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# Optimisation de l'utilisation du tacrolimus chez le transplanté hépatique

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Spécialité : « Epidémiologie, Analyse de risque, recherche clinique »

Par **Arunkumar Michel RAYAR**

# Optimisation de l'utilisation du tacrolimus chez le transplanté hépatique

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## **CHAPITRE 1 :**

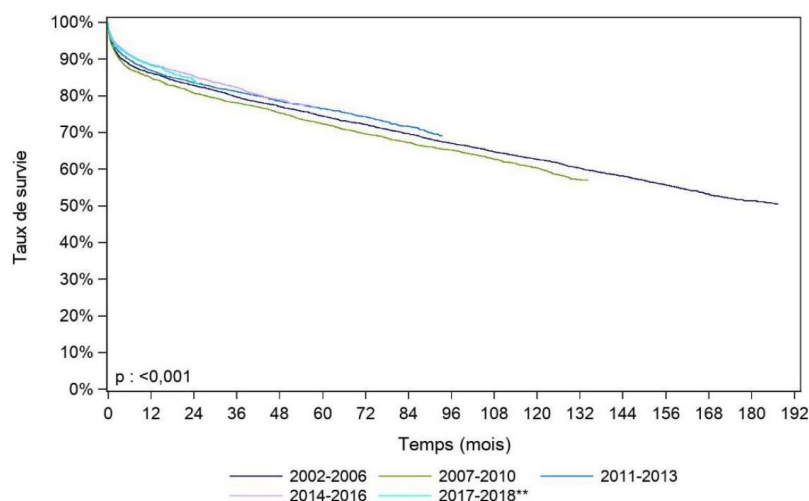
### **GENERALITES ET OBJECTIFS DE LA THESE**

## D) GENERALITES

### A) La transplantation hépatique

Initialement perçue comme un exploit chirurgical, la transplantation hépatique (TH) est désormais considérée comme une intervention de « routine ». Ainsi, depuis la première tentative réalisée par Thomas Earl Starzl en 1963<sup>1</sup> de nombreuses avancées ont été réalisées tant dans le domaine chirurgical que médical permettant la réalisation de plus de 1300 greffes de foie chaque année en France.<sup>2</sup>

Ces progrès ont permis à la TH de se positionner comme le meilleur traitement des insuffisances hépatiques aiguës, des hépatopathies chroniques décompensées mais aussi des tumeurs primitives hépatiques. Ainsi, la survie des patients transplantés ne cesse d'augmenter malgré la gravité et la complexité croissante des tableaux cliniques et est, à l'heure actuelle, de l'ordre de 70-80% à 5 ans (Figure 1). Par ailleurs, de nouvelles indications émergent essentiellement oncologiques telles que les cholangiocarcinomes péri-hilaires<sup>3</sup> et les métastases hépatiques de cancers colo-rectaux.<sup>4</sup>



**Figure 1 : Survie des transplantés hépatiques en France selon la période de greffe (d'après<sup>2</sup>)**



## B) L'immunosuppression en transplantation hépatique

Les bons résultats de la TH obtenus à partir des années 80 sont indissociables de l'amélioration des traitements immunosuppresseurs (IS). En effet, si la difficulté technique de l'acte chirurgical a pu être rapidement surmontée grâce à l'ingéniosité des pionniers de la greffe,<sup>5-7</sup> les complications immunologiques sont longtemps restées un frein à l'essor de la TH. Les premiers schémas thérapeutiques associant généralement de l'azathioprine, des corticostéroïdes et des immunoglobulines anti-lymphocytes<sup>1</sup> ne permettaient pas de prévenir efficacement l'apparition d'un rejet aigu cellulaire et encore moins de le traiter lorsqu'il survenait. De plus, ces traitements étaient responsables de complications infectieuses sévères. Ainsi, le décès du patient en postopératoire était fréquent et la survie ne dépassait guère les 30% à 1 an.<sup>8</sup>

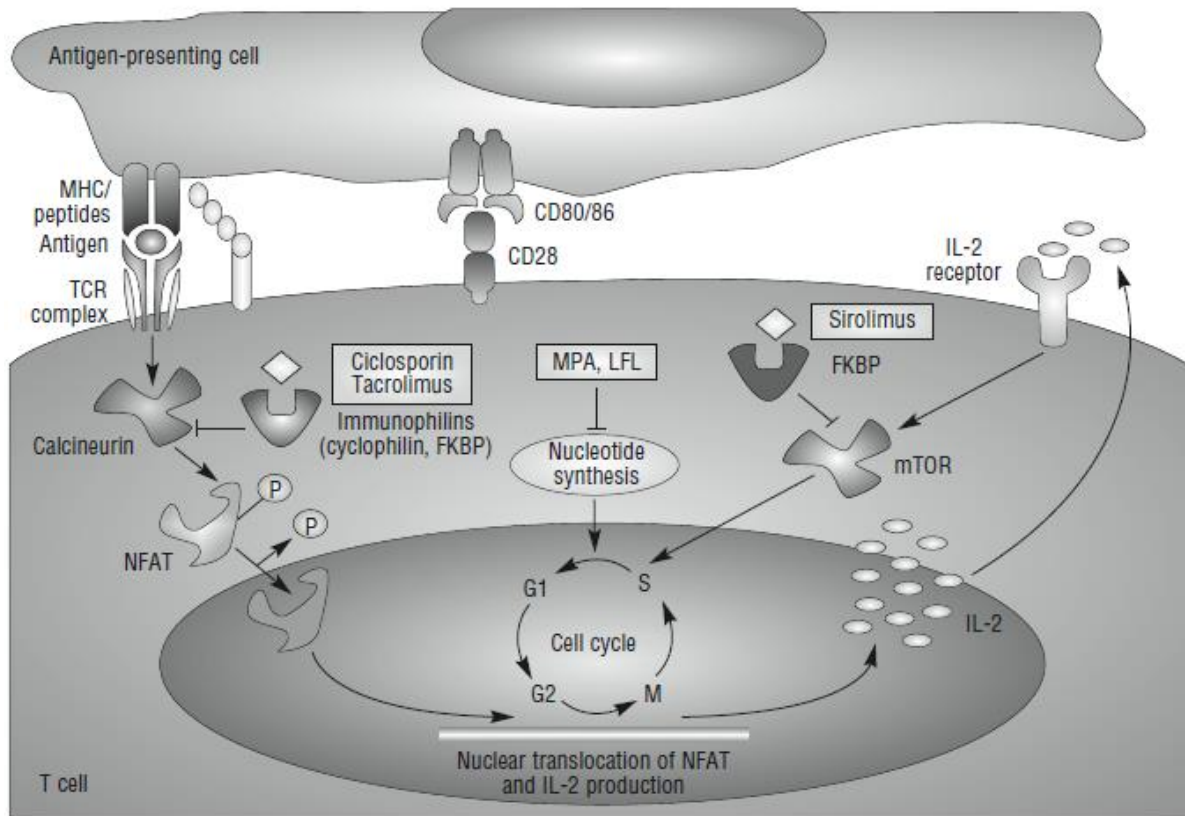
Une des avancées majeures a donc été la découverte de la ciclosporine en 1973 par Jean-François Borel<sup>9</sup> et son utilisation clinique par Sir Roy Calne,<sup>10</sup> permettant d'améliorer considérablement la survie des malades et par conséquent le nombre de greffes réalisées. Par la suite, la découverte du tacrolimus (TAC) a été la deuxième évolution majeure dans le domaine de l'immunosuppression en transplantation hépatique (et d'organe solide de façon générale).

## II) LE TACROLIMUS

### A) Généralités

La tacrolimus (TAC), aussi appelé FK 506, a été découvert en 1984 au Japon, à partir d'un champignon (*Streptomyces tsukubaensis*). Il appartient à la famille des macrolides (Figure 2) et agit en inhibant l'activité de la calcineurine (CNI). Cette enzyme est une sérine-thréonine





**Figure 3 : mécanisme d'action du tacrolimus (d'après <sup>12</sup>)**

### C) Utilisation en transplantation hépatique

Le tacrolimus a une activité cent fois plus puissante que la ciclosporine A et a montré sa supériorité dans la prévention du rejet dans 2 études prospectives randomisées<sup>13,14</sup>. Son utilisation a été approuvée par la FDA (Food and Drug Administration) en 1994 bien que son utilisation chez l'homme ait été initiée dès 1989 par l'équipe de STARZL dans le traitement du rejet aigu cellulaire.<sup>15</sup>

En transplantation hépatique, le tacrolimus est administré par voie orale. La posologie habituelle est de 0,1 mg/kg/jour en 2 prises. En effet, la forme galénique la plus utilisée en post-TH immédiat reste le tacrolimus à libération immédiate (Prograf®, laboratoire Astellas). Des formes à libération prolongée ont été développées, initialement dans le but d'améliorer

l'observance puis dans l'hypothèse, non vérifiée à ce jour, de diminuer l'incidence des effets indésirables (EI) en diminuant l'intensité du pic de concentration sanguine. Le TAC est généralement le médicament principal d'une triple thérapie IS associant un inhibiteur de la synthèse des purines (mycophénolate mofétil) et un corticostéroïde (prednisone).<sup>16</sup>

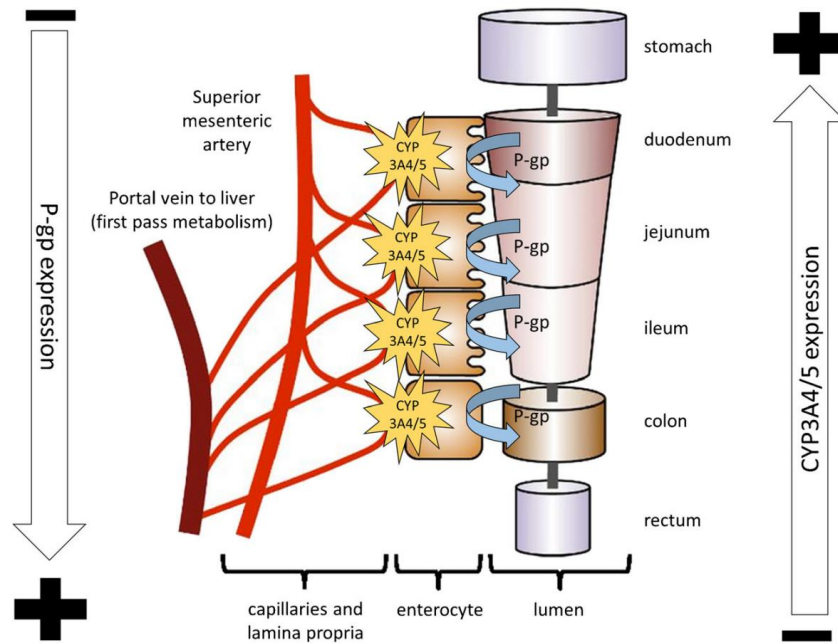
Actuellement, les recommandations préconisent une concentration sanguine résiduelle cible (C0) entre 6-10 ng/mL durant le premier mois lorsqu'il fait partie d'une triple thérapie IS puis entre 5-8 ng/mL par la suite. Lorsqu'il est utilisé en monothérapie ou sans administration concomitante de corticoïdes, la C0 recommandée est de 10-15 ng/mL puis 5-10 ng/mL par la suite.<sup>17</sup>

#### D) Métabolisme du tacrolimus

Bien que le métabolisme du TAC soit sujet à une grande variabilité inter- et intra-individuelle,<sup>18</sup> sa connaissance est primordiale dans l'étude de son efficacité et de sa toxicité.

Après administration sous forme orale, le TAC est absorbé au niveau de la partie haute du tube digestif (au niveau du duodénum et jéjunum). Lorsqu'il est administré sous cette forme, sa biodisponibilité est médiocre et de l'ordre de 25%.<sup>12,18</sup> La concentration maximale sanguine est généralement atteinte après 30 - 60 min<sup>19</sup> et est réduite en cas d'absorption alimentaire concomitante.

Le médicament est presque exclusivement métabolisé au niveau hépatique et éliminé dans la bile puis les selles.<sup>18</sup> Cependant, il existe un métabolisme pré-systémique au niveau de l'entérocyte où le médicament est métabolisé par le cytochrome P450 (CYP) 3A4 et la P-glycoprotéine (P-gp). Cette dernière est une pompe d'efflux capable de réexcréter le TAC dans la lumière digestive afin qu'il soit réabsorbé ultérieurement (figure 4).<sup>20-22</sup>



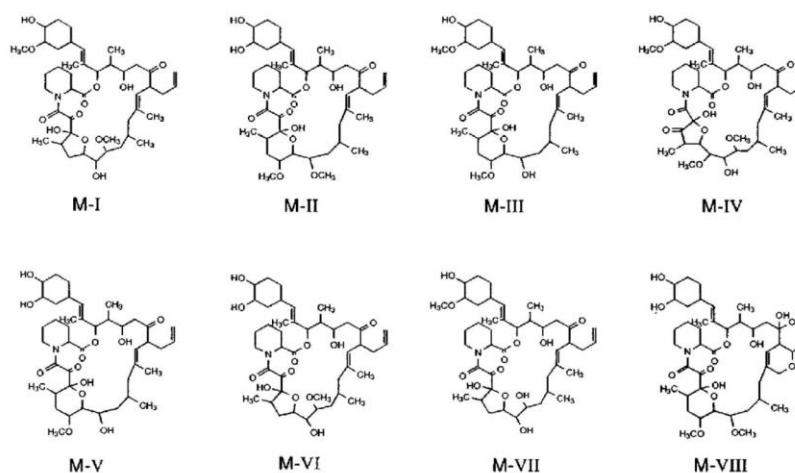
**Figure 4 : Absorption du Tacrolimus au sein du tube digestif (P-gp : P-glycoprotein ; CYP : cytochrome P450) d'après <sup>23</sup>**

Une fois présent dans la circulation sanguine, le TAC se fixe principalement aux FK-binding protéines présentes dans les érythrocytes. Ainsi la concentration de TAC est environ 15 fois plus importante dans le sang total que dans le plasma. De plus, au niveau plasmatique, près de 99% du TAC se fixe aux protéines plasmatiques (essentiellement l' $\alpha$ 1-glycoprotéine, les lipoprotéines, et l'albumine). En conséquence, moins de 1 % du TAC est présent sous forme libre dans le plasma.<sup>20-22</sup> Pour exercer son effet thérapeutique, le TAC doit pénétrer dans le cytoplasme du lymphocyte T. Au niveau de la membrane du lymphocyte, la P-gp est également exprimée et pourrait réguler la concentration intracellulaire, c'est-à-dire de TAC actif.

Au niveau hépatique, le TAC est intensément métabolisé au sein du microsome par les CYP 3A, avant d'être excrété dans la bile sous forme métabolisé. Il existe plusieurs isoformes du CYP3A mais les plus fréquentes et les plus exprimées sont le CYP3A4 et le CYP3A5. La

différence d'expression de ces isoformes explique en grande partie la variabilité inter-individuelle.<sup>18,24</sup>

Il existe 8 principaux métabolites du TAC<sup>25</sup> (Figure 5) dont les concentrations peuvent être mesurées dans le sang et certains possédant une activité pharmacologique similaire à la molécule mère (Figure 6). Cependant, leur rôle éventuel dans la toxicité liée au TAC reste inconnu.



**Figure 5 : métabolites du tacrolimus (d'après <sup>25</sup>)**

(M-I: 13-O-demethylated ; M-II: 31-O-demethylated ; M-III: 15-O-demethylated ; M-IV: 12-hydroxylated ; M-V: 15- 31- di-demethylated ; M-VI: 13- 31- di-demethylated ; M-VII: 13- 15 methoxy- di-demethylated ; M-VIII: 31-methoxy O-demethylation 10-membered ring structure through the 19- to 22)

Tacrolimus and metabolites	Immunocrossreactivity (%)	Pharmacological activity (IC <sub>50</sub> , ng/mL)
Tacrolimus	100	0.11
M-I	nil	1.71
M-II	109.0	0.11
M-III	90.5	> 1000
M-IV	8.8	3.13
M-V	92.2	> 1000
M-VI	nil	8.78
M-VII	nil	> 1000
M-VIII	nil	15.27

**Figure 6 : Activité des métabolites du Tacrolimus (d'après <sup>25</sup>)**

L'injection de TAC marqué au <sup>14</sup>Carbone chez des rats a permis de confirmer que 95% du TAC était éliminé dans les selles et moins de 0,5% était retrouvé sous forme inchangée. L'élimination rénale est mineure.

Par conséquent, toute pathologie biliaire ou altération de la fonction hépatique entraîne une altération du métabolisme et de l'élimination du TAC. Après transplantation hépatique, le greffon récupérant ses fonctions de façon progressive durant les premiers jours, le métabolisme du TAC évolue donc également durant cette période et tout retard de reprise de fonction du greffon peut être responsable d'une baisse de la clairance et d'une augmentation de la demi-vie du médicament<sup>26-28</sup> exposant donc à un risque de surdosage.

#### E) Méthodes de monitoring pharmacologique

La marge thérapeutique du TAC est étroite et tandis qu'un sous dosage expose le patient à un risque augmenté de rejet aigu, à l'inverse un surdosage expose au risque de survenue d'effets indésirables. Par conséquent, le suivi thérapeutique pharmacologique qui réside en la mesure des concentrations sanguines et l'adaptation de la posologie dans le but de maintenir ces concentrations dans la zone thérapeutique, a une importance majeure.

En pratique courante, le suivi thérapeutique pharmacologique repose sur la mesure, dans le sang total, de la concentration résiduelle du TAC (molécule mère) communément appelé « C0 ». Le choix du sang total comme matrice (plutôt que le plasma ou les urines) se justifie par le fait qu'une très grande partie du TAC est fixée sur les érythrocytes rendant plus simple sa détection. Le choix de la concentration résiduelle (plutôt que le pic observé à un autre moment suivant la prise) se justifie car il semble être le meilleur reflet de l'exposition au TAC

29 .

Cette approche conventionnelle représente donc un substitut acceptable de l'évaluation de l'aire sous la courbe de la concentration en fonction du temps (Area Under the Curve, (AUC)) du TAC qui, afin d'être calculée nécessiterait au moins 6 à 8 mesures réalisées après la prise du médicament. Cette approche paraît donc difficile à mettre en œuvre en pratique quotidienne pour des raisons évidentes de confort du patient et de logistique. Il faut cependant noter que le développement des dispositifs de micro-prélèvements cutanés pourrait prochainement partiellement lever cette limite.<sup>30</sup>

D'autres marqueurs pharmacologiques, supposés plus directement liés à l'effet immunosuppresseurs du médicament, ont également été développés. Ainsi, on peut mentionner le dosage intracellulaire du TAC dans les cellules mononuclées sanguines périphériques (PBMC), fraction enrichie en lymphocyte T, qui est une méthode ayant montré sa supériorité par rapport à la mesure de la concentration sanguine résiduelle comme marqueur de l'efficacité du TAC à la phase très précoce.<sup>31</sup> D'autres méthodes plus complexes, consistant à mesurer directement l'activité de phosphorylation de la calcineurine dans les cellules mononuclées (témoin direct de l'action du TAC au niveau de la cellule cible)<sup>32</sup> ou encore la quantité d'ARN messager transcrite par les gènes de l'IL-2, de l'IFN- $\gamma$  et du GM-CSF (dont les voies de signalisation sont inhibées par le TAC) ont été proposées.<sup>33</sup>

Bien que performantes, ces méthodes sont malheureusement trop complexes et souvent trop coûteuses pour pouvoir être applicables en pratique clinique quotidienne.

## F) Toxicité

La TAC est un médicament dont la marge thérapeutique est étroite et dont le métabolisme (réalisé par le greffon) est dynamique durant les premiers jours suivant la TH. Ceci explique la difficulté à obtenir un équilibre des concentrations sanguines durant les premiers jours ainsi



que la fréquence importante des effets indésirables durant cette phase, et dont la survenue impacte significativement la durée d'hospitalisation.

Le TAC est responsable de multiples effets indésirables. Les principaux sont : la néphrotoxicité, la neurotoxicité, les complications métaboliques et cardio-vasculaires et l'augmentation du risque de cancer. Evidemment, de par son mécanisme d'action, il est également responsable d'une leucopénie (voir d'une pancytopenie) et augmente le risque infectieux.

