THERAPEUTIC AND PHARMACOLOGICAL MONITORING OF TACROLIMUS IN RENAL TRANSPLANT PATIENTS IN C.H.U TIZI OUZOU, ALGERIA.

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I- INTRODUCTION

Immunosuppressants are now an essential treatment in the prevention of transplant rejection. Tacrolimus a calcineurin inhibitor, works by blocking the production of interleukin 2 by T cells and is currently the cornerstone of immunosuppressive treatment, but its side effects are many and varied (hypertension, diabetes , nephrotoxicity,...). The nephrotoxicity of tacrolimus is a particularly serious problem. Therapeutic and pharmacological monitoring of tacrolimus is a key element in the therapeutic management of renal transplant patients, allowing individual adaptation of prescribing to maintain drug exposure within a predefined concentration range (therapeutic targets) and thus improve efficacy while limiting toxicity. The determination of residual concentrations, is still widely used as a guide for individualizing tacrolimus doses.

II- OBJECTIF

The objective of this study is to put forward the interest of therapeutic monitoring of tacrolimus in the optimization of the management of patients after renal transplantation. This study investigates the variations of the residual level of tacrolimus according to the dose and the patients (variability intra-individual and inter-individual).
The monitoring is initially based on the determination of residual levels of tacrolimus by an immunological technique (CMIA); secondarily on the evaluation of biological profiles.

III- MATERIELS AND METHODS

Type of study and population studied

▪This study is a descriptive prospective study conducted on a random sample of 34 renal transplant patients followed by the Nephrology Department of the Nedir Mohamed Hospital in Tizi Ouzou over a 4-month period from December 2017 to April 2018.
▪ The population consisted of 18 men and 16 women with age extremes ranging from 15 to 55 years and depending on the post-transplant delay, these patients were divided into two groups: post-transplant delay of 0 to 6 weeks (n=7); post-transplant delay of over 6 weeks (n=27). the total number of samples is 160.

Parameters studied

TACROLIMUS

- Whole blood
- E.D.T..A. Tube
- Equilibrium time: 48 hours on average.
- Sampling just before taking tacrolimus **(Residual concentration)**

BIOLOGICAL PARAMETERS

- Determination of urea, creatinine, uric acid, ASAT, ALAT and total bilirubin and calculation of G.F.R.
- Heparinized tube
- Sampling in the morning at the same time as tacrolimus and on an empty stomach.

Techniques

TACROLIMUS

- **Manual pre-treatment step:**
- Extraction of tacrolimus from the whole blood sample using a precipitation reagent and centrifugation. The supernatant is decanted into a pre-treatment tube intended for this purpose.
- **CMIA method on immunoanalysis automaton Architect I1000SR:**
- Paramagnetic microparticles coated with anti-Tacrolimus or anti-Ciclosporin antibodies
- Competition between the molecule present in the sample and the molecule labelled with acridinium present in the reaction mixture.
- Washing, activation and dosage of the resulting chemiluminescence intensity.

BIOLOGICAL PARAMETERS

- The assays were performed on ARCHITECT plus ci4100.

IV- RESULTS

Table 1: Therapeutic intervals of tacrolimus

Post-transplant period	Residual concentration of tacrolimus C0 (ng/ml)
From 0 to 6 weeks	10-15
Beyond 6 weeks	5-10
Toxicity	>20

Table 02: Parameters of tacloremia distribution by post-transplant delay.

Post-transplant period	From 0 to 6 weeks	Beyond 6 weeks
Number of patients (n)	6	29
Number of samples	32	128
Averages	13.89	7.02
Deviations	4.5	3
1st quartile	11.2	5
Median	12.8	6.25
3rd quartile	16.8	8.22
Minimum	6	3
Maximum	23.3	19.4

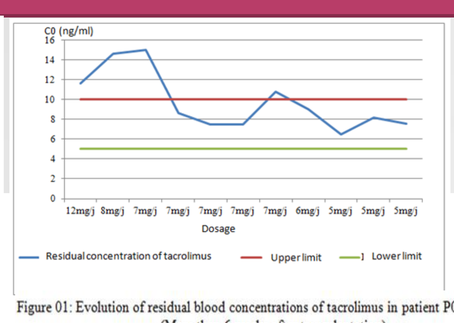
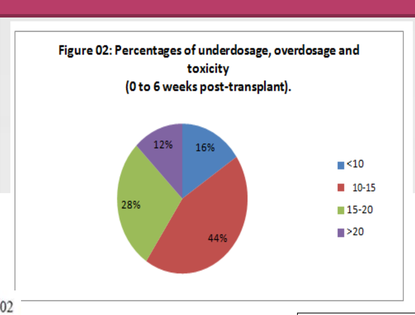
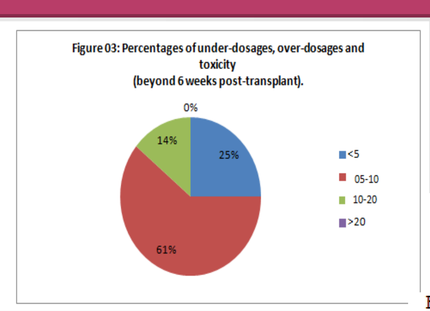


Figure 01: Evolution of residual blood concentrations of tacrolimus in patient P02 (More than 6 weeks after transplantation).





