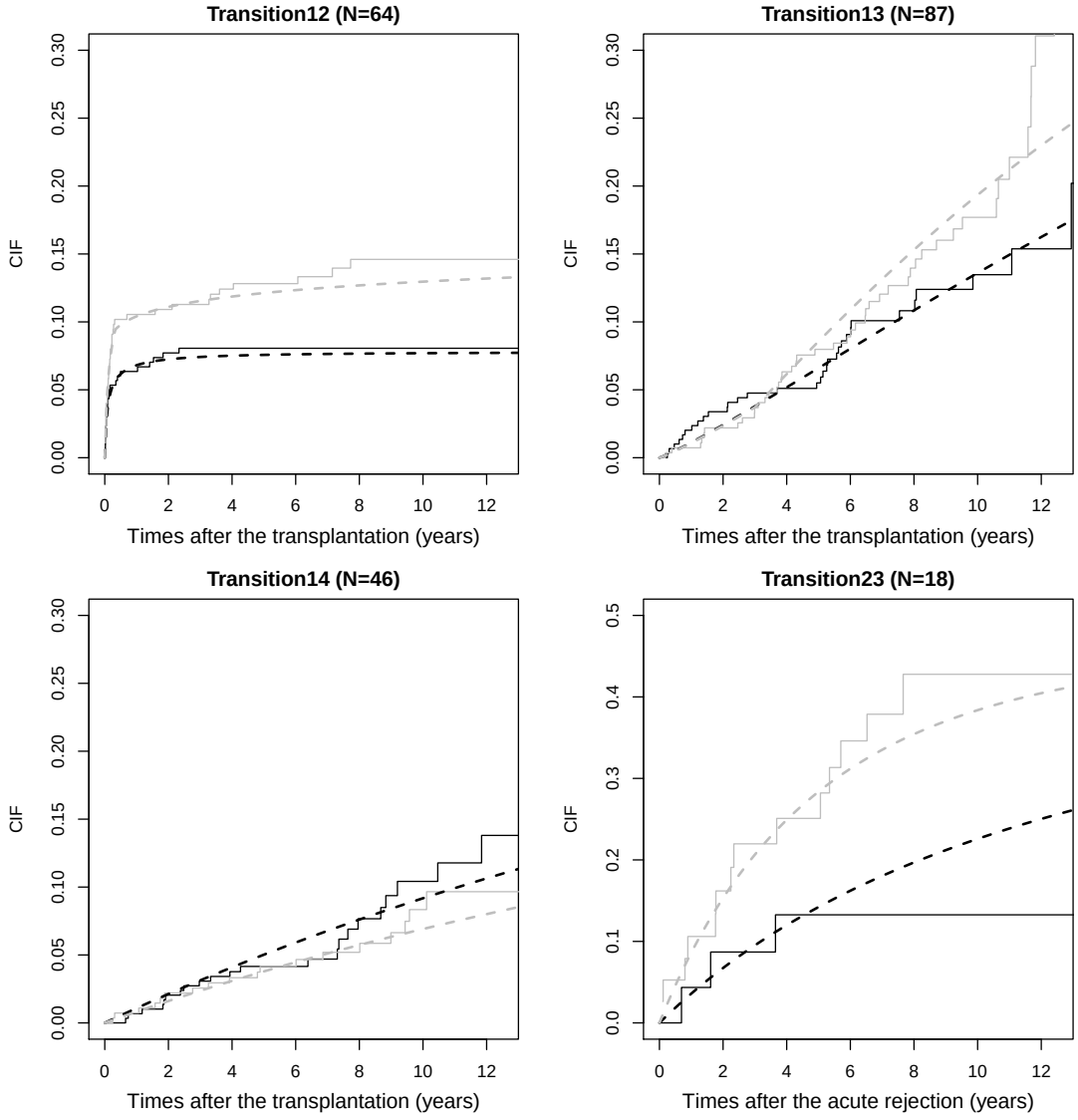

Figure 2: Estimations of the cumulative incidence functions associated to transitions from the non-parametric Aalen-Johansen estimator and from the SMM.
Black color corresponds to AT_1R-Abs<10U and gray color corresponds to AT_1R-Abs $\geq$10U. Steps are the non-parametric Aalen-Johansen estimations of the cumulative incidence functions (CIFs). Dash lines are the estimations of the CIFs from the univariate SMM including AT_1R-Abs.