Néphrotoxicité : Les inhibiteurs de la calcineurine sont des médicaments néphrotoxiques et le TAC semble avoir une toxicité rénale supérieure à celle de la ciclosporine A.<sup>13</sup> Le TAC peut être responsable d'une insuffisance rénale aigüe secondaire à une vasoconstriction au niveau de l'artériole afférente. Cette atteinte est présente chez 20-50% des patients transplantés hépatiques au cours de la première année post-TH. Elle est généralement réversible et dépend du niveau d'exposition au médicament. Elle est donc variable selon les doses administrées et le schéma d'immunosuppression mais est surtout liée aux concentrations sanguines de TAC.<sup>16,34</sup> Le TAC peut aussi entraîner une insuffisance rénale chronique (définie par un débit de filtration glomérulaire <30mL/min) chez 18% des patients à 5 ans de la TH, qui est associée à une augmentation de la mortalité de près de 5 fois.<sup>35</sup> Le mécanisme de cette atteinte est souvent multifactoriel et associe des lésions liées à l'hypertension artérielle (HTA) secondaire, une hyalinose artériolaire, une fibrose interstitielle, une atrophie tubulaire ou une microangiopathie thrombotique.

Neurotoxicité : Les complications neurologiques après TH secondaires aux CNI sont très fréquentes durant les premières semaines<sup>36-38</sup> puisque près de 30 % des patients vont présenter une atteinte neurologique dont le spectre clinique est très large. En effet, les atteintes peuvent se manifester comme un simple tremblement des extrémités, des paresthésies, des céphalées, une confusion, une crise convulsive ou encore une encéphalopathie postérieure réversible

(PRES syndrome). Dans la grande majorité des cas, ces atteintes sont réversibles et ne sont pas associées à des lésions organiques du système nerveux central ou périphérique. Le mécanisme de cette toxicité n'est pas connu mais plusieurs hypothèses ont été avancées comme l'induction d'un vasospasme cérébral suite à une production excessive d'endothéline ou encore des altérations mitochondriales responsables de perturbations métaboliques.<sup>39-42</sup> Il est à noter que ni la survenue de ces effets indésirables neurologiques, ni leur intensité ne sont corrélées à la concentration résiduelle sanguine de TAC.

Néanmoins, le traitement consistera généralement en une réduction ou une suspension temporaire du traitement. En cas d'atteinte majeure, une contre-indication à la reprise du TAC et un switch à une autre molécule (comme l'évérolimus) peuvent être décidés. Un traitement symptomatique peut également être nécessaire dans les formes graves (traitements antiépileptique ou antihypertenseur). Dans certains cas extrêmes, une réhospitalisation en réanimation avec mise en place de suppléance respiratoire peut être nécessaire et parfois malheureusement conduire au décès du patient.

Effets indésirables métaboliques et cardiovasculaires : Les complications métaboliques et cardiovasculaires sont particulièrement fréquentes chez le transplanté hépatique. En effet, les évènements cardiovasculaires représentent la première cause de mortalité « non-hépatique » chez ces patients avec une fréquence de l'ordre de 13-15 % à 5 ans.<sup>43,44</sup> Par ailleurs, on estime la prévalence du syndrome métabolique<sup>45</sup> après TH à 40-60% avec une incidence durant la première année post-TH de 35%.<sup>46,47</sup> Le syndrome métabolique sera à son tour responsable d'une augmentation du risque cardiovasculaire, d'insuffisance rénale, de maladie métabolique au niveau du greffon ou de cancer.<sup>48-51</sup> Bien que le mécanisme physiopathologique reste méconnu, l'implication du TAC dans la survenue du syndrome métabolique et des évènements cardiovasculaires semble majeure. En effet, le TAC agit sur la plupart des composantes du syndrome métabolique<sup>44,52</sup> puisqu'il a un effet diabétogène (par toxicité

directe sur les cellules  $\beta$  pancréatiques, diminution de la sécrétion d'insuline et augmentation de l'insulino-résistance), hyperlipémiant (par stimulation du récepteur du LDL-cholestérol et diminution de l'excrétion biliaire du cholestérol) et hypertenseur (par vasoconstriction artérielle au niveau rénal). Le traitement consiste en une prise en charge symptomatique des différents facteurs de risques.

Cancers : Le risque de cancer après TH est 2 à 3 fois plus élevé que celui de la population générale.<sup>53-55</sup> Les inhibiteurs de la calcineurine (TAC et ciclosporine) ont été identifiés comme facteur de risque dans la survenue d'un syndrome lymphoprolifératif,<sup>56</sup> d'un cancer cutané<sup>57</sup> mais aussi de cancers solides.<sup>58</sup>

### **III) OBJECTIFS DU TRAVAIL DE THESE**

La transplantation hépatique n'est donc plus une activité d'exception. Les évolutions chirurgicales et médicales (notamment dans le domaine de l'immunosuppression) ont permis d'améliorer son efficacité et la sécurité des patients.

Ainsi, le constat actuel est que les complications postopératoires les plus fréquentes sont désormais les effets indésirables des traitements immunosuppresseurs avec au premier plan le tacrolimus.

Par conséquent, l'objectif de ce travail de thèse est de tenter d'optimiser l'utilisation du tacrolimus chez le patient transplanté hépatique à l'aide de méthodes innovantes et d'apporter de nouveaux éléments permettant de comprendre les mécanismes physiopathologiques de sa toxicité.

Les différentes pistes de recherches que nous allons explorer sont : l'optimisation des concentrations résiduelles cibles et la recherche de nouveaux marqueurs pharmacologiques de suivi thérapeutique à la fois dans le sang et dans une nouvelle matrice : la bile.

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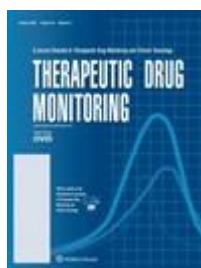
## CHAPITRE 2 :

### REDEFINITION DE LA CONCENTRATION RESIDUELLE CIBLE DE TACROLIMUS EN POSTOPERATOIRE PRECOCE

**Redefining therapeutic drug monitoring of tacrolimus in liver transplantation: A target trough concentration of 4-7 ng/mL during the first month is safe and improves graft and renal function**

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## **D) CONTEXTE :**

La survenue de certains effets indésirables du TAC est précoce (durant les premières semaines après son introduction) et il est usuellement admis qu'elle est « concentration-dépendante ». Ainsi, les objectifs de concentrations résiduelles du TAC ont, durant ces dernières années, volontairement été diminués. En effet, dans notre pratique, nous avons progressivement acceptés des concentrations résiduelles aux environs de 5 ng/mL durant le premier mois après la greffe (en l'absence de retentissement sur le bilan hépatique), jusqu'à ce que cela devienne aujourd'hui notre objectif.

Cependant, cette volonté de minimisation précoce peut paraître périlleuse au premier abord car pourrait exposer le patient à un risque important de rejet aigu cellulaire durant cette période précoce.

Il n'existe, par ailleurs, dans la littérature aucune étude évaluant l'intérêt d'une minimisation des concentrations sanguines résiduelles en dessous de 6 ng/mL durant le premier mois après transplantation hépatique. Notre étude était donc la première à évaluer la sécurité et l'efficacité de cette stratégie.

## II) MANUSCRIT DE L'ARTICLE PUBLIE (en Anglais)

### 1 | INTRODUCTION

Tacrolimus (TAC) is the cornerstone of immunosuppressive regimen in liver transplantation (LT). Its therapeutic margin is narrow, so low TAC exposure increases the occurrence of acute cellular rejection (ACR) while high TAC exposure is associated with an increased risk of adverse events, in particular, nephrotoxicity.<sup>1,2</sup> Therapeutic drug monitoring (TDM) of TAC, which consists in measuring TAC trough whole blood concentrations (Cmin), has significantly participated in improving the postoperative outcomes of liver transplant patients.

The current recommendation for TAC Cmin is 6 to 10 ng/mL (in combination with mycophenolic acid or an m-TOR inhibitor and corticosteroids) to ensure sufficient immunosuppressive efficacy while decreasing its renal toxicity.<sup>3-5</sup> However, most clinical trials have targeted the higher part of this range,<sup>6</sup> and in clinical practice, few centers can accept an early postoperative Cmin below 7 ng/mL given the threat of ACR.

Nevertheless, LT epidemiology has evolved in the last few decades with more marginal donors (especially older), sicker recipients (with pre-existing renal dysfunction) and a higher proportion of hepatocellular carcinoma indications (with risk of recurrence related to immunosuppression level). TAC minimization therefore needs to be explored beyond the current recommendation, especially since the occurrence of ACR has been shown to lead to minimal consequences in the early postoperative period after LT.<sup>7</sup> To date, few studies have evaluated the feasibility of minimizing the Cmin target below 7 ng/ml.

The aim of our study was to retrospectively evaluate the efficacy and tolerance of a minimization of TAC Cmin below 7 ng/ml during the first month after the procedure in a large cohort of liver transplant patients.

## **2 | MATERIALS AND METHODS**

### **2.1 | Patient selection**

All transplant patients in our department between January 2002 and December 2017 (n=1,677) were retrospectively analyzed.

Retransplant patients (n=169), patients transplanted with a split graft (n=55), with other associated organs or already on a TAC regimen (n=66) and in emergency for acute liver failure (n=54) were excluded from the analysis. Patients treated with cyclosporine or receiving induction treatment (i.e. anti-lymphocyte or anti-interleukine-2) (n=290), who died or were retransplanted within the first month after LT (n=64) or whose TAC treatment had to be withdrawn during this period (n=54) were also excluded. Ultimately, patients who had very low (i.e. <4 ng/ml) TAC median Cmin owing to major drug discontinuation (n=13) and patients with missing data (n=8) were also excluded.

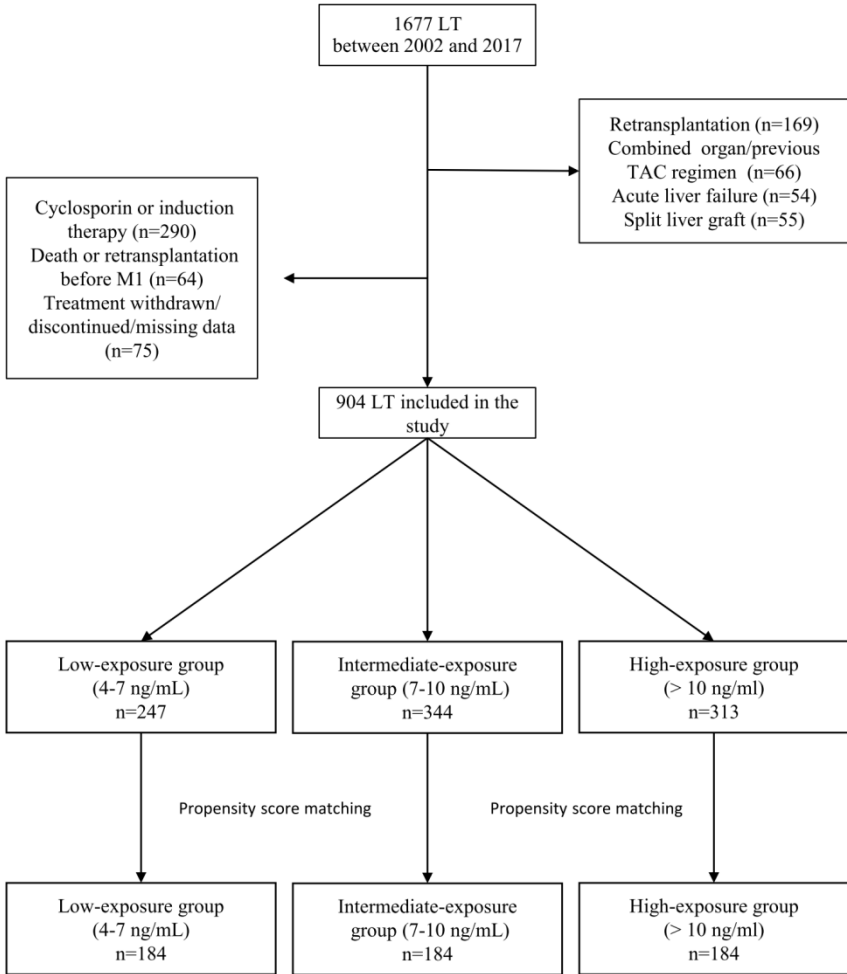
Finally, our study population consisted of 904 liver transplant patients treated by oral TAC given twice daily, started between POD1 or 3, and maintained until at least one month after LT (Figure1).

### **2.2 | Surgical procedure and medical care**

All patients had orthotopic liver transplantation (OLT) with inferior vena cava preservation. After the procedure, standardized immunosuppression associating TAC, mycophenolate mofetil and a short course of corticosteroids was administered. Tacrolimus whole blood trough concentration was measured daily during the first 7 POD and then three times a week during the hospital stay or more frequently according to clinical necessity in case of complications. After hospital discharge, patients were seen weekly until at least 3 months after LT and had TAC trough level measurements in outpatient consultations. Whole-blood

TAC levels were measured with a fully validated liquid chromatography tandem mass spectrometry adapted from a previously published method.<sup>8</sup>

No significant modification regarding surgical procedure or postoperative medical care was observed during the study period except for the TAC-minimizing policy.

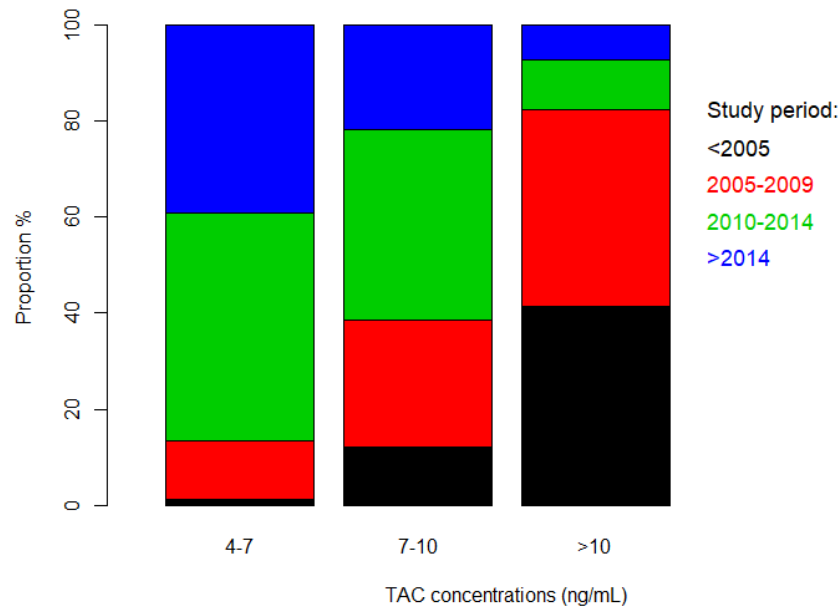


**Figure 1: Flow chart**

**2.3 | Tacrolimus target Cmin and group definition**

During the study period, the targeted TAC Cmin progressively evolved. Indeed, in the early period (mostly before 2010), the objective was to reach a Cmin value over 10 ng/ml. A

reduction policy was then progressively instituted with an objective of C<sub>min</sub> below 10 ng/ml and then (approximately since 2014) below 7 ng/ml (Figure 2). However, we did not intend to go below 4ng/ml (those patients were subsequently excluded from the analysis).



**Figure 2: Tacrolimus exposure over the study period**

We defined 3 groups based on the median TAC C<sub>min</sub> calculated during the first month after LT using all measured C<sub>min</sub> between POD8 (since we estimated that the steady state was not reached before this date) and POD30. In order to improve the precision of the model and reduce the effect of extreme values, an imputation method (mean value weighted by the number of days between 2 consecutive measured values) was used to estimate C<sub>min</sub> when dosage was not performed.

The low-exposure group (Low group) was made up of patients with a median C<sub>min</sub> between 4-7 ng/ml (i.e. >4 and <7) during the first month after LT, the intermediate-exposure group (Intermediate group) by patients with a median C<sub>min</sub> between 7-10 (i.e. ≥7 and <10) and the high-exposure group by patients with a median C<sub>min</sub> ≥10ng/ml.

## **2.4 | Clinical and biological parameter definitions**

Clinical and biological data were retrospectively collected from a prospective database. The following parameters were analyzed: donor and recipient characteristics, underlying liver disease and OLT indication, Child-Pugh grade and MELD score, cold ischemia time, post-transplant liver and renal function. The occurrence of TAC-related neurotoxicity (defined by confusion, convulsion, tremor, seizures and posterior reversible encephalopathy syndrome) and acute rejection defined as biopsy proven rejection or highly clinically suspected (i.e. elevated liver or cholestasis enzyme without other cause and rapidly resolved after increase of TAC concentration or corticosteroid bolus) during initial hospitalization were collected and analyzed.

Early allograft dysfunction (EAD) was defined according to Olthoff et al. criteria (i.e. the presence of one or more of the following criteria: bilirubin level  $\geq 10\text{mg/dL}$  on POD7; INR  $\geq 1.6$  on POD7; ALAT or ASAT level  $> 2000\text{ IU/L}$  before POD7).<sup>9</sup>

Patient and graft survival at M3, M6 and beyond (i.e. long-term survival) were calculated.

## **2.5 | Statistical analysis**

Quantitative variables were expressed as mean  $\pm$  standard deviation or by median with extreme values and compared using Kruskal-Wallis test. Qualitative variables were expressed as number and percentage and compared using Chi-squared test. Survival analysis was made with Kaplan-Meier curve and compared with log-rank test.

In order to minimize bias owing to the heterogeneity between groups, we performed a propensity score matching (1/1 ratio) analysis. The significantly different variables between the 3 groups were used in the calculation of the propensity score. Exact match was prioritized

and the maximum distance allowed between two matched patients was set at 0.2 (i.e. caliper restriction).

All statistical analyses were made with R software version 3.4.3. The propensity score analysis was performed using the “Matchit” package version 3.0.2. A  $P<0.05$  value was considered to be statistically significant.

### **3 | RESULTS**

#### **3.1 | Patient characteristics (Table 1)**

During the study period, 904 patients were analyzed. A median  $C_{min}$  between 4 and 7 ng/mL was achieved in 247 (27.3%) patients (low group), between 7 and 10 ng/mL in 344 (37.9%) patients (intermediate group) and higher than 10 ng/mL in 313 (34.5%) patients (high group). As expected, the repartition between groups was different over the study period (Figure 2) with a majority of patients with high  $C_{min}$  in the early period while almost all patients with low  $C_{min}$  were in the later period.

Men represented 79.4% ( $n = 718$ ) of the patients with a median age of 57 years with a significant difference between groups (60 vs. 57 vs. 56 in the low, intermediate and high groups respectively,  $P<0.001$ ). The MELD, CHILD-PUGH grade and creatinine level before the procedure were similar while the indication for LT ( $P<0.001$ ) and the proportion of hospitalized patients before LT ( $P=0.001$ ) was significantly different. Similarly, there were significant differences regarding donor sex ( $P=0.038$ ) and age ( $P<0.001$ ) with older donors in the low group.

	All patients (n= 904)	Low group (n=247)	Intermediate group (n=344)	High group (n=313)	P-value
<b><u>Recipient:</u></b>					
Gender (Male)	718 (79.4)	196 (79.4)	278 (80.8)	244 (78)	0.663
Age (year)	57 [16-73]	60 [17-73]	57 [16-71]	56 [16-69]	<0.001
BMI	26.7 [15.7-47.2]	26.5 [17.2-45.7]	27.4 [17.9-47.2]	26.6 [15.7-43.7]	0.444
Diagnosis					<0.001
HCC	316 (35)	108 (43.7)	119 (34.6)	89 (28.4)	
Alcohol	363 (40.2)	92 (37.2)	143 (41.6)	128 (40.9)	
Biliary diseases	64 (7.1)	11 (4.5)	21 (6.1)	32 (10.2)	
Viral hepatitis	69 (7.6)	7 (2.8)	22 (6.4)	40 (12.8)	
Other	94 (10.2)	29 (11.7)	9 (11.4)	24 (7.6)	
MELD score	13.2 [5.4-45.6]	12.9 [5.4-45.6]	14.3 [5.4-42.9]	12.0 [5.4-40]	0.104
Creatinine level (µmol/L)	72 [28-497]	71 [28-325]	71.5 [34-305]	72 [34-497]	0.788
CHILD PUGH grade					0.168
A	340 (37.6)	97 (39.3)	122 (35.5)	121 (38.7)	
B	248 (27.4)	66 (26.7)	86 (25)	96 (30.7)	
C	316 (35%)	84 (34)	136 (39.5)	96 (30.7)	
Medical state before LT					0.001
Home	799 (88.4)	216 (87.4)	293 (85.2)	290 (92.7)	
Hospital	51 (5.6)	21 (8.5)	18 (5.2)	12 (3.8)	
Intensive care unit	54 (6)	10 (4)	33 (9.6)	11 (3.5)	
Cold ischemia time (min)	539 [178-1148]	510 [178-930]	531 [196-904]	584 [183-1148]	<0.001
<b><u>Donor</u></b>					
Donor Gender (male)	519 (57.4)	139 (56.3)	183 (53.2)	197 (62.9)	0.038
Donor age	56 [10-93]	62 [10-92]	59 [11-93]	50 [13-86]	<0.001
Donor BMI	24.5 [14.0-54.4]	24.7 [14.3-40.4]	24.5 [14.0-54.4]	24.5 [14.8-43.6]	0.667
<b><u>Posoperative outcomes</u></b>					
Acute rejection	170 (18.8)	25 (10.1)	46 (13.4)	99 (31.6)	<0.001
TAC related toxicity	198 (21.9)	78 (31.6)	70 (20.3)	50 (16)	<0.001
EAD incidence	168 (18.6)	49 (19.8)	57 (16.6)	62 (19.8)	0.486
Graft survival					

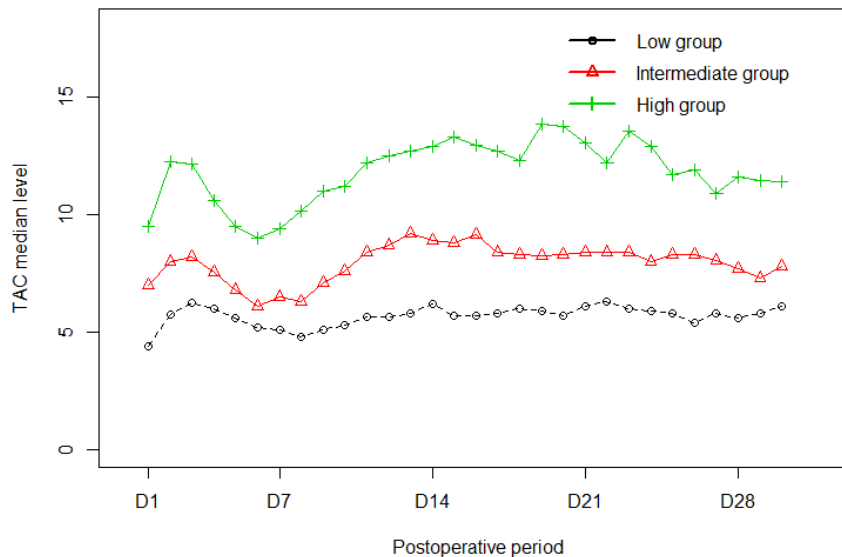


M3	883 (97.7)	242 (98)	334 (97.1)	307 (98.1)	0.657
M6	864 (95.6)	236 (95.5)	328 (95.3)	300 (95.8)	0.953
M12	843 (93.3)	227 (91.9)	321 (93.3)	295 (94.2)	0.546
Patient survival					
M3	891 (98.6)	242 (98)	340 (98.8)	309 (98.7)	0.657
M6	874 (96.7)	237 (96)	335 (97.4)	302 (96.5)	0.614
M12	859 (95)	230 (93.1)	329 (95.6)	300 (95.8)	0.27

BMI: Body mass index; EAD: Early allograft dysfunction; ECD: Extended criteria donor; HCC: Hepatocellular carcinoma; MELD: model for end-stage liver disease; TAC: tacrolimus; TIPS: Transjugular intrahepatic portosystemic shunts.

**Table 1: Study population characteristics (entire population)**

Daily TAC Cmin during the first month are shown in Figure 3. As expected, after limited fluctuation (between POD3 and POD7) secondary to therapeutic adaptation, the target concentration was mostly reached between POD8 and POD11 and maintained within the first month.



**Figure 3: Daily Tacrolimus trough concentration during the first month among groups**

The proportion of acute rejection was significantly higher in the high group ( $P<0.001$ ) while proportion of TAC-related toxicity was significantly higher in the low group ( $P<0.001$ ).

### 3.2 | Survival and biological outcomes

At month 1 (M1) (Table 2), the biological levels of ALT ( $P<0.001$ ) and AST ( $P<0.001$ ) were significantly lower in the low group. Cholestasis was also significantly improved in the low group with a significant difference in ALP ( $P<0.001$ ), GGT ( $P<0.026$ ) and bilirubin levels ( $P<0.001$ ). INR ( $P=0.002$ ) and creatinine levels ( $P<0.001$ ) were also significantly improved in the low-concentration group.

At M3, the difference still existed regarding ALT ( $P<0.001$ ), ALP ( $P<0.001$ ), bilirubin ( $P<0.001$ ), INR ( $P=0.046$ ) and creatinine ( $P<0.001$ ).

	All patients n= 904	Low group n=247	Intermediate group n=344	High group n=313	P- value
<b>ALT (UI/L)</b>					
M1	35 [4-820]	30 [4-356]	34 [4-803]	44 [4-820]	<b>&lt;0.001</b>
M3	23 [4-369]	21 [4-273]	23 [4-353]	27 [4-369]	<b>&lt;0.001</b>
<b>AST (UI/L)</b>					
M1	21 [5-870]	19 [6-112]	21 [5-870]	23 [7-272]	<b>0.002</b>
M3	20 [4-431]	19 [9-329]	20 [4-190]	19.5 [7-431]	0.416
<b>ALP (UI/L)</b>					
M1	209 [42-2292]	151 [42-1389]	195 [51-2292]	259 [64-1598]	<b>&lt;0.001</b>
M3	125 [27-7215]	86 [28-2188]	118 [27-1500]	160.5 [37-7215]	<b>&lt;0.001</b>
<b>GGT (UI/L)</b>					
M1	134 [18-1667]	116 [28-1605]	136 [18-1667]	143 [28-1265]	<b>0.026</b>
M3	47 [5-2610]	48 [11-2172]	45 [10-844]	47.5 [5-2610]	0.242
<b>Bilirubin (µmol/L)</b>					
M1	13 [3-453]	11 [3-412]	13 [3-227]	15.7 [5-453]	<b>&lt;0.001</b>
M3	8 [1.17-466]	7 [2-209]	8 [2-466]	9 [1.17-143]	<b>&lt;0.001</b>

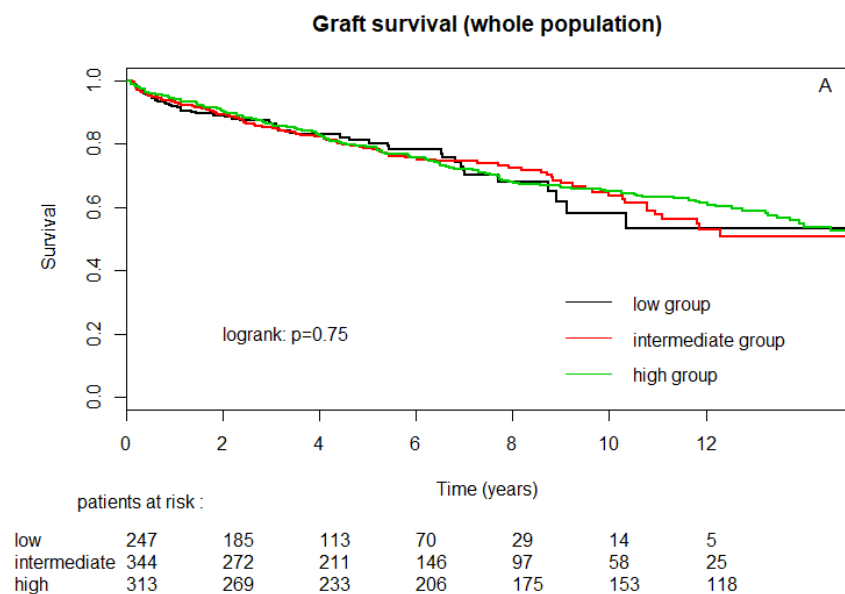
<b>INR</b>					
M1	1.11 [0.73-8.22]	1.09 [0.89-5.08]	[0.88-5.69]	1.14 [0.73-8.22]	<b>0.002</b>
M3	1.08 [0.76-6.9]	1.05 [0.85-3.51]	1.07 [0.89-6.9]	1.1 [0.76-5.5]	<b>0.046</b>
<b>Creatinine (µmol/L)</b>					
M1	91 [22-398]	83 [31-226]	88 [22-311]	102.5 [46-398]	<b>&lt;0.001</b>
M3	86.85 [38-302]	85 [46-237]	83 [38-219]	95 [43-302]	<b>&lt;0.001</b>

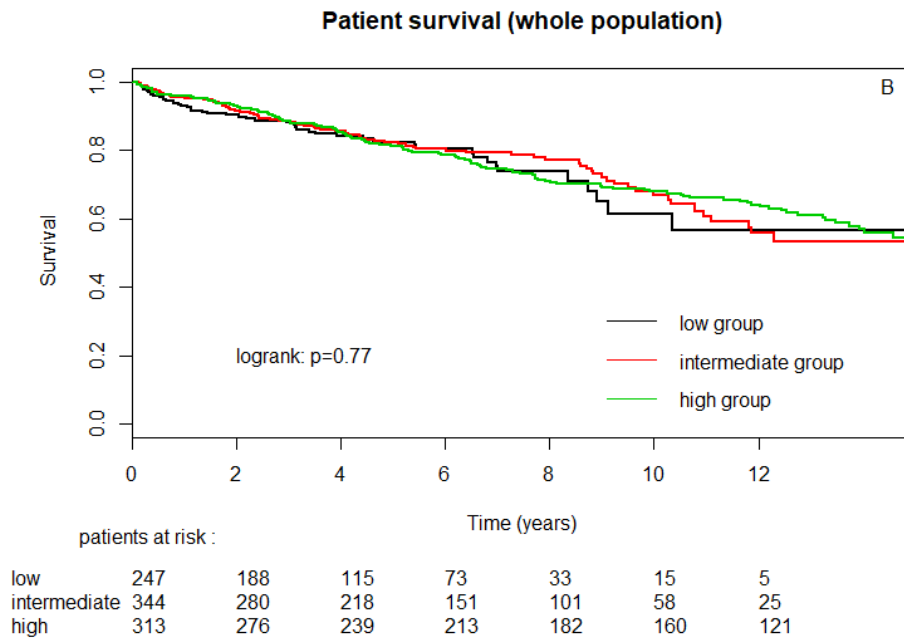
ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; FV: factor V; GGT: gamma-glutamyl transferase; INR: international normalized ratio; PR: prothrombin time; TAC: tacrolimus

**Table 2: Biological parameters (entire population) at M1 and M3**

The EAD rate was not different between all of the groups ( $P=0.486$ ) (Table 1). Graft survival (Table 1) was similar at M3 (98%, 97.1% and 98.1% in the low, normal and high groups respectively,  $P=0.657$ ) and M12 (91.9%, 93.3% and 94.2% in the low, normal and high groups respectively,  $P=0.546$ ). Patient survival was similar at M3 (98%, 98.8% and 98.7% in the low, normal and high groups respectively,  $P=0.657$ ) and M12 (93.1%, 95.6% and 95.8% in the low, normal and high groups respectively,  $P=0.27$ ).

Long-term survival was also similar for grafts ( $P=0.75$ ) (Figure 4-A) and patients ( $P=0.77$ ) (Figure 4-B).





**Figure 4: Grafts (A) and patients (B) survival on whole population**

### 3.4 | Propensity matching analysis

After propensity matching, 552 patients were analyzed (184 patients in each group). There was no difference regarding patient and donor characteristics, in particular, recipient age ( $P=0.125$ ), donor age ( $P=0.442$ ) and the proportion of hospitalized patients before LT ( $P=0.9$ ). (Table 3)

	All patients n= 552	Low group n=184	Intermediate group n=184	High group n=184	P-value
<b><u>Recipient:</u></b>					
Gender (Male)	450 (81.5)	146 (79.3)	146 (79.3)	158 (85.9)	0.177
Age (years)	57 [17-73]	58 [17-73]	57 [18-70]	56.5 [17-68].	0.125
BMI	27.1 [15.7-47.2]	26.4 [17.2-45.7]	27.8 [17.9-47.2]	27.4 [15.7-43.7]	0.258
Diagnosis					0.066
HCC	211 (38.2)	79 (42.9)	65 (35.3)	67 (36.4)	
Alcohol	218 (39.5)	66 (35.9)	76 (41.3)	76 (41.3)	
Biliary diseases	30 (5.4)	9 (4.9)	8 (4.3)	13 (7.1)	

Viral hepatitis	43 (7.8)	7 (3.8)	17 (9.2)	19 (10.3)	
Other	50 (9.1)	23 (12.5)	18 (9.7)	9 (4.8)	
MELD score	13.2 [5.4-45.6]	12.6 [5.4-45.6]	13.6 [5.4-40]	12.9 [5.4-40]	0.095
Creatinine level (μmol/L)	72 [34-497]	71 [42-325]	69.5 [34-216]	73 [34-497]	0.319
CHILD PUGH grade					0.46
A	217 (39.3)	73 (39.7)	65 (35.3)	79 (42.9)	
B	143 (25.9)	51 (27.7)	46 (25)	46 (25)	
C	192 (34.8)	60 (32.6)	73 (39.7)	59 (32.1)	
Medical state before LT					0.9
Home	496 (89.9)	164 (89.1)	164 (89.1)	168 (91.3)	
Hospital	32 (5.8)	12 (6.5)	12 (6.5)	8 (4.3)	
Intensive care unit	24 (4.3)	8 (4.3)	8 (4.3)	8 (4.3)	
old ischemia time (min)	527 [178-930]	522.5 [178-930]	522 [196-888]	533 [272-907]	0.814
<b><u>Donor</u></b>					
Donor Gender (male)	317 (57.4)	105 (57.1)	85 (46.2)	113 (61.4)	0.334
Donor age	57 [10-93]	59 [10-90]	57 [11-93]	55 [14-86]	0.442
Donor BMI	24.5 [14.0-54.4]	24.5 [14.3-38.5]	24.5 [14.0-54.4]	24.7 [16.0-43.6]	0.866
<b><u>Posoperative outcomes</u></b>					
Acute rejection	74 (13.4)	24 (13)	20 (10.9)	30 (16.3)	0.305
TAC related toxicity	130 (23.6)	48 (26.1)	47 (25.5)	35 (19)	0.206
EAD incidence	101 (18.3)	40 (21.7)	27 (14.7)	34 (18.5)	0.215
Graft survival					
M3	542 (98.2)	182 (98.9)	178 (96.7)	182 (98.9)	0.196
M6	528 (95.7)	178 (96.7)	173 (94)	177 (96.2)	0.401
M12	514 (93.1)	170 (92.4)	169 (91.8)	175 (95.1)	0.416
Patient survival					
M3	547 (99.1)	182 (98.9)	181 (98.4)	184 (100)	0.243
M6	533 (96.6)	178 (96.7)	177 (96.2)	178 (96.7)	0.947
M12	522 (94.6)	171 (92.9)	173 (94)	178 (96.7)	0.253

BMI: body mass index; EAD: early allograft dysfunction; ECD: extended criteria donor; HCC: hepatocellular carcinoma; MELD: model for end-stage liver disease; TAC: tacrolimus; TIPS: transjugular intrahepatic portosystemic shunts.

**Table 3: Study population characteristics (matched population)**

The incidences of acute rejection ( $P=0.305$ ) and TAC-related toxicity ( $P=0.205$ ) were also similar between the groups.

At M1, the level of ALT was still significantly lower in the low group ( $P=0.029$ ) as well as ALP ( $P<0.001$ ), bilirubin ( $P=0.013$ ), INR ( $P=0.032$ ) and creatinine levels ( $P<0.001$ ) (Table 4). At M3, the difference still persisted for ALP ( $P<0.001$ ), bilirubin ( $P<0.001$ ) and creatinine levels ( $P=0.001$ ).

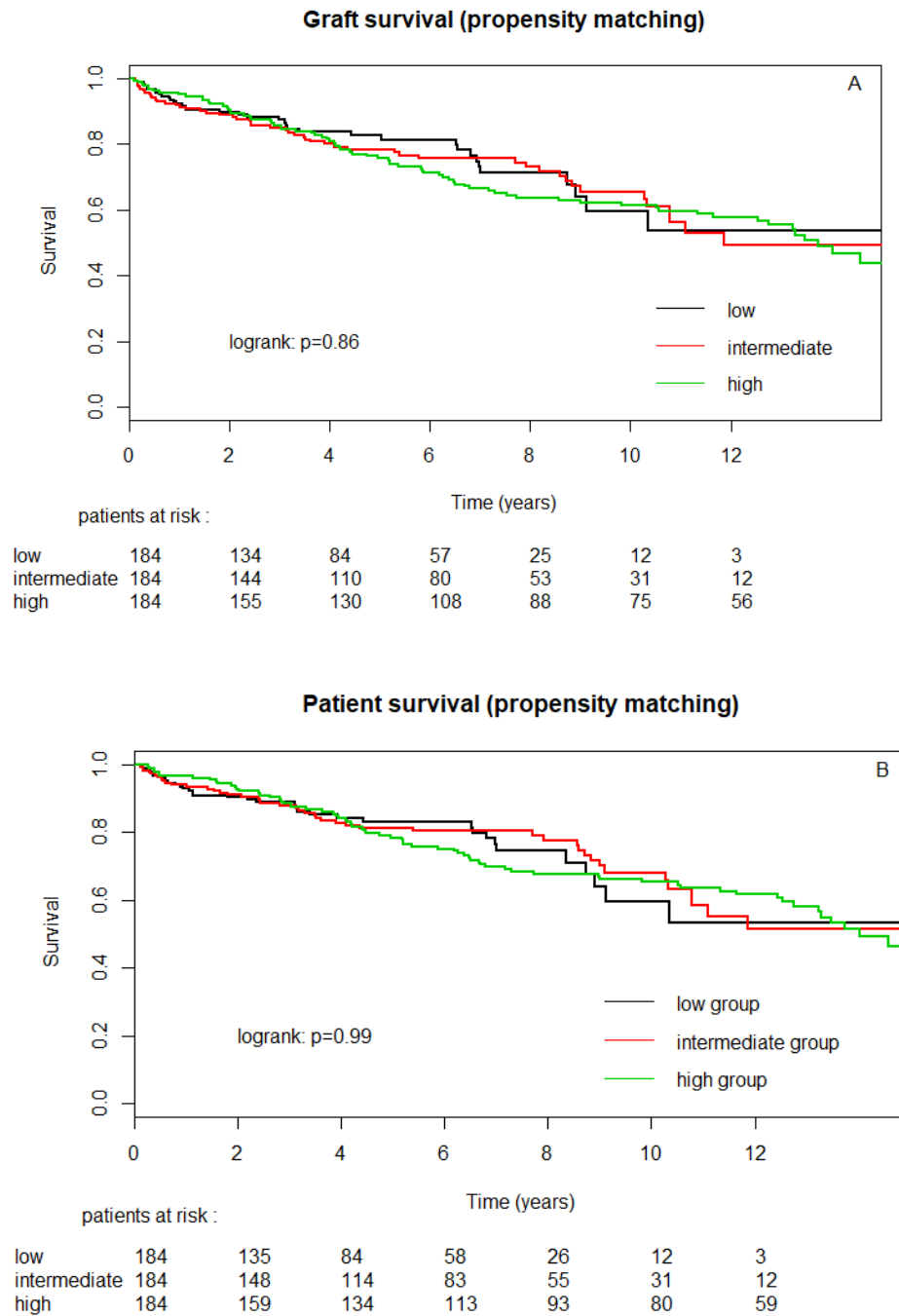
	All patients n= 552	Low group n=184	Intermediate group n=184	High group n=184	P-value
<b>Creatinine (<math>\mu\text{mol/L}</math>)</b>					
M1	91 [31-398]	83 [31-226]	85 [34-311]	106 [46-398]	<b>&lt;0.001</b>
M3	85 [38-237]	85 [46-237]	81 [38-219]	90.5 [48-154]	<b>0.001</b>
<b>ALT (UI/L)</b>					
M1	37 [4-820]	32 [4-356]	38 [8-803]	41 [4-820]	<b>0.029</b>
M3	23 [4-369]	21 [4-273]	23.5 [6-233]	25 [6-369]	0.063
<b>AST (UI/L)</b>					
M1	21 [6-870]	19 [6-99]	23 [6-870]	22 [7-141]	0.053
M3	20 [7-431]	20 [9-329]	21 [7-190]	20 [9-431]	0.43
<b>ALP (UI/L)</b>					
M1	200.5 [42-2292]	155 [42-1389]	200 [52-2292]	245 [64-1521]	<b>&lt;0.001</b>
M3	126 [27-7215]	88.5 [28-2188]	123.5 [27-1500]	160.5 [37-7215]	<b>&lt;0.001</b>
<b>Bilirubin (<math>\mu\text{mol/L}</math>)</b>					
M1	13 [3-453]	11 [3-412]	14 [4-126]	14 [5-453]	<b>0.013</b>
M3	8 [1.17-466]	7 [2-209]	8 [3-466]	9 [1.17-96]	<b>&lt;0.001</b>
<b>GGT (UI/L)</b>					
M1	133 [23-1667]	114 [29-1512]	140 [23-1667]	139 [28-1265]	0.097
M3	48 [5-2363]	46.5 [11-2172]	49.5 [10-844]	46 [5-2363]	0.713
<b>INR</b>					
M1	1.11 [0.73-8.22]	1.08 [0.89-5.08]	1.11 [0.88-5.69]	1.12 [0.73-8.22]	<b>0.032</b>
M3	1.08 [0.76-6.9]	1.06 [0.89-3.16]	1.09 [0.89-6.9]	1.09 [0.76-5.5]	0.281

ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; FV: factor V; GGT: gamma-glutamyl transferase; INR: international normalized ratio; PR: prothrombin ratio; TAC: tacrolimus

**Table 4: Biological parameters (matched population)**

The EAD rate was not different between all of the groups (21.7%, 14.7% and 18.5% for low, normal and high group respectively,  $P=0.215$ ) (Table 3).