 ISSN: 2102-6238

3.6. Simulations

Table 4: Performances of the SMM according to three censoring rates (100 simulated samples of 600 patients)

Censoring rate	Coefficient[1]	True effect	Mean estimate	Absolute bias	% Relative bias	RMSE[2]	Empiric SE	Asymptotic SE	Coverage rate (%)
≈ 30%	γ_{13} AT_1R-Abs≥10U	-1.41	-1.45	-0.04	2.70	0.25	0.25	0.29	98
	γ_{14} AT_1R-Abs≥10U	-1.74	-1.77	-0.03	2.00	0.32	0.32	0.34	97
	γ_{13} Recipient Age>55 years	-0.52	-0.57	-0.05	9.60	0.31	0.31	0.31	95
	γ_{14} Recipient Age>55 years	1.67	1.65	-0.01	-0.90	0.31	0.31	0.32	97
	β_{12} AT_1R-Abs≥10U, t<4 months	-1.16	-1.20	-0.04	3.10	0.35	0.35	0.30	94
	β_{12} AT_1R-Abs≥10U, t≥4 months	-2.70	-2.80	-0.11	4.00	0.48	0.47	0.45	97
	β_{12} HLA-ABDR incompatibilities>5	1.16	1.09	-0.07	-6.20	0.78	0.78	0.63	93
	β_{13} AT_1R-Abs≥10U, t<3 years	0.10	0.06	-0.05	-44.30	0.38	0.38	0.38	99
	β_{13} AT_1R-Abs≥10U, t≥3 years	0.86	0.85	-0.01	-0.80	0.17	0.17	0.17	94
	β_{13} Recipient Age>55 years	1.90	1.94	0.03	1.80	0.20	0.20	0.20	93
	β_{13} PRA on T cells>25%	0.49	0.51	0.01	2.40	0.22	0.22	0.22	97
	β_{14} PRA on B cells>25%	0.94	0.97	0.03	3.10	0.31	0.31	0.28	92
	β_{23} AT_1R-Abs≥10U	1.38	1.36	-0.02	-1.70	0.55	0.55	0.49	91
	β_{23} Donor Age>55 years	1.71	1.66	-0.06	-3.30	0.58	0.58	0.47	92
≈ 50%	γ_{13} AT_1R-Abs≥10U	-1.41	-1.48	-0.06	4.50	0.39	0.39	0.39	94
	γ_{14} AT_1R-Abs≥10U	-1.74	-1.80	-0.07	3.90	0.45	0.45	0.43	94
	γ_{13} Recipient Age>55 years	-0.52	-0.53	-0.01	1.60	0.35	0.35	0.34	94
	γ_{14} Recipient Age>55 years	1.67	1.67	0.01	0.30	0.35	0.35	0.37	94
	β_{12} AT_1R-Abs≥10U, t<4 months	-1.16	-1.21	-0.04	3.70	0.42	0.42	0.37	92
	β_{12} AT_1R-Abs≥10U, t≥4 months	-2.70	-2.80	-0.10	3.90	0.59	0.58	0.58	95
	β_{12} HLA-ABDR incompatibilities>5	1.16	1.10	-0.06	-5.40	0.85	0.85	0.65	90
	β_{13} AT_1R-Abs≥10U, t<3 years	0.10	0.08	-0.02	-19.60	0.40	0.40	0.41	99
	β_{13} AT_1R-Abs≥10U, t≥3 years	0.86	0.90	0.04	4.60	0.24	0.23	0.25	95
	β_{13} Recipient Age>55 years	1.90	1.91	0.00	0.10	0.27	0.27	0.26	92
	β_{13} PRA on T cells>25%	0.49	0.49	0.00	0.20	0.27	0.27	0.28	96
	β_{14} PRA on B cells>25%	0.94	1.00	0.06	6.60	0.34	0.33	0.33	93
	β_{23} AT_1R-Abs≥10U	1.38	1.44	0.06	4.00	0.66	0.66	0.59	96
	β_{23} Donor Age>55 years	1.71	1.71	0.00	0.00	0.73	0.74	0.55	91
≈ 70%	γ_{13} AT_1R-Abs≥10U	-1.41	-1.43	-0.01	0.90	0.69	0.70	0.61	84
	γ_{14} AT_1R-Abs≥10U	-1.74	-1.77	-0.03	1.80	0.63	0.63	0.57	87
	γ_{13} Recipient Age>55 years	-0.52	-0.51	0.02	-3.60	0.42	0.42	0.42	93
	γ_{14} Recipient Age>55 years	1.67	1.65	-0.02	-1.10	0.42	0.42	0.44	95
	β_{12} AT_1R-Abs≥10U, t<4 months	-1.16	-1.11	0.05	-4.50	0.59	0.59	0.51	86
	β_{12} AT_1R-Abs≥10U, t≥4 months	-2.70	-2.65	0.04	-1.60	0.91	0.91	0.79	88
	β_{12} HLA-ABDR incompatibilities>5	1.16	1.07	-0.09	-7.80	1.01	1.01	0.69	89
	β_{13} AT_1R-Abs≥10U, t<3 years	0.10	0.11	0.01	7.60	0.53	0.53	0.51	97
	β_{13} AT_1R-Abs≥10U, t≥3 years	0.86	0.90	0.04	4.80	0.48	0.48	0.45	92
	β_{13} Recipient Age>55 years	1.90	1.90	-0.01	-0.30	0.50	0.50	0.45	91
	β_{13} PRA on T cells>25%	0.49	0.52	0.03	6.30	0.36	0.36	0.38	95
	β_{14} PRA on B cells>25%	0.94	0.99	0.05	4.90	0.45	0.45	0.43	93
	β_{23} AT_1R-Abs≥10U	1.38	1.45	0.07	5.30	0.85	0.85	0.71	92
	β_{23} Donor Age>55 years	1.71	1.70	-0.01	-0.80	0.81	0.82	0.65	89