At M3, M6 and M12, graft and patient survival was similar as was long-term graft ( $P=0.86$ ) (Figure 5-A) and patient ( $P=0.99$ ) survival (Figure 5-B).



**Figure 5: Grafts (A) and patients (B) survival after propensity score matching**

## 4 | DISCUSSION

Minimization of TAC treatment reduces the occurrence of calcineurin inhibitor adverse events and has been increasingly explored during the last two decades. A TAC trough blood concentration threshold of 6 to 10 ng/mL is currently recommended since it has been found to ensure sufficient treatment efficacy while decreasing toxicity.<sup>3,5</sup> However, the lower part of this range was rarely reached in clinical trials aimed at minimizing TAC exposure. In the present study, we found that patients in the low-exposure group (i.e. 4-7 ng/mL) had similar M3, M6, M12 and long-term graft and patient survival compared with the intermediate (7-10 ng/mL) and high-exposure groups (>10 ng/mL) despite the fact that the recipients in these groups were older, more frequently hospitalized before LT and transplanted with older donors. As for renal function, while the creatinine level was not different between the groups before LT, patients in the higher-exposure group presented higher creatinine levels at M1 and M3. These results are consistent with previous studies reporting that high TAC exposure is associated with more renal dysfunction.<sup>4</sup> Moreover, liver enzymes and cholestasis were significantly improved in the low-exposure group which led us to hypothesize that TAC could also have direct toxicity on liver parenchyma. This is in accordance with previous clinical reports of TAC liver toxicity in transplanted patients.<sup>10,11</sup>

After propensity score matching patients, 552 patients were evaluated with similar patient and graft survival and improved liver and renal function. We therefore believe that patients could be safely exposed to TAC trough concentrations as low as 4-7 ng/mL.

This finding was also observed by Jia et al. who found similar outcomes with TAC trough concentrations between 5-7, 7-10 or 10-15 ng/mL.<sup>12</sup> However, in their study, TAC trough concentrations were only evaluated after M1. Early minimization could therefore not be evaluated from their study unlike ours. Moreover, Jia et al. found that the low-exposure group



(composed of a small number of patients (n=21) with TAC trough concentrations between 0 and 5 ng/mL) was associated with poorer patient survival. In our experience, patients with very low TAC Cmin usually presented major postoperative complications (not necessarily related to TAC) requiring immunosuppressive treatment withdrawal and poorer outcomes. This finding should therefore be interpreted with caution.

Similarly, Aguiar et al. retrospectively analyzed data from 661 liver transplant recipients among whom 221 received TAC-based minimized immunosuppressive treatment (defined as a TAC trough concentration below 5 ng/mL) and reported improved renal function. However, this target was achieved late with a median time to minimization since transplantation of 32 months.<sup>13</sup>

Initially, the first consensus on TAC optimization recommended targeting Cmin between 15 to 20 ng/mL, later adapted to 10 to 20 ng/mL during M1 then 10 to 15 ng/mL until the end of M3 with a minimization to 5 to 10 ng/mL thereafter.<sup>14,15</sup> With the development of multitherapy regimen, attempts to minimize TAC have been made and several randomized controlled trials have been conducted with the assumption that this strategy could lead to better tolerability of the drug, especially in terms of renal function. Since 2012, new data have emerged leading to a meta-analysis which concluded that a de novo TAC Cmin target of 6 to 10 ng/mL (in combination with mycophenolic acid or an m-TOR inhibitor and corticosteroids) ensures sufficient immunosuppressive potency while decreasing the renal toxicity of the drug.<sup>3,4</sup> However, most studies leading to the 6-10 ng/mL recommendation usually reached the higher range of this objective with a mean Cmin frequently above 7 ng/mL during M1 or had a low number of patients.<sup>16-18</sup>

On the contrary, in our study, patients precociously reached the range of 4-7 ng/mL, testifying to our will to voluntarily keep Cmin under 7ng/mL and stay in this range during M1. Our

study is the therefore first to demonstrate the feasibility of a TAC minimization policy immediately after the procedure in daily clinical practice.

The main limitation of our study is its retrospective design. Indeed, the absence of randomization and the long study period inevitably induce differences between groups and thus potential bias. Firstly, we observed a clear difference in group repartition over the study period which can be responsible for a temporal bias. Indeed, almost no patients were exposed to TAC trough concentrations below 7 ng/mL before 2005 while approximately 40% of the patients were in this group after 2014 – reflecting our will to decrease patient TAC exposure throughout the study period. However, there were no other major changes in surgical or medical care during the study period and the increasing experience of our medical and surgical teams could be balanced by the increased severity of the recipients and donors. We therefore believe that the temporal bias does not discredit our findings. Secondly, we observed a higher rate of patients with rejection in the high-concentration group while patients with TAC-related toxicity were higher in the low group. It could be said that this repartition is paradoxical and could explain the differences observed regarding the biological parameters. However, we explained this repartition as a consequence rather than a cause of the occurrence of clinical events. Indeed, in case of the onset of acute cellular rejection, high TAC exposure must be achieved. Consequently, patients were assigned to the high-exposure group. On the contrary, patients presenting an early TAC-related adverse event presented decreased concentrations and were therefore assigned to the low-exposure group. However, after propensity score matching, these differences vanished (thereby decreasing the potential bias secondary to heterogeneity between groups) while the renal and liver biological parameters were still improved in the low-exposure group.

## 5 | CONCLUSION

In conclusion, in a large cohort of liver transplant recipients, we showed that most patients could benefit from immediate TAC exposure minimization protocol targeting 4-7 ng/mL. This minimization led to similar patient and graft survival and ensured better renal function as well as liver biological parameters. However, this result should be confirmed in a prospective randomized controlled trial to definitely establish this therapeutic range. Newer approaches should also be developed to identify the subpopulation of patients who would benefit from even lower TAC exposure.

### III) SYNTHÈSE

Notre étude a permis de démontrer qu'une concentration résiduelle de TAC entre 4 et 7 ng/mL, durant le premier mois après la TH, permettait une amélioration de la fonction rénale ainsi que du bilan hépatique, sans sur risque sur la survie du greffon ou du receveur.

Bien que rétrospective, cette étude fournit des arguments forts confortant cette attitude qui est désormais notre pratique quotidienne. En effet, l'utilisation d'un score de propension a permis de partiellement corriger les biais inhérents à la nature rétrospective de cette étude et la large taille de notre effectif confère une puissance importante à nos résultats.

Il est à souligner que cette attitude n'est rendue possible que grâce à l'instauration initiale d'une triple thérapie immunosuppressive associant un inhibiteur de la calcineurine (le tacrolimus), un anti-métabolite (le mycophénolate mofétil) et un corticostéroïde (la prednisone).

En revanche, nous pouvons constater que la survenue des effets indésirables du TAC reste importante, y compris dans le groupe bénéficiant de la minimisation. Cette observation nous a donc amené à admettre que la concentration sanguine résiduelle du TAC était un marqueur imparfait de sa tolérance et à rechercher d'autre marqueur pharmacologique plus performant. C'est dans cette optique que nous proposons d'étudier l'intérêt de la variabilité intra-individuelle des concentrations sanguines de tacrolimus.

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## CHAPITRE 3 :

ETUDE DE L'INTERET DE LA VARIABILITE INTRA-INDIVIDUELLE DES  
CONCENTRATIONS DE TACROLIMUS EN TRANSPLANTATION HEPATIQUE

**High inpatient variability of tacrolimus exposure in the early period after liver  
transplantation is associated with poorer outcomes**

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## **D) CONTEXTE**

La variabilité intra-individuelle (Inpatient variability, IPV) des concentrations de tacrolimus (TAC) est un paramètre pharmacologique qui a principalement montré son intérêt dans le monitoring du traitement immunosuppresseur en transplantation rénale. Cependant, il pourrait surtout être le reflet de l'observance des patients car dans la plupart des travaux publiés sur le sujet, ce paramètre est évalué à distance de l'intervention (généralement au-delà de 6 mois) et donc dans un état de stabilité clinique et thérapeutique. En transplantation hépatique (TH), l'IPV a été étudiée essentiellement chez l'enfant où il est également un témoin de l'observance.

En transplantation hépatique chez l'adulte, la période précoce étant marquée par une reprise progressive des fonctions du greffon, l'IPV pourrait être un excellent marqueur de la variabilité pharmacocinétique du TAC non liée à l'observance (du fait du suivi hospitalier et péri-hospitalier rapproché qui limite le risque d'inobservance). Cette variabilité précoce si elle est importante pourrait être associée à la survenue des effets indésirables (EI) du TAC lesquels apparaissent volontiers précocement en post-TH. A ce jour, il n'existe pas de travaux ayant étudié l'IPV précoce en transplantation d'organe solide.

L'objectif de notre étude était donc d'étudier l'intérêt de l'IPV du TAC, durant la phase précoce post TH, dans la prédiction de l'efficacité et de la sécurité du TAC.



## II) MANUSCRIT DE L'ARTICLE PUBLIE (en Anglais)

### 1 | INTRODUCTION

Tacrolimus (TAC) is the cornerstone of immunosuppressive regimen in organ transplantation and in particular in liver transplantation (LT). Its pharmacokinetics is characterized by a high inter- and inpatient variability leading to an unpredictable dose-response relationship. Therapeutic drug monitoring (TDM) of TAC, usually based on whole blood trough concentration, enables the maintenance of blood concentrations within the therapeutic range and hence reduces the incidence of allograft rejection as well as the occurrence of adverse events <sup>1</sup>. However, despite appropriate TDM, these events still occur and subsequently reveal the limits of TAC trough concentration measurement.

Recent studies have shown that the inpatient variability (IPV) of TAC concentration is a key factor in immunosuppressive treatment and is associated with poorer outcomes in renal transplant recipients. Indeed, a high TAC IPV has been associated with graft loss, onset of donor specific antibodies <sup>2</sup> as well as the risk of fibrosis and tubular atrophy <sup>3</sup>. Other teams have reported that a high TAC IPV could result in poor long-term outcomes (defined as a composite endpoint consisting of graft loss, biopsy-proven acute rejection, chronic allograft nephropathy and doubling in plasma creatinine concentration) <sup>4-6</sup>.

While IPV appears to be a relevant biomarker of TAC efficacy and therefore a potential target for TDM in renal transplantation, few data are available for liver transplantation. The aim of our study was to evaluate the impact of IPV in TAC whole blood trough concentrations in the early period after liver transplantation (reflected by the coefficient of variation of drug levels) on patient early and long-term outcomes.

## 2 | PATIENTS AND METHODS

### *Patient selection*

All transplanted patients in our department between January 2002 and December 2014 (n = 1,297) were retrospectively analyzed. Grafts were all harvested from brain-dead donors and were usually preserved with Celsior®.

Patients transplanted with a split graft (n = 45) or with other associated organs (n = 51) were excluded from the analysis, as were retransplanted patients (n = 137) owing to the fact that these patients already received calcineurin inhibitor. Since our objective was to efficiently evaluate the influence of the inpatient variability of TAC exposure during the first month after LT on postoperative outcomes, we decided to exclude from the analysis the patients who died or were retransplanted within the first 15 postoperative days (POD) (n = 65) since the cause is usually related to surgical complications, primary allograft malfunction or recipient-related complications and not to the immunosuppressive regimen.

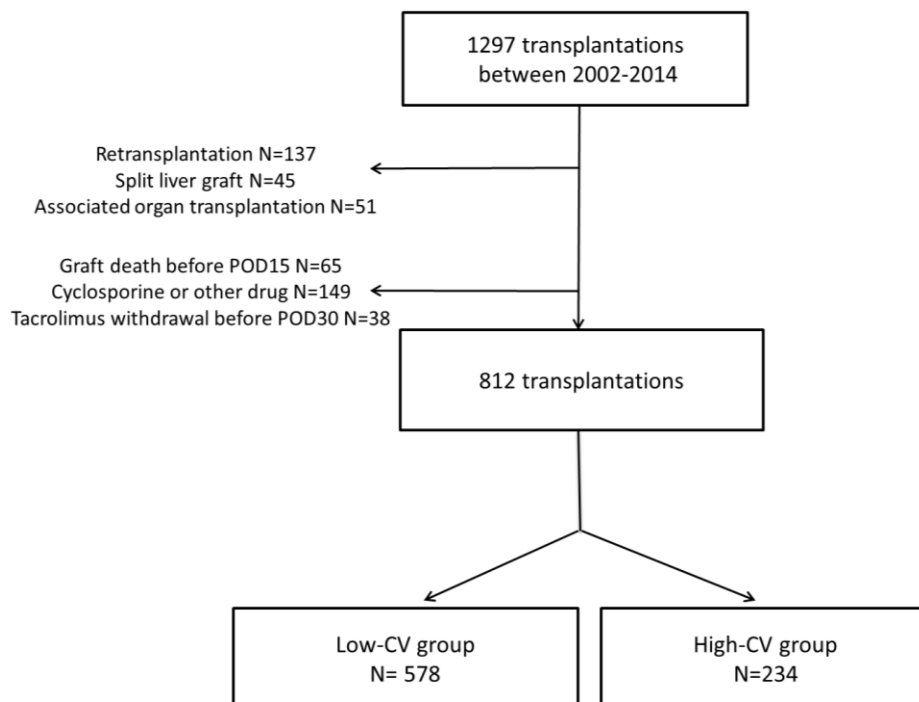
Patients treated with cyclosporine or receiving induction treatment (i.e. anti-lymphocyte or anti-interleukine-2) were also excluded (n = 149). Patients who had TAC regimen stopped before POD 30 (n = 38) were also excluded in order to have enough trough concentrations measured per patient for IPV estimation.

Ultimately, our study population consisted of 812 liver transplanted patients treated by oral TAC given twice daily and started on POD 1 or 2 until at least POD 30 (Fig.1).

### *Surgical procedure and medical care*

All patients had orthotopic liver transplantation with inferior vena cava preservation. After the procedure, standardized immunosuppression associating TAC, mycophenolate mofetil and a short course of corticosteroids was administered. Tacrolimus whole blood trough concentration was measured daily during the first 7 POD and then three times a week during

the hospital stay or more frequently according to the clinical necessity in case of complications. After hospital discharge, patients were seen weekly until at least 3 months after LT and had TAC trough level measurements in outpatient consultations. Whole-blood TAC levels were measured with a fully validated liquid chromatography tandem mass spectrometry adapted from a previously published method <sup>7</sup>.



**Figure1: Flow chart**

The other concomitant drugs administered were essentially esomeprazole (for gastric ulcer prophylaxis), trimethoprim-sulfamethoxazole (for pneumocystis prophylaxis) and fluconazole (for fungal infection prophylaxis). A CMV pre-emptive strategy was adopted for all patients. There was no difference in treatment administration between the two groups.

No significant modification regarding the surgical procedure or postoperative medical care was observed during the study period.

### *IPV and Coefficient of Variation (CV) of TAC trough concentration calculations*

Inpatient variability in TAC concentrations was estimated by calculating its coefficient of variation (CV) according to the following equation:  $CV = \text{standard deviation (SD) of TAC trough concentrations} / \text{mean TAC trough concentrations}$  <sup>2</sup>.

Mean concentrations were calculated using all measured values from POD 8 to 30. Trough concentrations measured before POD 8 were excluded since steady-state concentrations are usually reached after the first week of treatment.

We decided to separate our study population into 2 groups (low-CV group and high-CV group) according to the cutoff of CV=40% (which approximatively correspond to round value of the third quartile of CV distribution in order to get a value easily usable in practice).

### *Clinical and biological parameter definitions*

Clinical and biological data were retrospectively collected from a prospective database. The following parameters were analyzed: donor and recipient characteristics, underlying liver disease and OLT indication, Child-Pugh grade and MELD score (retrospectively calculated for patients transplanted before 2007, date on which a MELD score based graft allocation system was instituted in France), cold ischemia time, post-transplant liver and renal function (estimated by CKD-EPI value) <sup>8</sup>. The main postoperative complications collected and analyzed within the 3 first months after LT, were arterial complications (i.e. thrombosis and stenosis), biliary complications (i.e. biliary leakage and stenosis), neurologic complications (i.e. encephalopathy appearing or persisting after POD 7, confusion, tremor, seizures and posterior reversible encephalopathy syndrome), cardiovascular complications (i.e. acute coronary disease, acute pulmonary edema, arrhythmia) and acute rejection defined as biopsy proven rejection or highly clinically suspected (i.e. elevated liver or cholestasis enzyme associated with TAC concentration < 4 ng/mL without other cause and rapidly resolving after

increase in TAC concentration).

An extended criteria graft (ECD) was considered when the donor matched at least one of the following criteria: age > 65 years; BMI > 30; ICU stay > 7 days prior harvesting; natremia > 155 mmol/L; liver enzymes 3 times higher the normal value (i.e. aspartate aminotransferase (ASAT) blood level > 150 IU/mL, alanine aminotransferase (ALAT) blood level > 170 IU/mL).

Early allograft dysfunction (EAD) was defined according to Olthoff et al.<sup>9</sup> criteria (i.e. the presence of one or more of the following criteria: bilirubin level  $\geq$  10 mg/dL on POD 7; INR  $\geq$  1.6 on POD 7; ALAT or ASAT level > 2000 IU/L before POD 7).

Patient and graft survival at 3 months, 12 months and beyond (i.e. long-term survival) were calculated.

### *Statistical analysis*

Quantitative variables were expressed as mean  $\pm$  standard deviation or by median with extreme values and compared using Student's *t*-test or Wilcoxon test as appropriate. Qualitative variables were expressed as number and percentage and compared using Chi-squared or Fisher's exact test, as appropriate.

Survival analysis was performed with Kaplan-Meier curve and compared with log-rank test. All variables with a *p*-value < 0.1 were included in a multivariate cox model as well as significant variables or confusion variables (i.e. significantly different between the 2 groups). The best multivariate model was selected using a descendant stepwise method including only significant variables. A *p* < 0.05 value was considered to be statistically significant. All statistical analyses were performed with R software version 3.1.3.

### 3 | RESULTS

#### *Variation of tacrolimus trough concentrations*

A total of 812 patients were analyzed with a majority of men (n = 622, 76.6%) and a median age of 56 [15-73]. The median follow up was 61.8 [2.3-167.6] months. The main characteristics of the study population are summarized in Table 1.

Between POD 8 and 30, the TAC mean trough concentration was  $9.8 \pm 6.0$  ng/ml with a median CV of 31.4% (first quarter limit of 23.5% and a third quarter limit of 41.8%). The low-CV group therefore corresponded to patients with a CV < 40% (n = 578, 71.2%) while the high-CV group corresponded to patients with a CV  $\geq$  40% (n = 234, 28.8%).

	<b>Entire population n= 812 (%)</b>	<b>CV&lt;40 n=578 (%)</b>	<b>CV<math>\geq</math>40 n=234 (%)</b>	<b>p-value</b>
<b>Gender</b>				
Male	622 (76.6%)	455 (78.7%)	167 (71.4%)	0.03
Female	190 (23.4%)	123 (21.3%)	67 (28.6%)	
<b>Age</b>	56 [15; 73]	56 [15; 72]	56 [16; 73]	0.86
<b>Medical history</b>				
Diabetes mellitus	153 (18.8%)	114 (19.7%)	39 (16.7%)	0.36
Arterial hypertension	86 (10.6%)	68 (11.8%)	18 (7.7%)	0.11
TIPS	35 (4.3%)	26 (4.5%)	9 (3.8%)	0.82
Hepato-renal syndrome	37 (4.6%)	25 (4.3%)	12 (5.1%)	0.76
<b>BMI</b>	26.4 [15.7 ; 47.2]	26.5 [15.7 ; 45.7]	26.0 [17.4 ; 47.2]	0.29
<b>Diagnosis</b>				0.31
HCC	221 (27.2%)	165 (28.5%)	56 (23.9%)	
Alcohol	358 (44.1%)	250 (43.3%)	108 (46.2%)	
Acute liver failure	34 (4.2%)	19 (3.3%)	15 (6.4%)	
Biliary and immunologic	62 (7.6%)	44 (7.6%)	18 (7.7%)	
Viral hepatitis (B+C)	66 (8.1%)	47 (8.1%)	19 (8.1%)	
Other	71 (8.7%)	53 (9.2%)	18 (7.7%)	
<b>CV infection</b>	79 (9.7%)	52 (9%)	27 (11.5%)	0.33

<b>Child Pugh class</b>				<0.001
A	266 (32.7%)	216 (37.4%)	50 (21.4%)	
B	217 (26.7%)	151 (26.1%)	66 (28.2%)	
C	331 (40.7%)	211 (36.5%)	118 (50.4%)	
<b>MELD score (median value)</b>	14.5 [5.41; 40]	13.2 [5.4; 40]	17.8 [5.4; 40]	<0.001
<b>CKD-EPI value (preoperative)</b>	95.9 [7.5; 170.5]	95.9 [11.5; 146.4]	96.6 [7.5; 170.5]	0.66
<b>Dialysis (preoperative)</b>	13 (1.6%)	7 (1.2%)	6 (2.6%)	0.21
<b>Cold ischemia time (min)</b>	564.5 [183; 1148]	568.5 [183; 1148]	556.5 [19; 930]	0.34
<b>Surgical duration (min)</b>	360 [134; 800]	360 [134; 743]	370 [175; 800]	0.44
<b>Bilioenteric anastomosis</b>	46 (5.7%)	34 (5.9%)	12 (5.1%)	0.80
<b>Donor Gender</b>				0.29
Male	477 (58.6%)	346 (59.9%)	130 (55.6%)	
Female	337 (41.4%)	232 (40.1%)	104 (44.4%)	
<b>Donor age (median value)</b>	53 [10; 90]	53.5 [11; 87]	53 [10; 90]	0.73
<b>Donor BMI (median value)</b>	24.49 [14.02; 54.4]	24.49 [14.02; 54.4]	24.69 [14.27; 48.89]	0.49
<b>ECD graft</b>	451 (55.5%)	322 (55.7%)	127 (54.3%)	0.75

BMI: Body mass index; CKD-EPI: Chronic Kidney Disease - Epidemiology Collaboration; CV: Coefficient of variation; ECD: Extended criteria donor; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; TIPS: Transjugular intrahepatic portosystemic shunts

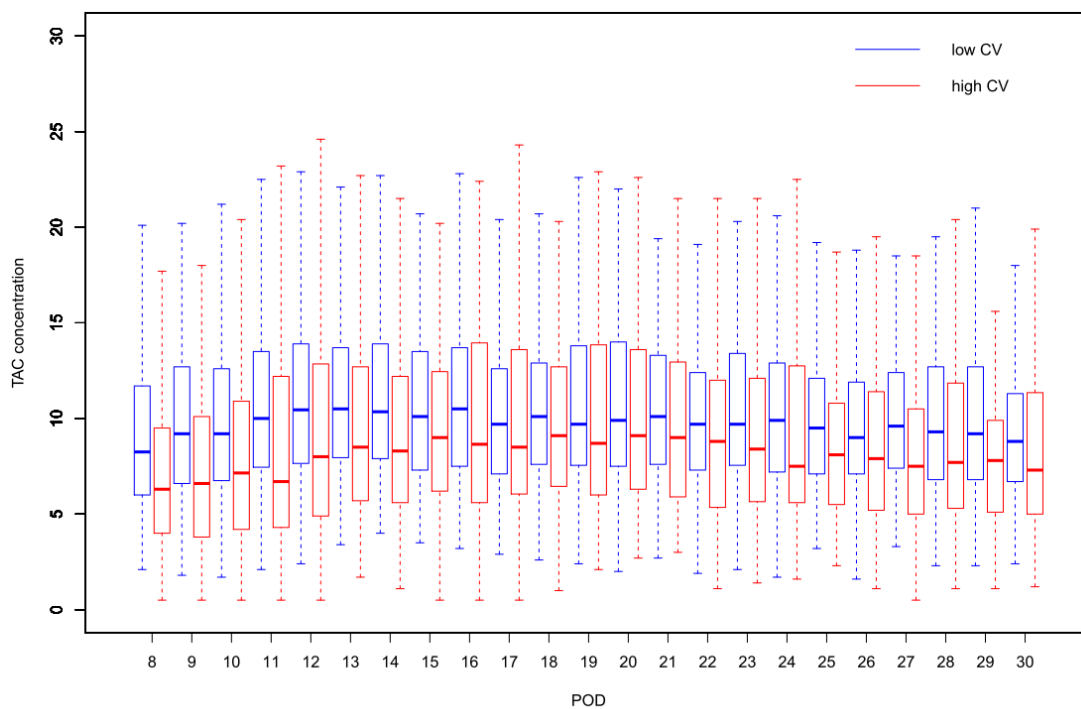
**Table 1: Study population characteristics**

*Risk factor for high coefficient of variation of tacrolimus concentrations (table1)*

The high and low-CV groups were similar regarding recipient age and indication for transplantation including the incidence of HCV infection and the presence of liver malignancy. Cold ischemia and duration of operation were also not different between the 2 groups as were the characteristics of the liver graft (i.e. donor age, sex and incidence of ECD grafts). However, MELD score ( $p < 0.001$ ) and Child-Pugh grade ( $p < 0.001$ ) were significantly different between the 2 groups and were then identified as being risk factors for a high CV while renal function was not.

*Relation between coefficient of variation of tacrolimus concentrations and postoperative outcomes (table2)*

The mean trough concentration of TAC was significantly lower in the high-CV group than in the low-CV group ( $9.3 \pm 5.9$  vs.  $10.0 \pm 5.9$  ng/mL,  $p = 0.009$ ), but still remained within the normal therapeutic range (figure 2) which was confirmed by a similar acute rejection rate (19.7% in the high-CV group vs. 17.0% in the low-CV group,  $p = 0.417$ ).



**Figure 2: Evolution of tacrolimus blood trough concentration**

There were no differences in the incidence of biliary (9.0% vs. 6.4%,  $p = 0.26$ ) and arterial complications (2.6 vs. 1.9%  $p = 0.83$ ) between the high and low-CV groups.

There were significantly more neurologic (31.2% vs. 16.6%,  $p < 0.001$ ), cardiovascular (19.7% vs. 9.7%,  $p < 0.001$ ) complications and acute renal failure requiring dialysis (8.5% vs. 2.2%,  $p < 0.001$ ) while the median CKD-EPI value was not different (71.4 vs 75.6 mL/min,  $p=0.34$ ) in the high-CV group than in the low-CV group. As a result, median hospital stay was significantly increased in the high-CV group (23 days vs. 18 days,  $p < 0.001$ ).



	Entire population n= 812 (%)	CV<40 n=578 (%)	CV≥40 n=234 (%)	p-value
<b>Cardio-vascular complications</b>	104 (12.9%)	56 (9.7%)	46 (19.7%)	0.001
<b>Neurologic complications</b>	171 (21.1%)	96 (16.6%)	73 (31.2%)	<0.001
<b>Dialysis</b>	33 (4.1%)	13 (2.2%)	20 (8.5%)	<0.001
<b>CKD-EPI value (at POD30)</b>	74.8 [7.3 ; 184.3]	75.6 [14.0 ; 151.3]	71.4 [7.3 ; 184.3]	0.34
<b>Acute rejection</b>	144 (17.7%)	98 (17%)	46 (19.7%)	0.42
<b>EAD incidence</b>	156 (19.2%)	104 (18%)	52 (22.2%)	0.21
<b>Arterial complications</b>	17 (2.1%)	11 (1.9%)	6 (2.6%)	0.83
<b>Biliary complications</b>	58 (7.1%)	37 (6.4%)	21 (9%)	0.26
<b>ICU stay</b>	4 [1; 199]	4 [1; 199]	5 [1; 148]	<0.001
<b>Hospitalization duration</b>	19 [8; 333]	18 [8; 333]	23 [9; 232]	<0.001
<b>TAC trough concentration (ng/mL)</b> (mean value within first POD 30)	9.8 ±6.3	10.0 ±5.9	9.3 ±5.9	0.009
<b>Mean number of dosages</b> (within first POD 30)	14 [4; 29]	14 [5; 29]	15 [4; 29]	0.10
<b>Outcomes</b>				
3-month graft survival	785 (96.7%)	563 (97.4%)	222 (94.9%)	0.108
1-year graft survival	742 (91.4%)	536 (92.7%)	206 (88%)	0.043
3-month patient survival	794 (97.8%)	568 (98.3%)	226 (96.6%)	0.224
1-year patient survival	760 (93.6%)	547 (94.6%)	213 (91%)	0.081

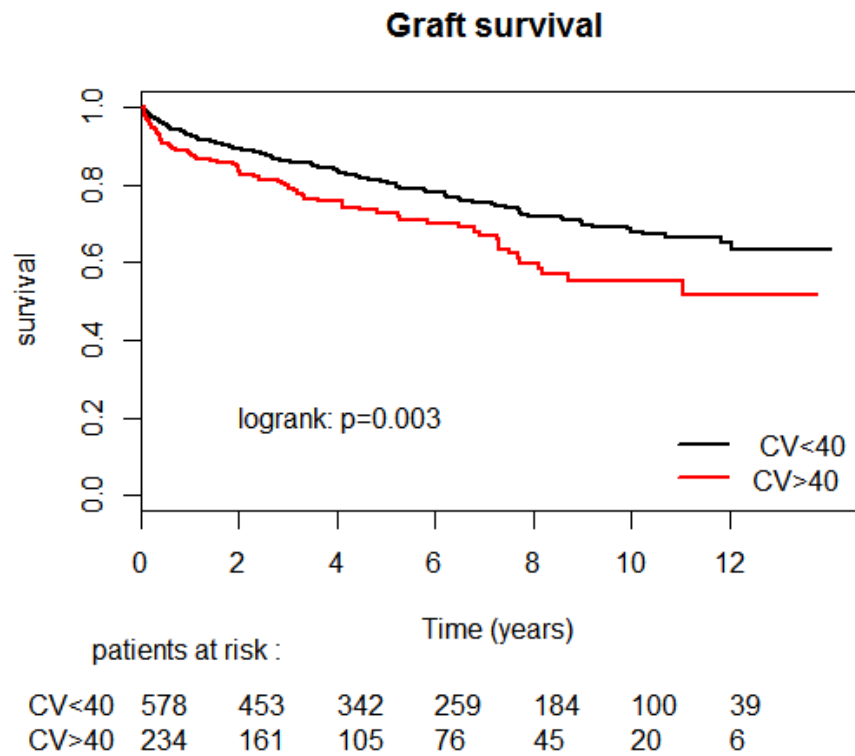
CKD-EPI: Chronic Kidney Disease - Epidemiology Collaboration; CV: Coefficient of variation; EAD: early allograft dysfunction; ICU: intensive care unit; POD: Post-operative day; TAC: tacrolimus

**Table 2: Postoperative outcomes**

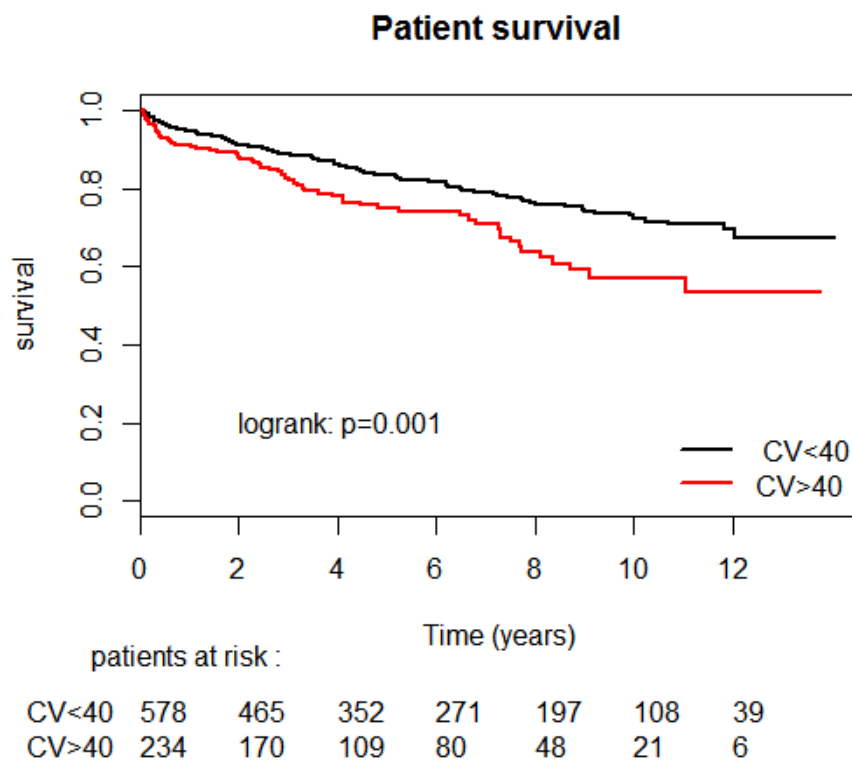
*Relation between coefficient of variation of tacrolimus concentrations and graft and patient survival*

Three-month graft survival was not different between the 2 groups (94.9% in high-CV group vs. 97.4% in low-CV group,  $p = 0.108$ ). However, this difference reached statistical significance for 1-year graft survival (88.0% vs. 92.7%,  $p = 0.043$ ) (table2). Moreover, long-

term graft survival was significantly worse in the high-CV group than in the low-CV group ( $p = 0.003$ ) (Fig. 3a) as was patient survival ( $p = 0.001$ ) (Fig. 3b).



**A**



**B**

**Figure 2 : Patient and graft survival**

In multivariate analysis, a CV  $\geq$  40% was significantly associated with poorer survival (HR = 1.57 [1.18; 2.01],  $p = 0.002$ ) as well as presence of an HCV infection (HR = 1.49 [1.01; 2.19],  $p = 0.04$ ) and liver malignancy (HR = 1.36 [1.03; 1.79],  $p = 0.03$ ) (table3).

Variables	Univariate (Logrank Test)	Multivariate (Cox Model)		
	p	p	Hazard Ratio	[CI 95%]
Recipient gender	0.09	ns		
Age (years)	0.64	-		
BMI*	0.78	-		
HCV infection	0.02	0.04	1.49	[1.01; 2.19]
Liver malignancy	0.03	0.03	1.36	[1.03; 1.79]
Child-Pugh grade	0.63	ns		
MELD score	0.71	ns		
Donor age	0.70	-		
ECD graft	0.11	ns		
EAD	0.38	ns		
CV $\geq$ 40	0.003	0.002	1.57	[1.18; 2.01]
Mean TAC concentration	0.6	-		

BMI: Body mass index; CV: Coefficient of variation; ECD: Extended criteria donor; EAD: early allograft dysfunction; TAC: tacrolimus

**Table 3: Univariate and multivariate analysis of graft survival**

## 4 | DISCUSSION

Tacrolimus is the cornerstone of immunosuppressive regimen in organ transplantation. Its use requires close therapeutic monitoring which in daily clinical practice relies on dosage of TAC trough concentration despite the fact that it could be an imperfect surrogate marker of its activity<sup>10</sup>. Recently, the coefficient of variation of TAC concentrations, calculated beyond 6 months post transplantation, was found to be a more relevant biomarker of TAC toxicity and immunosuppressive efficacy, especially in kidney transplantation<sup>2,3,5</sup>.

In the present study, we found that a coefficient of variation of TAC trough concentration in the early period above 40% was significantly associated with higher incidences of neurologic, cardiologic complications and postoperative acute renal failure which are well-known calcineurin inhibitor-related adverse events<sup>11</sup>. Interestingly, we observed that the mean trough concentration of tacrolimus was significantly lower in the high-CV group than in the low-CV group but remained within the therapeutic range (as confirmed by the same incidence of acute rejection in the 2 groups). This finding raises the hypothesis that a high CV of TAC could be a relevant determinant of the onset of the drug's effects (its toxicity as well as its activity) rather than the absolute value of the trough concentration. Variation of TAC concentration could therefore subsequently result in organ toxicity more than constant exposure.

Moreover, we found that a high CV was a risk factor for poorer long-term patient and graft survival in both univariate and multivariate analyses. The possible mechanism is still unknown. However, we could speculate that a high CV would probably induce sub-acute rejection with subclinical liver parenchyma lesion which would slowly increase over time and be clinically relevant in the long-term. It would have been of interest to analyze the occurrence of donor specific antibodies between the high and low-CV groups but unfortunately, these data were not available.

We also found that a high pre-operative liver failure (i.e. high Child-Pugh grade and MELD score) was associated with high CV after liver transplantation unlike that of preoperative renal function (which is, however, partly included in the MELD calculation). Since patient with high MELD score (i.e. with a more severe liver disease) have a longer graft function restoration and as TAC is metabolized by the graft and eliminated through biliary excretion our findings are in accordance with TAC pharmacokinetics<sup>12</sup>.

To our knowledge, our study is the first to evaluate the role of early TAC CV and found a significant relation with TAC related toxicity (i.e. neurological, cardiac and renal

complications). Recently, the inpatient variability of TAC concentrations was recognized as a significant predictor of poorer outcomes in kidney transplantation. Indeed, Van Hove et al. reported that renal transplant recipients with a high TAC CV were at higher risk of developing histologic kidney lesions 2 years after transplantation<sup>3</sup>. In the same way, Rodrigo et al. reported that in 310 renal transplant recipients, a CV > 30% was responsible for a 2.6 higher risk of graft loss while TAC trough levels alone were not a risk factor<sup>2</sup>. Other respective studies have shown that CV, calculated between 6 and 12 months after kidney transplantation, was associated with poor long term outcomes (expressed as a composite endpoint combining graft loss, rejection and decrease in renal function)<sup>4,5,13</sup>. Regarding liver transplantation, Christina et al. showed, in a retrospective study including 150 pediatric liver transplant recipients, the relationship between the TAC blood level standard deviation (called medication level variability index (MLVI)) and biopsy-proven acute rejection<sup>14</sup>. Recently, the same team confirms their results, on a prospective study evaluating 379 pediatric liver transplantations, the association between MLVI and late acute rejection<sup>15</sup>. However, the authors failed to demonstrate a relation between MLVI and worst graft or patient survival<sup>15</sup>.

Nevertheless, our results must be interpreted with caution. Indeed, the retrospective nature of our analysis as well as the long study period could induce a bias. However, our policy regarding immunosuppressive regimen was constant over the study period and the strict selection criteria should decrease the bias owing to the retrospective nature. Indeed, we voluntarily chose to exclude patients who died or were transplanted prematurely in the postoperative period (i.e. before POD 15) since the cause is usually related to primary graft dysfunction or to surgical complications (usually vascular). This selection process could explain why the MELD score, the Child-Pugh grade and the occurrence of EAD were not found to be risk factors for poorer outcomes in our study since these risk factors usually lead to early death or retransplantation<sup>9,16</sup>. On the other hand, we found that HCV infection and

liver malignancy (i.e. the 2 main etiologies of initial disease recurrence on grafts) were significantly associated with poorer graft survival. These results validate our multivariate analysis and strengthen our findings.

Moreover, the relationship between high-CV and the occurrence of postoperative complications should be interpreted cautiously as it is hard to determine if a high CV was a cause or a consequence. Indeed, occurrence of early neurologic complications or renal failure usually results in interruption or decrease in TAC dosage which result in increasing TAC CV. However, adverse events usually occurred soon after initiating TAC or increasing its dosage while drug level remains within the therapeutic range, we believe that a rapid increase in TAC blood trough concentration (witnessed by a high CV of TAC) could rather be responsible of postoperative complications than an elevated TAC blood trough concentration. This hypothesis is supported by the fact that mean TAC blood trough concentration was paradoxically lower in the high-CV group than in the low-CV group while remaining within the therapeutic range. Moreover, the causality effect could not be questioned regarding the long term outcomes as we found that a high CV of TAC during the first 30 POD was associated with worst outcomes beyond 3 months.