[1] See Table 2 for details.
[2] RMSE: Root mean square error

Table 4 summarizes the results obtained from the 100 simulation data sets with 600 subjects regarding the three censoring rates: 30%, 50% and 70%. Regardless the censoring rate and parameters, the absolute bias in the SMM estimates was lower than 0.10. For only one of the fourteen effects (β_{13} AT_1R-Abs≥10U, t<3 years), the relative bias exceeded 10% but this could be partly explain by a true effect close to zero. As indicated by the RMSE, which includes both

 ISSN: 2102-6238

components of standard error and bias, the variability of estimation of covariate effects around the true value increased with censoring rates. This variability was also observed on standard errors. For most parameters, empiric and asymptotic standard errors were close, reflecting correct asymptotic estimation of variance from the Hessian matrix. Exception can be observed for time-varying regression coefficients and for the regression coefficient associated to HLA-ABDR incompatibilities, a binary covariate with large unbalanced distribution (5% of patients with more than 5 incompatibilities). All the coverage rates of the 95% CIs obtained from the SMM ranged between 84% and 97% for the censoring rate of 70% and were higher than 90% for lower censoring rates.

4. Discussion

In this paper, we have presented a methodology to analyse time to multiple events. The SMM was adapted to study the relation between the pre-graft level of AT_1R-Abs and the evolution of KTR. We modelled two parts in the SMM: the sequence of the observed states and the sojourn time distributions given sequences. The interest of this decomposition is to assess the effects of covariates on the risk to do a transition and on the speed of transition. We used parametric distributions to have a simple writing and easy computable likelihood. In our application, the generalized Weibull functions seemed adapted to model the baseline duration distributions.