One of the most point open for criticism of this study is our choice to calculate CV in the early postoperative period (i.e. between POD 8 and POD 30) while most studies in renal transplantation and previous studies in liver transplantation opted for a more distant period (usually beyond 6 months after transplantation)<sup>2-5,13-15</sup>. Indeed, in their studies, Christina et al.<sup>14</sup> and Shemesh et al.<sup>15</sup> calculated MLVI on at least 3 TAC standard deviation measured beyond 6 months after LT<sup>14</sup>. Therefore, MLVI reflect the patient medical nonadherence<sup>14,17</sup> which is known to be more elevated in pediatric recipient than in adult recipient<sup>18</sup>, especially in the early postoperative period<sup>19</sup>. Moreover, MLVI was predictive of occurrence of late acute rejection beyond 6 months which has limited consequence on liver graft survival

compared to other solid organ transplantation<sup>5,13,15</sup>. Therefore, as most severe complications and especially TAC related complications occur in the early period, we then choose to evaluate the TAC CV (which might be a better pharmacokinetic parameter of its efficacy and tolerance than its standard deviation) in the early post transplantation period and we demonstrated, in a very large cohort of adult liver transplant recipients (n=812), the negative impact of a high TAC CV in early period on efficacy (i.e. decreased graft survival) as well as on safety (i.e. increased cardiovascular, neurological and renal complications).

Our finding could therefore make it possible to identify patients at higher risk and consequently to propose them more effective therapeutic drug monitoring methods than whole blood TAC trough concentration measurement which did not take in consideration variation of the drug metabolism related to evolution of the graft function, blood cell and plasmatic protein variation which are known factor to influence TAC concentration and activity<sup>10,20</sup>. In fact, newer methods for TAC drug monitoring are currently being explored which integrate TAC distribution in lymphocytes and elimination via hepatocytes through biliary excretion. Capron et al. demonstrated the relationship between peripheral blood mononuclear cells concentrations, intrahepatic concentrations and rejection during the first week post-transplantation<sup>21</sup>.

Moreover, patients at risk of high CV should be screened of having a higher TAC clearance (by a *CYP3A5* genotyping and/or with an early measurement of AUC) in order to be assigned to intensive therapeutic monitoring and education for limiting TAC variability.

Alternatively, our team is currently evaluating the interest of dosing the biliary excretion of tacrolimus collected through the biliary T-tube (STABILE study, NCT02820259)<sup>22,23</sup>. These newer drug monitoring options could improve the management of patients at higher risk of poor outcome and acting at this early stage could therefore improve patient outcome.

## 5 | CONCLUSION

In conclusion, our study highlighted the role of coefficient of variation of TAC whole-blood trough concentrations in TAC-related toxicity and poorer graft outcomes. Patients with severe pre-transplantation hepatic failure who are at higher risk of high CV should be proposed for closer therapeutic drug monitoring to reduce TAC variability.

## 6 | SUPPLEMENTAL MATERIALS

	CV 1 <sup>st</sup> Q n=203 (%)	CV 2 <sup>nd</sup> Q n=203 (%)	CV 3 <sup>rd</sup> Q n=203 (%)	CV 4 <sup>th</sup> Q n=203 (%)	p-value
<b>Cardio-vascular complications</b>	19 (9.4%)	18 (8.9%)	23 (11.3%)	42 (20.7%)	0.001
<b>Neurologic complications</b>	30 (14.8%)	31 (15.3%)	45 (22.2%)	63 (31%)	<0.001
<b>Dialysis</b>	2 (1.0%)	5 (2.5%)	8 (3.9%)	18 (8.9%)	0.001
<b>CKD-EPI value (at POD30)</b>	73.9	78.3	74.0	73.4	0.30
<b>Acute rejection</b>	25 (12.3%)	35 (17.2%)	44 (21.7%)	40 (19.7%)	0.08
<b>EAD incidence</b>	39 (19.2%)	38 (18.7%)	34 (16.7%)	45 (22.2%)	0.61
<b>Arterial complications</b>	30 (14.8%)	39 (19.2%)	28 (13.8%)	35 (17.2%)	0.55
<b>Biliary complications</b>	4 (2.0%)	17 (8.4%)	18 (8.9%)	19 (9.4%)	0.01
<b>ICU stay</b>	3 [1 ; 76]	4 [1 ; 143]	4 [1 ; 199]	5 [1 ; 148]	<0.001
<b>Hospitalization duration</b>	12 [ 8; 53]	15 [9 ; 90]	15 [9 ; 134]	17 [9 ; 216]	<0.001
<b>TAC trough concentration (ng/mL)</b> (mean value within first POD 30)	9.9 ±5.3	9.9 ±5.3	10.3 ±5.6	9.3 ±5.9	0.03
<b>Mean number of dosages</b> (within first POD 30)	13 [5 ; 29]	15 [5 ; 27]	16 [6 ; 28]	15 [4 ; 29]	<0.001
<b>Outcomes</b>					
3-month graft survival	3 (1.5%)	6 (3%)	8 (3.9%)	10 (4.9%)	0.25
1-year graft survival	13 (6.4%)	12 (5.9%)	20 (9.9%)	25 (12.3%)	0.07
3-month patient survival	1 (0.5%)	3 (1.5%)	8 (3.9%)	6 (3%)	0.09
1-year patient survival	10 (4.9%)	8 (3.9%)	16 (7.9%)	18 (8.9%)	0.13

1<sup>st</sup> Q : <23.53 ; 2<sup>nd</sup> Q: ≥23.53 and <31.37 ; 3<sup>rd</sup> Q: ≥31.37 and <41.82 ; 4<sup>th</sup> Q: ≥41.82; CKD-EPI: Chronic Kidney Disease - Epidemiology Collaboration; CV: Coefficient of variation; EAD: early allograft dysfunction; ICU: intensive care unit; POD: Post-operative day; TAC: tacrolimus



**Table S1: Postoperative outcomes according to CV quartile**

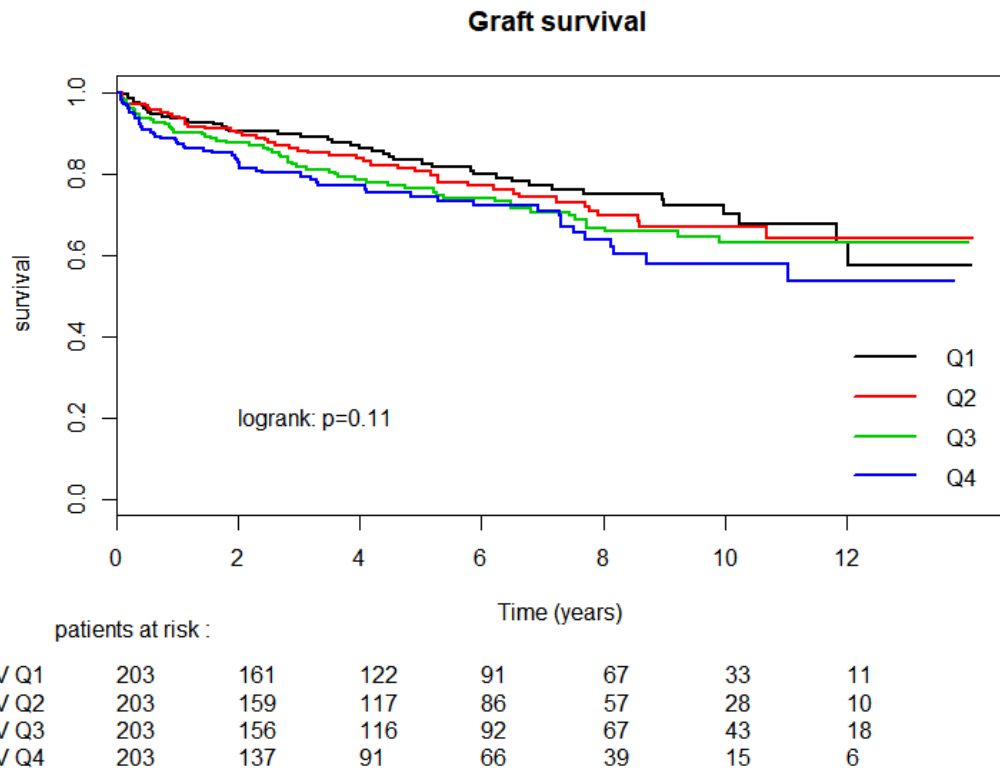
Causes of loss	Low-CV group		High-CV group	
	Graft loss only (retransplantation)	Graft loss with patient's death	Graft loss only (retransplantation)	Graft loss with patient's death
Graft's vascular	10	0	4	0
Biliary	1	0	2	0
Infection	0	12	0	10
Rejection	3	0	1	1
Initial disease recurrence	2	0	0	0
Cardiovascular	0	4	0	6
Malignancy	0	7	0	2
Other	0	3	0	2

**Table S2: Causes of graft and patient death before 1 year after transplantation.**

Causes of loss	Low-CV group		High-CV group	
	Graft loss only (retransplantation)	Graft loss with patient's death	Graft loss only (retransplantation)	Graft loss with patient's death
Graft's vascular	5	0	2	0
Biliary	2	0	0	0

Infection	0	14	0	0
Rejection	3	1	1	5
Initial disease recurrence	3	0	1	0
Cardiovascular	0	9	0	7
Malignancy	0	46	0	17
Other	3	9	0	9

*Table S3: Causes of graft and patient death beyond 1 year after transplantation.*



Legend of figure S1 : 1<sup>st</sup> Q : <23.53 ; 2<sup>nd</sup> Q: ≥23.53 and <31.37 ; 3<sup>rd</sup> Q: ≥31.37 and <41.82 ; 4<sup>th</sup> Q: ≥41.82

**Figure S1: Graft survival according to CV quartile**

### III) SYNTHÈSE

Notre étude a permis de mettre en évidence l'intérêt de la variation intra-individuelle des concentrations de TAC évaluée au travers du calcul du coefficient de variation (CV) comme marqueur associé à son efficacité et sa toxicité. En effet, les patients présentant un CV élevé étaient plus à risque de survenue d'un EI du TAC et avaient une survie à long terme diminuée. Ainsi, le CV calculé dès le 1<sup>er</sup> mois post TH, permet d'identifier précocement les patients à risque et d'adapter la surveillance. Dans ce travail, nous avons identifié un seuil de 40% légèrement supérieur aux valeurs usuellement rapportées en transplantation rénale. Néanmoins, la plupart des travaux s'accordant autour d'une valeur seuil de 30% ont été effectués dans une population de patients transplantés stables, traités depuis au moins six mois. Il est probable que les variations de concentrations soient plus importantes à la phase précoce post-transplantation en raison de l'instabilité des fonctions d'épuration du patient notamment en transplantation hépatique puisqu'il s'agit de l'organe majeur de biotransformation et d'excrétion du tacrolimus.

La question de savoir si un CV élevé est la cause ou la conséquence de l'apparition d'une toxicité du TAC reste non résolue de façon formelle. Les dysfonctions hépatique et cardiaque pourraient ainsi être à l'origine d'une modification de la pharmacocinétique du TAC. Le foie étant l'organe majeur du métabolisme et de l'excrétion du TAC tandis que le cœur impacte le débit de l'ensemble des organes (dont le foie et l'intestin) avec un effet direct sur l'extraction vasculaire des médicaments et donc sur les phases d'absorption, de métabolisme et d'excrétion. En revanche, les dysfonctions rénales rapportées dans l'étude n'ont vraisemblablement pas d'impact sur la pharmacocinétique du TAC ce qui pourrait impliquer une relation de causalité plus probable.

De façon intéressante, un CV élevé n'était pas synonyme de surexposition comme en témoigne la valeur médiane de la concentration résiduelle de TAC qui n'était pas plus élevée dans le groupe à haut CV, confirmant ainsi les limites du dosage sanguin de la concentration résiduelle de TAC. La mesure du CV paraît donc être un marqueur plus performant permettant d'identifier les patients à risque de présenter des EI à court, moyen ou long terme.

Par conséquent, il nous paraît important d'étudier de façon plus précise le métabolisme hépatique du TAC donc au sein du greffon. L'étude du TAC excrété dans la bile chez le patient transplanté hépatique pourrait être un meilleur reflet de son exposition et de son profil de sécurité.

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## CHAPITRE 4 :

### INTERET DU DOSAGE BILIAIRE DU TACROLIMUS : ETUDE STABILE

**Interest of tacrolimus concentrations measured in excreted bile in liver transplant recipients: the STABILE study**

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## **D) CONTEXTE**

Comme nous l'avons vu dans les chapitres précédents, la concentration résiduelle sanguine (C<sub>0</sub>) de tacrolimus (TAC) est un marqueur imparfait de son profil de sécurité en transplantation hépatique (TH), particulièrement durant la période post-opératoire précoce.

Par conséquent, de nouvelles méthodes de monitoring doivent être développées afin de mieux prédire la survenue des effets indésirables (EI) du TAC.

Le TAC étant métabolisé dans le foie et éliminé dans la bile, nous avons donc émis l'hypothèse que la mesure de sa concentration dans la bile excrétée par le greffon pouvait être un marqueur intéressant. Cette mesure de concentration pourrait tout à la fois être un meilleur reflet de la fraction libre de TAC, c'est-à-dire la fraction disponible pour exercer l'effet thérapeutique du médicament en raison du faible niveau de concentrations de protéines dans la bile et également un reflet de la concentration tissulaire dans le greffon. Tous les patients transplantés au CHU de Rennes ayant un drain biliaire (drain de Kehr), il est donc aisé d'avoir accès à la bile excrétée par le foie.

Après un travail initial de mise au point de la technique de dosage, l'étude STABILE (Suivi thérapeutique pharmacologique des concentrations de tacrolimus dans la bile chez le patient transplanté) était une étude prospective (clinicaltrials.gov #NCT02820259) ayant pour objectif d'évaluer l'intérêt du dosage biliaire du TAC chez les patients transplantés hépatiques.

## II) MANUSCRIT DE L'ARTICLE PUBLIE (en Anglais)

### 1 | INTRODUCTION

Tacrolimus (TAC) is the cornerstone of immunosuppressive therapy after solid organ transplantation, especially in liver transplantation (LT). However, its narrow therapeutic range requires a strict dosage adjustment in order to avoid the occurrence of acute cellular rejection in case of insufficient drug exposure or conversely, the occurrence of adverse effects (i.e., infection, diabetes mellitus, hypertension, neurotoxicity) in case of overdose.<sup>1</sup>

In clinical practice, TAC therapeutic drug monitoring (TDM) relies on whole blood trough concentration ( $TAC_{\text{blood}}$ ). However, this surrogate marker is known to imperfectly reflect TAC immunosuppressive activity or toxicity. Indeed, in liver transplant patients, it has been shown that  $TAC_{\text{blood}}$  was not able to predict the occurrence of either acute cellular rejection<sup>2</sup> nor TAC-related adverse effects,<sup>3,4</sup> especially during the early postoperative days (POD) since the liver graft is progressively recovering its metabolic function.<sup>5</sup> This is particularly true considering TAC neurotoxicity which occurs in approximately 20%<sup>4</sup> of the patients after LT. In fact, this cannot be predicted nor treated except by temporarily TAC suspension and subsequent spontaneous regression.

Novel approaches in drug monitoring are mandatory to improve post-operative outcomes. Alternative methods such as measuring TAC concentration in peripheral blood mononuclear cells ( $TAC_{\text{PBMC}}$ ), or directly in the liver graft<sup>6,7</sup> have been explored. Although these two methods have proved to be more efficient than monitoring  $TAC_{\text{blood}}$  to assess TAC efficacy, they are more difficult to implement, are expensive and too invasive to be routinely applicable at this time.

Tacrolimus is metabolized in the liver and excreted into the bile in both unchanged and metabolized forms.<sup>8,9</sup> We then hypothesized that measuring the TAC eliminated through the bile could represent an alternative and efficient surrogate marker of the drug effect.

The aim of our study was to evaluate the interest of measuring TAC concentration in the excreted bile, collected through a T-tube during the early POD after LT, as a potential new TDM marker and to predict TAC toxicity.

## **2 | PATIENTS AND METHODS**

### **Study Design**

The STABILE study (therapeutic drug monitoring of tacrolimus biliary concentrations for liver transplanted patients) is a prospective, nonrandomized, non-interventional, single-institution study (clinicaltrials.gov#NCT02820259). The ethics committee of the Rennes University Hospital approved the study protocol (N°16.45\_20160421) and all patients were provided oral and written information regarding the study and gave their written consent.

### **Patients**

From May 2016 to April 2017, adult candidates for liver transplantation or retransplantation from a whole liver graft were recruited. Patients with associated transplantation of another organ (kidney, lung, pancreas, heart), with hepaticojejunostomy, with a contraindication for TAC, with late introduction of TAC (i.e. beyond POD 5) or with immunosuppressive treatment induction by anti-lymphocyte serum were not included.

All patients had orthotopic liver transplantation with inferior vena cava preservation. Biliary anastomosis was duct-to-duct anastomosis with systematic insertion of a T-Tube which is usually left open until POD 7-10 and then clamped and removed 4 months after LT.

After the procedure, standardised immunosuppression associating TAC, mycophenolate mofetil and a short course of corticosteroids was started usually on POD 0 or 1. TAC dosage was initiated at 0.1 mg/kg daily or at 0.06 mg/kg daily in case of concomitant administration of fluconazole, split into 2 daily intakes. TAC dosage was then adapted according to TAC<sub>blood</sub> with a target trough concentration between 6 and 10 ng/ml.

### **Study objective and endpoints**

The primary objective was to show that the tacrolimus concentration excreted into the bile (TAC<sub>bileC</sub>) could be considered as a good marker of TAC immunosuppressive activity in the early post-operative period. Therefore, the primary endpoint was to assess a correlation between TAC<sub>bileC</sub> and TAC<sub>PBMC</sub> which is currently considered as one of the best surrogate markers of TAC immunosuppressive activity,<sup>2</sup> during the first 7 days after the first administration of TAC.

The secondary objectives of the study were: i) to show that TAC<sub>bileC</sub> could be a better surrogate marker than TAC<sub>blood</sub> in clinical practice, ii) to show that TAC<sub>bileC</sub> is representative of liver graft function restoration, iii) to determine if TAC<sub>bileC</sub> could predict TAC's neurotoxicity before it occurs.

The secondary endpoints were therefore: i) to compare the correlation between TAC<sub>bileC</sub> and TAC<sub>PBMC</sub> with the correlation between TAC<sub>blood</sub> and TAC<sub>PBMC</sub>, ii) to assess the correlations between TAC<sub>bileC</sub> and liver graft function parameters, iii) to compare the TAC<sub>bileC</sub> level measured before the occurrence of neurologic complications with TAC<sub>bileC</sub> levels in patients who did not present TAC-related neurotoxicity in the very early POD (i.e. during the first 3 days after the first administration of TAC).

### **Bile collection and TAC quantification in bile**

The study period included the first 7 days after the first administration of TAC. All of the patients had daily clinical and biological examinations. The excreted bile was collected through the T-Tube over a period of 12 hours between 7 p.m. (just after the evening administration of TAC) and 7 a.m. (just before the morning administration of TAC). The collected bile therefore represented TAC excretion following the evening administration. Bile volume was also noted.

A 5-mL sample of the homogenized 12-hour bile collection was collected for  $TAC_{bileC}$  measurement. TAC bile levels were measured according to a recently published liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method.<sup>9</sup> Since the lower detection threshold was 0.2 ng/ml, patients with undetectable levels (i.e. with no excreted TAC in bile or with a concentration inferior to 0.2 ng/ml) were considered as having a concentration of 0.19 ng/ml.

The excreted TAC quantity in bile ( $TAC_{bileQ}$ ) was calculated as follows:  $TAC_{bileQ} =$  volume of bile (during the 12-hour period) \*  $TAC_{bileC}$ .

### **Blood collection and TAC quantification in blood and PBMC**

Blood samples were drawn, concomitantly with the bile, at 7 a.m. (just before administration of the drug) and enabling the measurement of both  $TAC_{blood}$  and  $TAC_{PBMC}$ .  $TAC_{blood}$  was measured with a fully validated liquid chromatography tandem mass spectrometry adapted from a previously published method.<sup>10</sup> PBMC were isolated from the same sample used for whole-blood TDM and TAC intracellular concentration was measured according to a previously published method.<sup>11</sup>

Aberrant measurements were identified by graphical visual analysis (i.e. values > 2.5 standard deviation), verified (i.e. comparison with values measured the day before and the day after) and removed if confirmed.