The relationship between the pre-graft level of AT_1R-Abs and KTR outcomes was previously analysed using two independent Cox PH models traditionally applied in renal transplantation. These analyses indicated that the pre-graft level of AT_1R-Abs was 1) an independent risk factor of ARE and 2) was associated to a higher risk of long-term graft failure. Analyses on the same data using a SMM confirmed that patients with high pre-graft levels of AT_1R-Abs had an increased risk of ARE.

However, additional information were provided by the SMM. Firstly, regardless the occurence of ARE, the pre-graft level of AT_1R-Abs was associated to the time-to-dialysis. Among patients who returned to dialysis, those with high pre-graft levels of AT_1R-Abs seemed more likely to do earlier failures. In addition, the pre-graft level of AT_1R-Abs seemed to accelerate the return to dialysis after an ARE. One hypothesis to explain this new results might be that AT_1R-Abs would be a complementary risk factor for identification of patients with high immunological risk. In addition AT_1R-Abs may bind to the allograft immediately following transplantation and initiate pathological pro inflammatory action on endothelial cells that could induced ARE and displayed serious irreversible lesions leading to accelerate graft failure (Le Bas-Bernardet et al., 2003). Secondly, there was a time-varying effect of the pre-graft AT_1R-Abs on the time-to-dialysis without previous ARE. Indeed, there was no evidence that the pre-graft level of AT_1R-Abs influenced the time-to-dialysis in the 3 years post-transplantation, whereas an high pre-graft level of AT_1R-Abs seemed to increase the risk afterwards. Following the same hypothesis, this might be due to subclinical rejections that were not diagnosed in our cohort according that surveillance biopsies were not systematically realized during the survey period of the study.

The main limit of our study is the few number of events, especially the number of deaths after ARE. We could not evaluate if the pre-graft level of AT_1R-Abs was associated to the probability of return to dialysis or die after an ARE ($p_{2j}, j \in \{3,4\}$) or to the speed of transition from ARE to death (λ_{24}), even if it would be unlikely to have such clinical associations with

 ISSN: 2102-6238

death. Despite a median follow-up time of 7 years, two thirds of patients were right-censored. Consequently, the estimated probabilities p_{ij} could not be interpreted as proportions of transitions (when the time tends to infinity). Our simulations showed that the mean biases of estimations of covariate effects from our SMM model were low but variability was important in presence of high censoring rate, particularly for the binary covariate with large unbalanced distribution and for time-varying regression coefficients whose estimates are made per time period and therefore with fewer observations. Another limit could be the parametric distributions for durations. Although these assumptions seemed reasonable with the KTR data, there may be some diseases where the non-parametric distributions best fit (Joly and Commenges, 1999).

As a conclusion, this study shows the SMMs are particularly adapted to investigate the relationship between a biomarker and the evolution of disease. It offers additional results to traditional survival models about the interpretation of the mechanistic associated to a biomarker. The SMMs can be extended to the analysis of many markers which can be measured at various times during follow-up and can integrate interval-censored data (Foucher et al., 2010). The theoretical advantages of such models are well-established (informative censoring, transient states to model variable with time-dependent value,...) but perhaps more importantly the biostatistical community in translational and epidemiological researchs should use more frequently this methodology to offer original and more complete results to physicians or scientists.

5. Acknowledgments

The authors thank the laboratory Roche for support to DIVAT cohort.
Grant Support: This project is supported by a clinical research grant from the National Hospital Clinical Research Project (2011) and the French National Research Agency (2011).

References

Aalen, O. (1978). Nonparametric Inference for a Family of Counting Processes. *Ann Statist*, 6(4):701–726.

Aalen, O., Farewell, V., De Angelis, D., Day, N., and Gill, O. (1997). A Markov model for HIV disease progression including the effect of HIV diagnosis and treatment: application to AIDS prediction in England and Wales. *Stat Med*, 16(19):2191–2210.

Andersen, P. and Keiding, N. (2002). Multi-state models for event history analysis. *Statistical methods in medical research*, 11(2):91–115.

Christensen, E. (1987). Multivariate survival analysis using Cox regression-model. *Hepatology*, 7(6):1346–1358.

Commenges, D. (1999). Multi-state models in epidemiology. *Lifetime data analysis*, 5(4):315–327.

Foucher, Y., Giral, M., Soulillou, J., and Daures, J. (2007). A semi-Markov model for multistate and interval-censored data with multiple terminal events. Application in renal transplantation. *Statistics in medicine*, 26(30):5381–5393.

Foucher, Y., Giral, M., Soulillou, J. P., and Daures, J. P. (2010). A flexible semi-Markov model for interval-censored data and goodness-of-fit testing. *Statistical methods in medical research*, 19(2):127–145.

Foucher, Y., Mathieu, E., Saint-Pierre, P., Durand, J., and Daures, J. (2005). A semi-Markov model based on generalized Weibull distribution with an illustration for HIV disease. *Biometrical journal*, 47(6):825–833.

Giral, M., Foucher, Y., Dufay, A., Van Huyen, J. P., Renaudin, K., Moreau, A., Philippe, A., Hegner, B., Dechend, R., Heidecke, H., Brouard, S., Cesbron, A., Castagnet, S., Devys, A., Soulillou, J. P., and Dragun, D. (2013. [Epub ahead of print]). Pretransplant Sensitization Against Angiotensin II Type 1 Receptor Is a Risk Factor for Acute Rejection and Graft Loss. *Am. J. Transplant.*

Hothorn, T. and Zeileis, A. (2008). Generalized Maximally Selected Statistics. *Biometrics*, 64(4):1263–1269.

Huzurbazar, A. (2004). Multistate models, flowgraph models, and semi-Markov processes. *Communications in statistics-theory and methods*, 33(3):457–474.

 ISSN: 2102-6238

Joly, P. and Commenges, D. (1999). A penalized likelihood approach for a progressive three-state model with censored and truncated data: application to AIDS. *Biometrics*, 55(3):887–890.

Lagakos, S., Sommer, C., and Zelen, M. (1978). Semi-markov models for partially censored data. *Biometrika*, 65(2):311–317.

Lau, B., Cole, S., and Gange, S. (2009). Competing risk regression models for epidemiologic data. *Am. J. Epidemiol.*, 170(2):244–256.

Le Bas-Bernardet, S., Hourmant, M., Coupel, S., Bignon, J. D., Soulillou, J. P., and Charreau, B. (2003). Non-HLA-type endothelial cell reactive alloantibodies in pre-transplant sera of kidney recipients trigger apoptosis. *Am. J. Transplant.*, 3(2):167–177.

Meira-Machado, L., de Una-Alvarez, J., Cadarso-Suarez, C., and Andersen, P. (2009). Multi-state models for the analysis of time-to-event data. *Statistical methods in medical research*, 18(2):195–222.

Nelder, J. and Mead, R. (1965). A simplex-method for function minimization. *Computer journal*, 7(4):308–313.

Nikulin, M. and Haghighi, F. (2009). On the power generalized Weibull family: model for cancer censored data. *Metron*, LXVII(1):75–86.

Perez Ocon, R., Ruiz-Castro, J., and Gamiz-Perez, M. (1999). *Semi-Markov Models for Life Data Analysis*. Kluwer Academic Publishers, Dordrecht, Hardound, The Nederlands.

R Core Team (2012). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0.