Since our study was qualified as non-interventional, we could not maintain the T-tube open only for the purpose of the study. However, if a patient's condition improved rapidly, the T-tube was clamped before collecting the 7<sup>th</sup> bile sample in order to avoid delaying hospital discharge. Moreover, in order to accurately measure  $TAC_{PBMC}$ , a minimal number of 500,000 PBMC were required for the cell-isolation procedure. Patients in whom the amount of PBMC in the blood sample was lower than this threshold therefore could not have their  $TAC_{PBMC}$  determined. For all of these reasons, patients could not have all 7 of the expected dosages in most cases. Finally, only patients with at least 3 analysable triplets of concentrations of  $TAC_{bileC}$ ,  $TAC_{PBMC}$  and  $TAC_{blood}$  collected the same day were included in the study.

### **Data collection**

The following data were prospectively gathered: age, gender, body mass index (BMI), indication of transplantation, MELD score at the time of transplantation.

Donor characteristics: age, gender, BMI, cause of death, cold ischemia duration.

Biologic data:  $TAC_{blood}$ ,  $TAC_{PBMC}$ ,  $TAC_{bileC}$ , bile volume (collected during the 12-h period),  $TAC_{bileQ}$ , alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), total and conjugated bilirubin, serum protein and albumin, blood ionogram (sodium, potassium, chloride), urea, creatinine level, white blood cells count, red blood cells count and haemostasis parameters (prothrombin time, activated partial thromboplastin time, INR, factor V level).

Post-operative clinical events: occurrence of TAC neurologic complication (i.e. encephalopathy, confusion and tremor) occurring after TAC introduction and improving or resolving after therapeutic adjustment, during the study period.

### **Statistics**

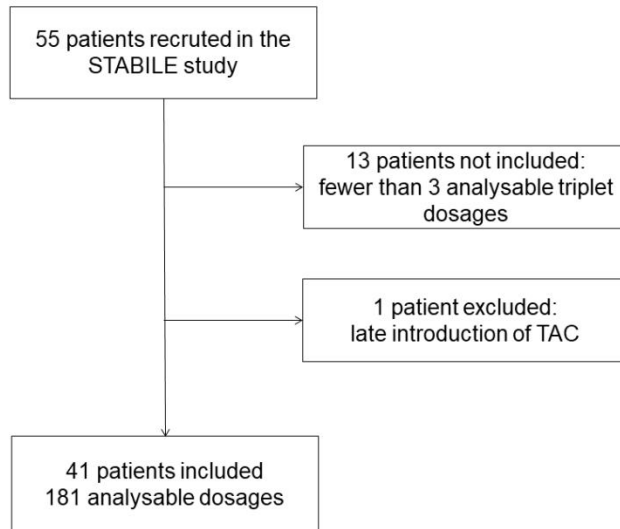
Quantitative variables were expressed as mean  $\pm$  standard deviation or by median with extreme values. Qualitative variables were expressed as number and percentage.

The correlations between TAC<sub>bileC</sub> and other parameters were evaluated with a repeated measures correlation method<sup>12</sup> in univariate analysis and with generalized linear mixed-effects models for univariate and multivariate analysis.<sup>13</sup> All variables with a p-value  $<0.1$  in univariate analysis as well as well-known clinically significant variables were included in multivariate analysis. The final model was selected using a stepwise descending procedure keeping only significant variables. Two separate multivariate models (one with TAC<sub>bileC</sub> and one with TAC<sub>blood</sub>) were created and compared by the Akaike information criterion (AIC) in order to find the best surrogate marker. A p-value  $<0.05$  was considered significant. All statistical analyses were performed with R statistical software (version 3.4.3) using the “Rmcorr” package (version 0.2.0) for repeated measures correlation, the “lme4” package (version 1.1-15) for generalized linear mixed-effects models and the ROCR package (version 1.0-7) for ROC curve analysis.

### **3 | RESULTS**

#### **Population characteristics**

Between May 2016 and April 2017, 55 patients were included in the STABILE study. Thirteen patients were excluded owing to fewer than 3 analysable concentrations (TAC<sub>bileC</sub>, TAC<sub>PBMC</sub> and TAC<sub>blood</sub> triplet collected the same day). One patient was excluded owing to late administration of TAC with induction therapy by basiliximab. Finally, 41 patients were analysed and constituted our study population (figure 1). Thirty-five (85%) were male. The median age was 58 [27-66] years. The indications for transplantation as well as the main characteristics of the recipients and donors are summarized in Table 1.



**Figure 1: flow chart of the STABILE study**

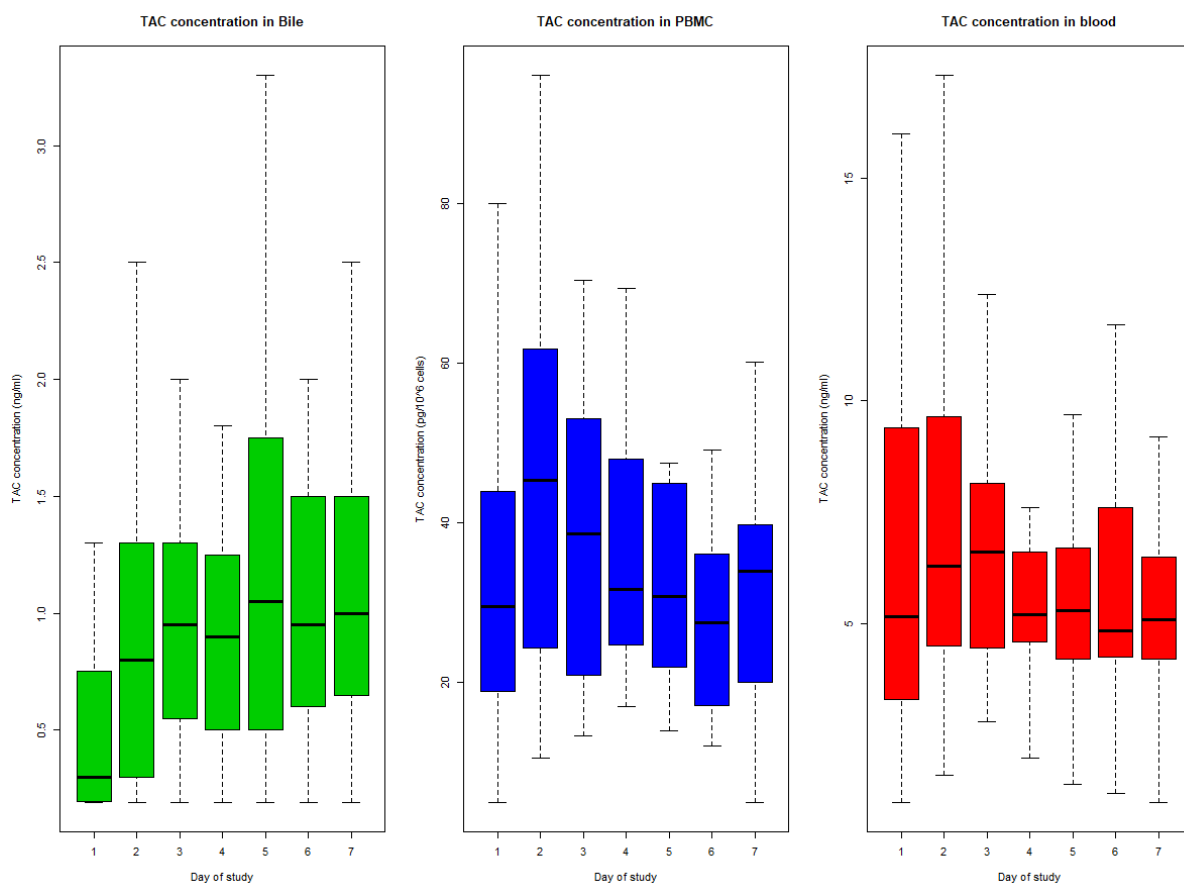
Variables	n=41
Age (years)	58 [27-65]
Sex ratio (M/F)	5.8 (35/6)
BMI	29 [19-46]
Indication for transplantation	
HCC	27 (65.9%)
Alcohol	8 (19.5%)
Other	6 (14.6%)
MELD	10 [5-35]
Donor Sex Ratio (M/F)	1.3 (23/18)
Donor age (years)	60 [10-88]
Donor BMI	24 [18-38]
Cause of donor death	
Vascular	21 (53.8%)
Trauma	9 (23.1%)
Other	9 (23.1%)
Cold ischemia time (min)	454 [279-700]
Duration of hospitalisation (days)	13 [9-56]

BMI: body mass index; HCC: hepatocellular carcinoma; MELD: model for end-stage liver disease

**Table 1: Population characteristics**



The median number of analysable triplet concentrations per patient was 5 [3-7] with a total of 182 triplet concentrations for the entire study population. One measurement of TAC<sub>bileC</sub> was considered as aberrant with a value of 6.7ng/ml and was excluded. Finally, the analysis was performed on 181 triplets. The evolution of TAC concentration in bile, blood and PBMC during the study period is shown in figure 2.



**Figure 2: Evolution of Tacrolimus concentration in bile, blood and PBMC**

### **Correlation between TAC<sub>bileC</sub> and TAC<sub>blood</sub> with TAC<sub>PBMC</sub>**

Regarding the primary endpoint, there was a significant correlation between TAC<sub>bileC</sub> and TAC<sub>PBMC</sub> with a correlation coefficient  $r=0.25$  ( $p=0.007$ ).

There was also a significant correlation relation between  $TAC_{\text{blood}}$  and  $TAC_{\text{PBMC}}$  with a correlation coefficient  $r=0.53$  ( $p<0.001$ ).

Moreover,  $TAC_{\text{bileQ}}$  was not significantly correlated with  $TAC_{\text{PBMC}}$  with a correlation coefficient  $r=0.17$  ( $p=0.08$ ).

Following the use of a generalized linear mixed-effect model, the variables significantly associated with  $TAC_{\text{PBMC}}$  are summarized in Table 2. Two variables (red blood cell count and white blood cell count) were tested in the multivariate analysis since they are well-known variables influencing TAC disposition). The 2 multivariate models are described in Table 3. The first multivariate model (Table 3a) created with the  $TAC_{\text{bileC}}$  ( $p<0.001$ , OR= 1.26) also included a TAC oral dose ( $p=0.003$ , OR=1.23) and GGT level ( $p=0.004$ , OR=0.998) and had an AIC value of 1247. The second multivariate model (table 3b) created with  $TAC_{\text{blood}}$  ( $p<0.001$ , OR=1.15) also included red blood cell count ( $p=0.005$ , OR=0.79) with an AIC of 1185. Thus, the model with  $TAC_{\text{blood}}$  better predicted  $TAC_{\text{PBMC}}$  than the model with  $TAC_{\text{bileC}}$ .

Variables	Univariate analysis (p)
<u>Recipient characteristics:</u>	
Age	0.35
Sex	0.83
BMI	0.48
MELD	0.91
<u>Donor characteristics:</u>	
Age	0.46
Sex	0.63
BMI	0.72
Cause of death	0.81
Cold ischemia time	0.40
<u>Tacrolimus:</u>	
TAC <sub>blood</sub>	<0.001
TAC <sub>bileC</sub>	<0.001
Oral dose	0.008
<u>Liver biological tests:</u>	
INR	0.38
Factor V	0.37
Total bilirubin	0.91
AST	0.09
ALT	0.06
ALP	0.37
GGT	0.01
<u>Blood tests:</u>	
Red blood cell count	0.67
White blood cell count	0.35
Neutrophils	0.17
Eosinophils	0.22
Basophils	0.24
Lymphocytes	0.65
Serum protein level	0.78
Albumin	<0.001
<u>Interracting drug:</u>	
Fluconazole	0.46
Amlodipin	0.33

ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; BMI: body mass index; GGT: Gamma-glutamyl transferase; INR: international normalized ratio; MELD: model for end-stage liver disease; TAC<sub>bileC</sub>: tacrolimus concentration excreted in the bile; TAC<sub>blood</sub>: tacrolimus whole blood trough concentration.

**Table 2: Univariate analysis of factors influencing tacrolimus concentration in peripheral blood mononuclear cells**

Variables	Multivariate (Generalized Linear Mixed Model)		
	p	estimator	[CI 95%]
TAC bile concentration	<0.001	1.26	[1.13-1.42]
TAC oral dose	0.003	1.23	[1.08-1.42]
GGT level	0.004	0.998	[0.997-0.999]

A

b)

Variables	Multivariate (Generalized Linear Mixed Model)		
	p	estimator	[CI 95%]
TAC trough concentration	<0.001	1.15	[1.12-1.17]
RBC count	0.005	0.79	[0.68-0.93]

B

TAC: tacrolimus; GGT: Gamma-glutamyltransferase; RBC: red blood cells;

**Table 3: Multivariate analysis of factors influencing tacrolimus concentration in PBMC. a) model created with tacrolimus concentration in bile with an AIC value of 1247, b) model created with tacrolimus whole blood trough concentration with an AIC value of 1185**

#### Relationship with graft function tests (Table 4)

TAC<sub>bileC</sub> was significantly correlated with the biological parameters of liver graft function such as factor V ( $r=0.40$ ,  $p=0.009$ ), INR ( $r= -0.31$ ,  $p=0.003$ ), prothrombin time ( $r=0.36$ ,  $p<0.001$ ) and total bilirubin level ( $r=0.21$ ,  $p=0.01$ ). TAC<sub>bileC</sub> was also significantly correlated

with biliary function tests such as ALP ( $r=0.19$ ,  $p=0.03$ ) and GGT ( $r=0.24$ ,  $p=0.004$ ). However, the correlation was not significant with liver injury parameters (i.e. liver enzymes) such as AST ( $r=-0.17$ ,  $p=0.052$ ) or ALT ( $r=-0.11$ ,  $p=0.17$ ).

Conversely, neither  $TAC_{\text{blood}}$  nor  $TAC_{\text{PBMC}}$  was significantly correlated with liver graft function parameters while  $TAC_{\text{blood}}$  was significantly correlated with both AST ( $r=0.24$ ,  $p<0.001$ ) and ALT ( $r=0.25$ ,  $p<0.001$ ).

Variables	TAC-bileC		TAC-blood		TAC-PBMC	
	r	p	r	p	r	p
Factor V	0.40	0.009	0.25	0.06	0.08	0.63
INR	-0.31	0.003	0.12	0.08	0.06	0.55
Prothrombin Time	0.36	<0.001	-0.07	0.35	-0.004	0.98
Total bilirubin level	0.21	0.01	0.12	0.10	-0.01	0.91
ALP	0.19	0.03	-0.04	0.60	-0.16	0.08
GGT	0.24	0.004	-0.14	0.04	-0.20	0.03
AST	-0.17	0.052	0.24	<0.001	0.14	0.13
ALT	0.11	0.17	0.25	<0.001	0.20	0.03

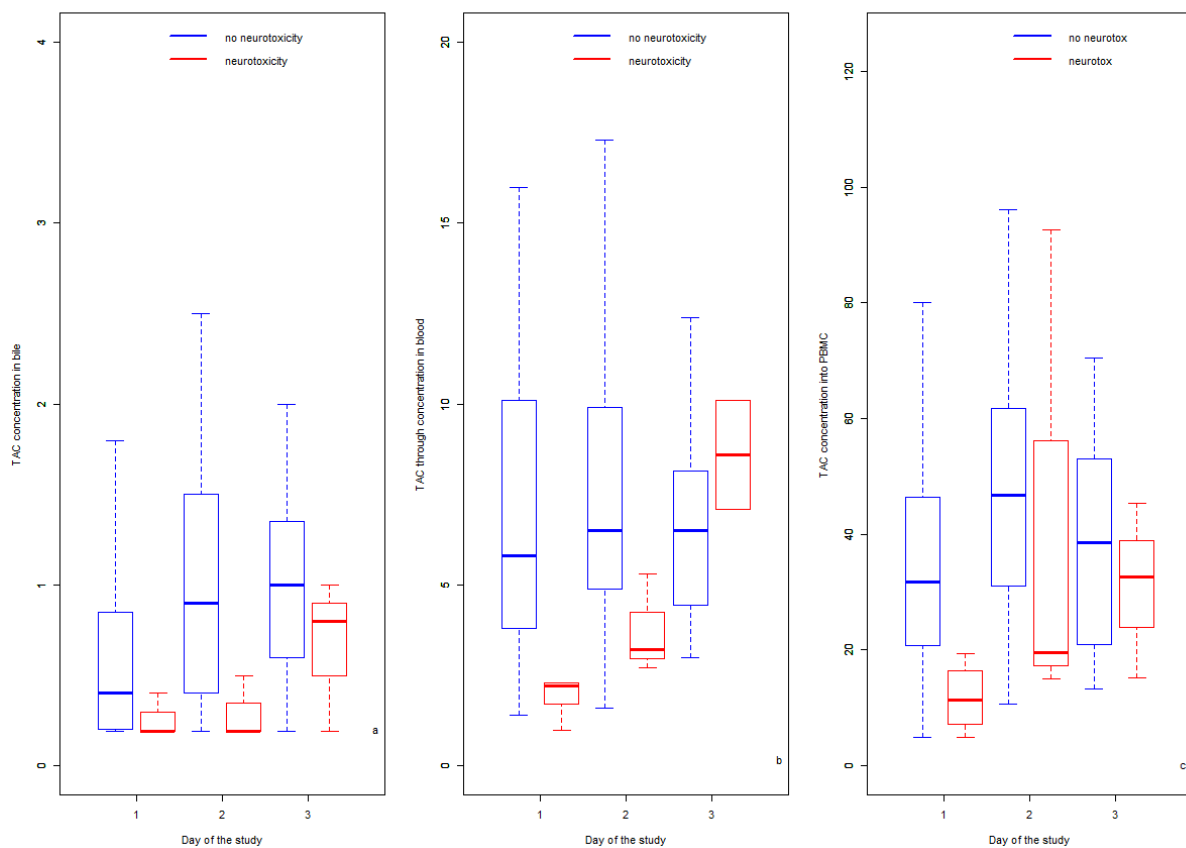
ALT: alanine aminotransferase; ALP: alkaline phosphatase; AST: aspartate aminotransferase; GGT: gamma glutamyl transferase; r: repeated measures correlation coefficient TAC-bileC: tacrolimus concentration in bile; TAC-blood: tacrolimus trough concentration in blood; TAC-PBMC: tacrolimus concentration in peripheral blood mononuclear cells.

**Table 4: correlation between TAC concentration in different matrices and post-operative liver parameters**

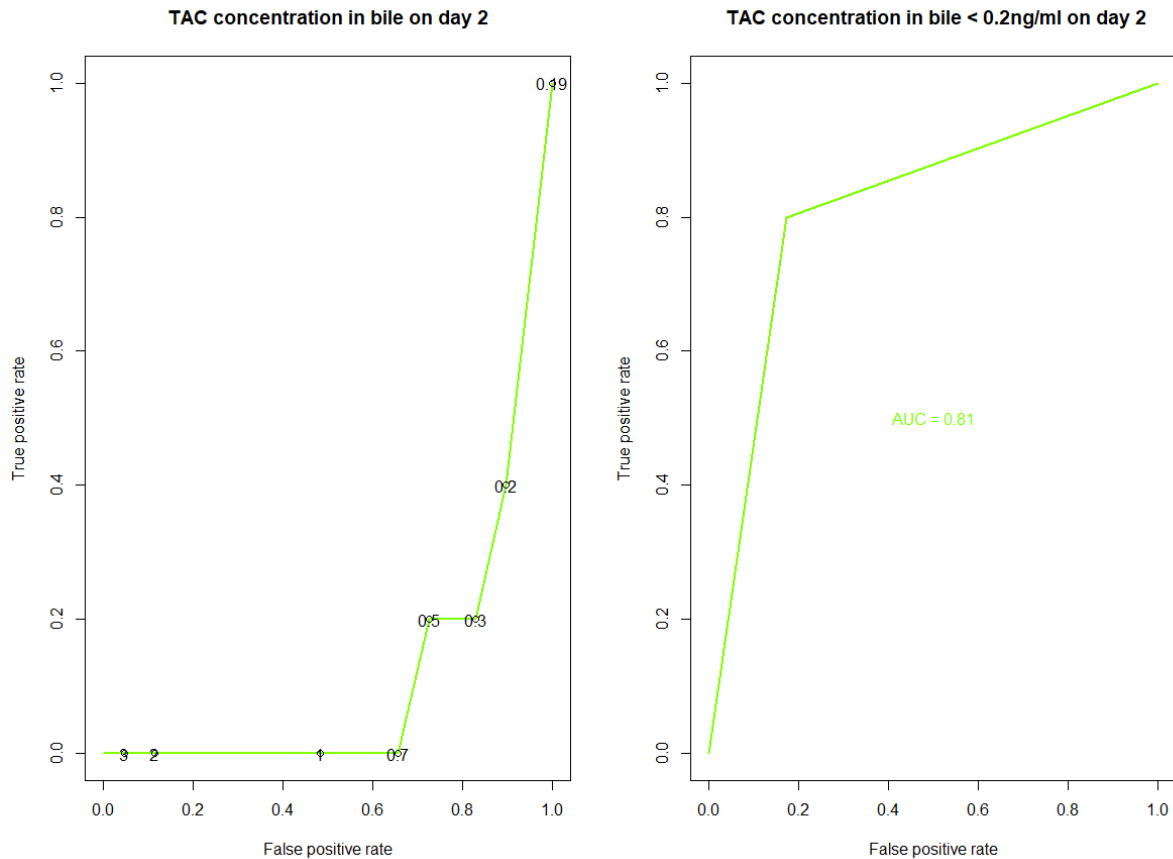
### Relationship with occurrence of TAC neurotoxicity

During the study period, 5 (12%) patients with a median age of 54 [36-64] years and a median MELD of 23 [5-35] presented TAC-related toxicity (mostly confusion and encephalopathy)

on days 1, 3, 4, 4 and 6 after the beginning of treatment. None of these patients had pre-LT encephalopathy higher than grade 2. Before the occurrence of neurological toxicity, the  $TAC_{bileC}$  level was significantly lower ( $p < 0.001$ ) in the patients presenting neurological complications compared with those who did not (figure 3a). Moreover, the levels of  $TAC_{blood}$  and  $TAC_{PBMC}$  were not increased in the patients presenting neurological complications (figure 3b-3c). The ROC curve analysis showed that a  $TAC_{bileC}$  level less or equal to 0.20 ng/mL (i.e., the lower detection threshold) day 2 after TAC administration was predictive of TAC neurologic complication occurrence with an AUC of 0.81 (figure 4).



**Figure 3: Tacrolimus concentration in different matrix in patients presenting neurotoxicity or not**



**Figure 4: ROC curves analysis of occurrence of neurological complication according to TAC concentration in bile at day 2 after treatment instauration**

#### 4 | DISCUSSION

The STABLE study aimed at evaluating the interest of measuring TAC concentration in bile. In the present study, we found that TAC concentration measured directly in a patient’s bile and collected non-invasively (i.e. through a T-tube) was significantly correlated with TAC concentrations in PBMC (considered as one of the best available biomarkers to assess TAC efficacy). One could therefore infer that  $TAC_{bileC}$  is also a good surrogate marker of TAC efficacy. Indeed,  $TAC_{bileC}$  could be a good reflection of both the intrahepatic concentration of TAC which has been shown to be significantly correlated with the incidence of acute cellular rejection<sup>2</sup> as well as to the unbound fraction of the drug (i.e. the active fraction of the drug). This finding is supported by the fact that TAC is eliminated through biliary excretion.<sup>8</sup>

Indeed, after intake, TAC is widely bound to proteins and the free fraction available to interact with the target cells (i.e. lymphocytes) is less than 1%. The free fraction may distribute to lymphocytes and hepatocytes where unbound TAC can be metabolized and minimally freely eliminated unchanged through biliary excretion. Since there are few proteins in bile and because free fraction in different cell types obeys pharmacokinetic equilibrium,  $TAC_{bileC}$  appears to be a good surrogate for free and intracellular TAC concentrations.

However, we also found that  $TAC_{blood}$  was significantly correlated with  $TAC_{PBMC}$ . Multivariate analysis showed that  $TAC_{blood}$  was better correlated with  $TAC_{PBMC}$  than  $TAC_{bileC}$ . This finding suggests that  $TAC_{bileC}$  could be of limited interest in TAC dosage adjustment compared with  $TAC_{blood}$ .

An interesting finding of our study is the fact that  $TAC_{bileC}$  was found to be correlated with liver graft function parameters which could help predict the occurrence of TAC neurologic toxicity. We also found that a  $TAC_{bileC}$  level lower than 0.20 ng/ml on day 2 (which reflects an absence of detection of TAC excretion in bile by our method) was highly predictive of the occurrence of neurologic complications with an AUC of 0.81. In our study, neurological toxicity mostly occurred beyond day 3 following TAC administration, therefore  $TAC_{bileC}$  levels measured on day 2 could represent the first biological parameters capable of predicting the occurrence of early TAC neurological toxicity. Thus, when a T-tube is placed, systematic measurement of  $TAC_{bileC}$  on day 2 may alert the clinician of the occurrence of neurological toxicity which often requires the discontinuation of treatment and exposes patients to TAC concentration variability which has previously been shown to be deleterious to patient outcome by our team.<sup>14</sup> On the contrary, we found that  $TAC_{blood}$  and  $TAC_{PBMC}$  were not correlated with liver graft function parameters and were not increased in patients presenting neurologic toxicity as one could have expected.



In addition, the absence of TAC excretion in bile associated with a lack of an increase in blood trough concentration led us to hypothesise that rather than TAC itself, neurologic toxicity could be related to accumulation of one of its metabolites. This hypothesis is probably the most interesting part of our study and is consistent with TAC pharmacokinetics. Indeed, since TAC is highly metabolized in the liver and then mostly eliminated through biliary excretion, a low TAC excretion in bile could also testify to an absence of TAC metabolite excretion which could accumulate in the blood. This accumulation could lead to neurotoxicity since it has already been shown that some metabolites could have pharmacological activities.<sup>15</sup> This hypothesis also explains why patients presenting neurological issues often have normal TAC blood trough concentrations. Indeed, since TAC whole blood trough concentration is mostly influenced by TAC fraction present into erythrocytes, an alteration of the excretion function of TAC (and its metabolites) resulting in an increase in TAC metabolites concentration in blood (potentially responsible for adverse events) may not result in TAC whole blood trough concentration modification. Unfortunately, we could not verify this hypothesis in our center since there is currently no analytical assay available for developing a TAC metabolite quantification method like previously reported<sup>15</sup>.

To our knowledge, this pilot study is the first to investigate the interest of measuring TAC<sub>bileC</sub> in liver-transplanted patients. Our results must therefore be interpreted with caution.

The limitations to our study are as follows: our population and the number of samples analysed were limited. Our results must therefore be confirmed in a larger cohort before being able to provide clinical recommendations. Secondly, we collected the bile over a 12-hour period with the risk of not collecting all of the bile owing to digestive lumen passage. However, Lenzen et al.<sup>16</sup> demonstrated that a T-tube is capable of collecting virtually all excreted bile. Moreover, as we used concentration and not quantity, we think that even if a small amount of bile was not collected, it may not significantly change our results. Thirdly,

the efficiency of the TAC drug monitoring in bile could be questionable since it was not applied in clinical practice. However, our team developed and validated a simple, efficient and reproducible method for the quantification of tacrolimus in human bile<sup>9</sup>.

Obviously, measuring TAC in excreted bile is only possible when a biliary drainage is placed, which is systematically performed in our centre, allowing us to have an easy, non-invasive and repeated access to excreted TAC in bile which we believed is a good reflect of TAC exposure in the liver graft. However, the purpose of our study was not to be a plea for T-tube insertion which is not the most widespread practice in liver transplantation procedure and is not possible in other solid organ transplantations. Therefore, using TAC<sub>bileC</sub> as a new TDM method may be limited to that kind of transplantation.

Another potential criticism is that we choose to only focus on neurological toxicity with clinical impact and not other adverse events. We justify this decision by considering that TAC-related neurotoxicity is the most specific and severe of its adverse events. Indeed, unlike neurological complications, other adverse TAC events (i.e. diabetes, hypertension, infection, haematologic toxicity, acute renal failure) could be related to other immunosuppressive treatments such as corticosteroids (for diabetes and hypertension) or mycophenolic acid (for infection or hematologic toxicity) or to surgical bleeding or insufficient post-operative fluid management (for acute renal failure). However, in order to avoid bias due to clinical examination, a standardized evaluation and classification of TAC-related neurotoxicity should be performed.

Following the same logic, we decided to choose TAC<sub>PBMC</sub> as the reference parameter instead of the incidence of acute cellular rejection because its incidence remains low and requires an invasive exam (i.e. liver biopsy) to establish the diagnosis.

## 5 | CONCLUSION

In conclusion, the STABILE study is the first clinical study to evaluate the potential interest of TAC concentration measured in bile after LT. Our results suggest that TAC concentration in bile could be used as a surrogate marker for the immunosuppressive effect of TAC but it is not as efficient as  $TAC_{\text{blood}}$  and is therefore of limited interest. Moreover, low TAC excretion in bile on day 2 could be the first biological parameter capable of predicting the occurrence of neurological toxicity and could then help the clinician adjust TAC dosage in order to prevent TAC toxicity.

### III) SYNTHÈSE

L'étude STABILE a permis de développer et valider une technique innovante de dosage du TAC dans la bile et a suggéré que la mesure de concentration biliaire de TAC pourrait présenter un intérêt dans la prédiction des effets indésirables du TAC. En revanche, en prenant la mesure de concentration de TAC dans les cellules mononuclées du sang périphérique comme marqueur substitutif de l'effet immunosuppresseur du médicament, la concentration biliaire s'est montrée moins bien corrélée aux effets que la concentration sanguine. L'hypothèse d'un lien entre concentration biliaire de TAC, concentration libre de TAC et/ou concentration tissulaire (intra-greffon) de TAC n'a pas été vérifiée. Les concentrations biliaires pourraient en revanche être reliées à la survenue d'évènements neurologiques.

L'observation la plus importante est probablement le fait que la survenue des EI du TAC est caractérisée par une absence de surexposition sanguine au TAC, puisque les concentrations résiduelles sanguines ne sont pas plus élevées chez les patients qui vont développer ces EI, ainsi qu'une diminution de son excrétion dans la bile. Ceci nous amène à formuler l'hypothèse que la toxicité du TAC n'est pas dû à la molécule mère mais possiblement à un de ses métabolites dont le dosage (à la fois dans le sang et la bile) présenterait un intérêt particulier. En effet, le TAC étant majoritairement excrété sous forme de métabolites, il est possible qu'une diminution de la clairance biliaire du TAC, laquelle ne conduit pas à une augmentation importante des concentrations sanguines soit, en revanche, associée à une accumulation plus importante des métabolites. Cette accumulation pourrait alors être à l'origine des effets indésirables neurologiques décrits chez le patient transplanté hépatique en post-greffe immédiat.

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## **CHAPITRE 5 :**

### **PERSPECTIVES ET CONCLUSION**

## **I) RESUME DES DIFFERENTS TRAVAUX ET PERSPECTIVES**

### **A) Rappel du contexte**

La découverte et l'utilisation de traitements immunosuppresseurs (IS) efficaces ont joué un rôle prépondérant dans l'essor et le succès de la transplantation hépatique (TH). Ainsi, parallèlement à l'amélioration des techniques chirurgicales, l'utilisation des inhibiteurs de la calcineurine, dont le tacrolimus (TAC) est actuellement le représentant principal, a permis de ne plus craindre les complications immunologiques.

Par conséquent, la morbidité post-transplantation est désormais principalement représentée par les complications liées au traitement IS et principalement au TAC. Le suivi thérapeutique pharmacologique du TAC repose sur la mesure des concentrations résiduelles sanguines du TAC qui est un marqueur imparfait de son efficacité et de sa toxicité. Durant la phase précoce, ceci pourrait être en partie dû à la reprise progressive de la fonction du greffon (et donc du métabolisme du TAC) au cours des premiers jours post-TH.

Partant de ce constat, notre travail avait pour objectif d'optimiser l'utilisation du tacrolimus chez le patient transplanté hépatique à l'aide de méthodes innovantes et d'apporter de nouveaux éléments permettant de comprendre les mécanismes physiopathologiques de sa toxicité.

### **B) Minimisation des objectifs de concentration résiduelle de tacrolimus**

La première piste d'amélioration était de minimiser la cible de concentration résiduelle de TAC. Ainsi, dans le chapitre 2, nous avons démontré qu'une concentration résiduelle cible comprise entre 4-7 ng/mL était un objectif atteignable dans le suivi des patients en permettant

le maintien de l'efficacité du traitement et que ce niveau d'exposition pouvait s'avérer bénéfique pour le patient en termes de réduction des effets indésirables. Cette étude rétrospective monocentrique ne faisait que transcrire les pratiques mises en œuvre dans notre service durant les dernières années. Il nous semblait cependant important d'évaluer et de valider cette attitude thérapeutique. Ce travail vient également combler un manque en termes de niveau de preuve puisque les travaux reliant un tel niveau de minimisation des concentrations de TAC à des évènements cliniques sont peu nombreux dans la littérature scientifique et ne concernent généralement qu'un nombre extrêmement réduit de patients.

Les résultats de notre étude devront être confirmés idéalement au travers d'une étude prospective randomisée afin de pouvoir évaluer efficacement l'incidence des effets indésirables du TAC ainsi que les conséquences immunologiques de cette stratégie de minimisation « extrême ».

### C) Etude de la variabilité intra-individuelle des concentrations de tacrolimus

Dans le chapitre 3, nous avons montré que la variabilité intra individuelle (IPV) représentée par le coefficient de variation (CV) des concentrations sanguines résiduelles de TAC était un marqueur intéressant dans le suivi thérapeutique pharmacologique du TAC. Partant du principe que la période postopératoire précoce (durant le premier mois post TH) était capitale, nous avons décidé d'étudier l'IPV durant cette période et montré son association avec la survenue des effets indésirables précoces du TAC ainsi que la survie à long terme.

Ces 2 observations vont être le point de départ d'explorations plus approfondies.

Ainsi, durant la période précoce, il semble important de tout mettre en œuvre afin de minimiser l'IPV. Les patients identifiés comme à risque d'IPV important (c.à.d. ceux présentant une insuffisance hépatique importante) pourraient bénéficier d'une stratégie



adaptée avec une titration progressive des posologies de TAC afin de diminuer le risque de survenue de ses complications. L'hypothèse avancée est que les patients dont l'IPV est élevée sont des patients pour lesquels le traitement doit être suspendu temporairement en raison d'effets indésirables, période à laquelle peut succéder une phase de sous-exposition en raison du délai de reprise du traitement. L'alternance de ces phases de surexposition, entraînant des lésions iatrogènes, et de phases de sous-exposition à l'origine d'une réactivation immunitaire et de nouvelles lésions, pourrait alors expliquer les complications cliniques et la moindre survie de ces patients. Ces variations de concentrations pourraient être liées au génotype des patients et notamment à celui du CYP3A5. Une analyse pharmacogénétique du CYP 3A5 pourrait ainsi permettre d'adapter les posologies de TAC initialement administrées au patient afin d'atteindre plus rapidement l'équilibre thérapeutique et ainsi diminuer le CV.

Concernant le retentissement à plus long terme, il serait intéressant d'étudier l'évolution du CV au-delà du 1<sup>er</sup> mois post transplantation ainsi que les conséquences immunologiques tardives sur le greffon ou encore en termes de récurrence de tumeur hépatique. Enfin, les patients ayant présentés un CV élevé durant le premier mois pourraient bénéficier d'une surveillance pharmacologique intensifiée avec calcul de l'AUC du TAC et détermination d'une cible de concentration personnalisée.

#### D) Intérêt du dosage biliaire du tacrolimus

Dans le chapitre 4, l'étude STABILE nous a permis de montrer qu'il était possible de mesurer les concentrations de TAC dans la bile. Ce nouveau marqueur pharmacologique apparaît peu intéressant dans le suivi pharmacologique de l'efficacité du TAC car moins bien corrélé à la concentration intra-PBMC que la concentration résiduelle sanguine. En revanche, il semble plus prometteur dans la prédiction de la neurotoxicité du TAC. De plus, les résultats de cette

étude nous amènent à nous poser la question de l'origine de la toxicité du TAC et notamment du rôle éventuel de ses métabolites.

Ceci sera prochainement exploré dans l'étude STABILE-2 (Intérêt du dosage sanguin et biliaire des métabolites du Tacrolimus dans la prévision de la survenue des effets indésirables liés à sa toxicité). Cette étude prospective, monocentrique, observationnelle, non randomisée, (financée par l'appel d'offre de recherche translationnel du CHU de Rennes, CORECT 2020) aura pour objectif d'étudier l'implication des métabolites du TAC dans sa toxicité. Pour cela les concentrations sanguines des métabolites du TAC (le 13-O-desmethyl-tacrolimus, le 15-O-desmethyl-tacrolimus et le 31-O-desmethyl-tacrolimus) seront mesurées dans le sang et dans la bile, durant les 5 premiers jours post-TH et comparées entre les patients présentant une toxicité neurologique et/ou rénale imputable au TAC et les patients ne présentant pas de complications.

## II) CONCLUSION

Ce travail de thèse avait pour objectif d'optimiser l'utilisation du TAC chez le patient transplanté hépatique.

Cet objectif est loin d'avoir été atteint.

Cependant, par l'intermédiaire de 2 études rétrospectives et une étude prospective, nous avons apporté des éléments permettant de modifier nos pratiques et ouvert plusieurs pistes dans la compréhension des mécanismes de la toxicité du TAC.

En conclusion, nous pouvons dire que bien que très largement utilisé depuis les années 1990, il existe encore une importante marge d'amélioration dans l'utilisation et le suivi pharmacologique du TAC du tacrolimus.

**Titre : Optimisation de l'utilisation du tacrolimus chez le transplanté hépatique**

**Mots clés :** Transplantation hépatique, Tacrolimus, Bile, variabilité intra individuelle, métabolites

**Résumé :** L'efficacité des traitements immunosuppresseurs a permis l'essor et le succès de la transplantation hépatique. Ainsi, l'utilisation des inhibiteurs de la calcineurine, dont le tacrolimus est le représentant principal, a permis une diminution drastique de la fréquence des complications immunologiques. En revanche, les effets indésirables liés à son utilisation représentent aujourd'hui une part importante de la morbidité post-transplantation. Partant de ce constat, l'objectif de notre travail était d'optimiser l'utilisation du tacrolimus chez les patients transplantés hépatiques à l'aide de méthodes pharmacologiques innovantes et d'explorer les mécanismes physiopathologiques de sa toxicité.

Un premier axe de recherche a été d'évaluer une stratégie de minimisation extrême de la cible de concentration résiduelle sanguine du tacrolimus permettant ainsi de conforter cette attitude thérapeutique. Une seconde piste a permis de mettre en évidence l'importance de la variabilité intra-individuelle des concentrations de tacrolimus durant la phase précoce comme marqueur de la survenue de ses effets indésirables et de la survie à long terme des patients. Enfin, l'étude de la mesure des concentrations de tacrolimus dans la bile nous a permis d'apporter des pistes sur les mécanismes potentiels de sa toxicité.

Les résultats de ces différents travaux nous ont donc permis d'améliorer notre pratique clinique et d'ouvrir plusieurs pistes dans la compréhension des mécanismes de la toxicité du tacrolimus.

**Title :** Optimisation of tacrolimus utilization in liver transplanted patients

**Keywords :** Liver transplantation, Tacrolimus, Bile, Inpatient variability, metabolites

**Abstract:** The efficacy of immunosuppressive drugs has led to the development and success of liver transplantation. Indeed, use of calcineurin inhibitors, of which tacrolimus is the main drug, has allowed an important decrease in the frequency of immunological complications. On the other hand, the adverse effects associated with its use now represent a significant proportion of post-transplant morbidity. Based on this observation, the objective of our work was to optimize the use of tacrolimus in liver transplant recipient using innovative pharmacological methods and to explore the mechanisms of its toxicity.

A first way of research was to evaluate a policy of extreme minimization of tacrolimus target blood trough concentration, thus confirming this therapeutic policy. The second part highlights the importance of the intra-patient variability of tacrolimus trough concentrations during the early phase as a marker of the occurrence of its adverse effects and of patient's the long-term survival. Finally, the measurement of tacrolimus concentrations in bile provides us new hypotheses on the mechanisms of its toxicity.

The results of these studies helped us to improve our clinical practice and to explore the mechanisms of tacrolimus toxicity.