 ISSN: 2102-6238

Appendix

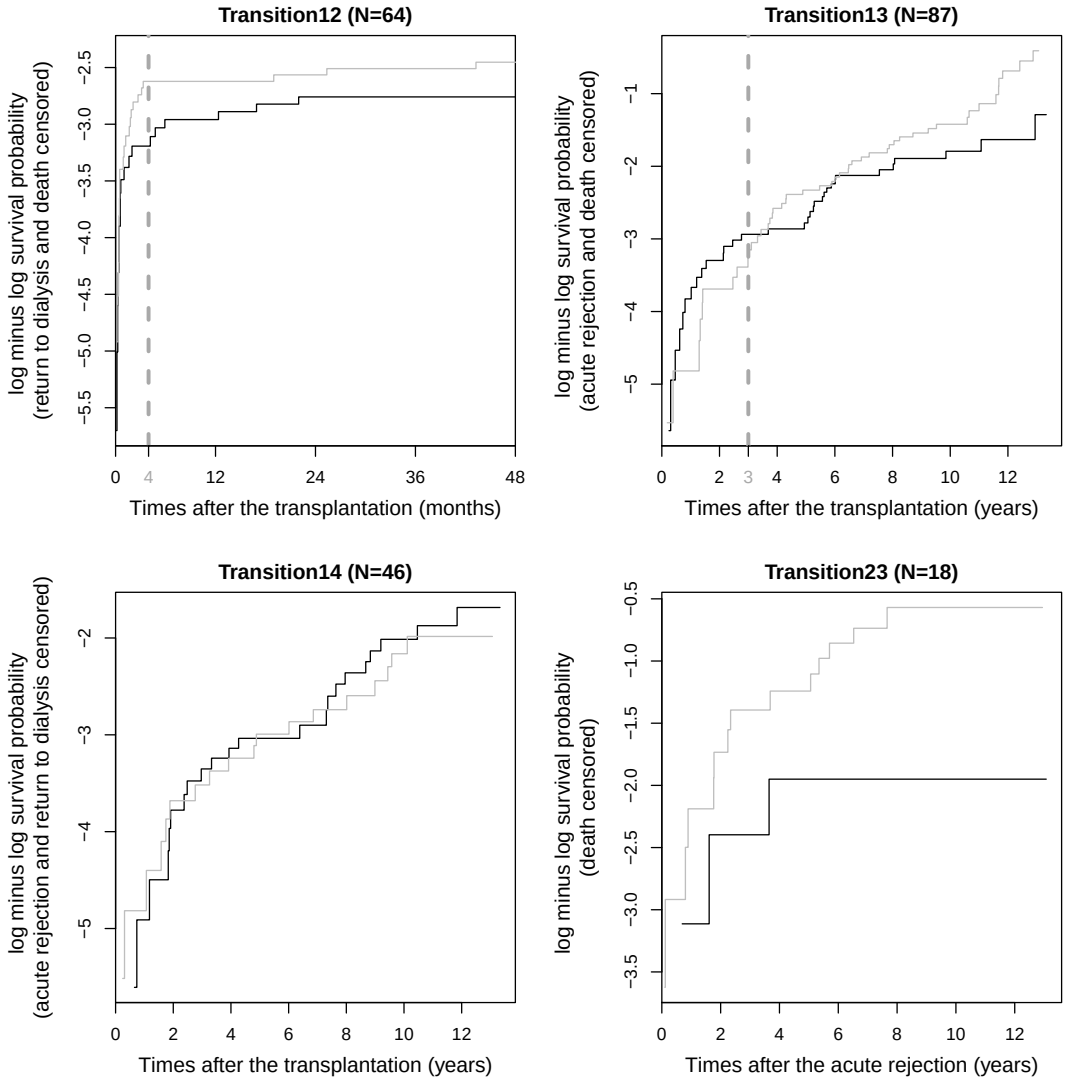

Figure 3: Log-minus-log plots of the survival probabilities according to AT_1R-Abs.
Vertical dash lines indicate that the variable AT_1R-Abs did not met PH assumptions for time-to-ARE (time-varying regression coefficients at 4 months post-transplantation) and time-to-return to dialysis (time-varying regression coefficients at 3 years post-transplantation).

 ISSN: 2102-6238