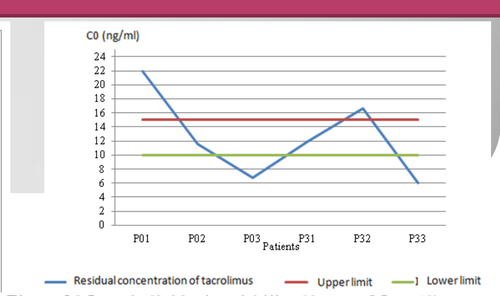


Figure 04: Inter-individual variability (dosage: 12 mg/d).

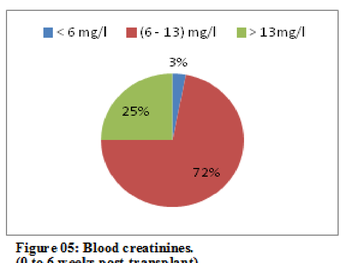


Figure 05: Blood creatinines. (0 to 6 weeks post-transplant)

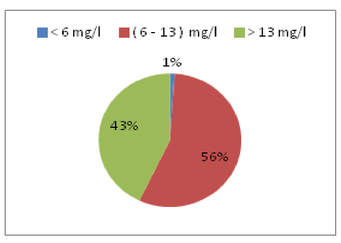


Figure 06: Blood creatinines. (After 6 weeks post-transplant).

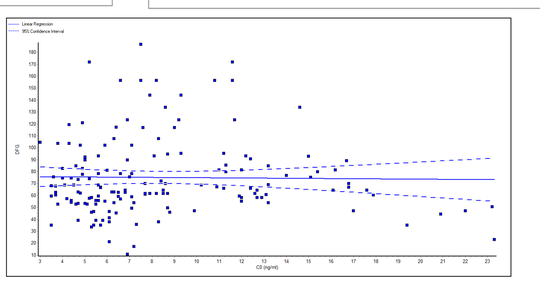


Figure 07: Correlation between residual blood concentrations of tacrolimus and GFR.

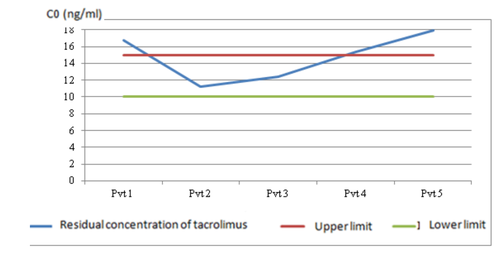


Figure 08: Intra-individual variability of the patient P32 (dosage: 3 mg/d).

V- DISCUSSION

Analysis of population
The population in this study is a young population aged 15 to 55 years old that meets the criteria for selecting patients for kidney transplantation and in which 97% of the patients showed a favorable evolution. Clinically, 68% of the patients in this study developed complications beyond 6 weeks post-transplant, including 18% hypertension, 12% diabetes and 15% anemia. These complications are major side effects of tacrolimus but direct imputability to tacrolimus remains difficult to determine. [1]

Therapeutic monitoring of tacrolimus
The study of all samples from patients in the 0-6 weeks post-transplant group shows that 43.75% of the results are within the norms and 60.94% of the results from patients over 6 weeks post-transplant are within the norms.
In the 0 to 6 week post-transplant group, 16% are below the norms and 28% are above the norms (toxic concentrations not included). On the other hand, 12% of the results corresponding to 4 samples from 2 patients exceed the toxicity level. The C0 of these 4 samples correspond to the first dosages after transplantation. A therapeutic adjustment was made and allowed a return to normal values.
In the group above 6 weeks post-transplant, no results above the toxicity threshold were observed. Indeed, 14% are below the standards and 25% are above the standards.
All these results reflect the efficiency of monitoring in adjusting doses to prevent any risk of underdosage (risk of rejection) and overdosage (risk of toxicity).

Study of inter-individual variability
The study of inter-individual variability confirmed the existence of fluctuations in the residual blood concentrations of tacrolimus between patients in the same dosage and in the same post-transplant period. The inter-individual variations are important, which is compatible with the idea that the genetic profile is one of the determinants of the response to treatments, that is their pharmacokinetic and pharmacodynamic characteristics (efficacy and undesirable effects). [2,3]

Study of intra-individual variability
Identification of the causes of unexpected concentrations can be multiple and can be explained in a relevant way for some patients. For the patient (P32) a sudden rise in the concentration of tacrolimus from sampling 3 can be explained the introduction of omeprazole which is an enzyme inhibitor that contributes to the increase in blood levels of tacrolimus.

Biological monitoring
Mean creatinine elevations have been observed in patients with infections that may be complicated by sepsis, septic shock and acute renal failure. It should be noted that tacrolimus concentrations in these patients at the time of these increases in creatinine levels were within the norms, which precluded the toxicity of tacrolimus being the cause. The coefficient of correlation between GFR and tacrolemia is not significant (P value: 0.83). Indicating that there is no correlation. However, due to the nephrotoxic potential of tacrolimus, it is recommended to closely monitor renal function. All patients had ASAT and ALAT blood concentrations in the norms, only one patient (P05) had 2 samples during his monitoring 5 times higher than the norm. This may be due to the metabolic effects of tacrolimus but the diagnosis of imputability is difficult to prove.

Study of special cases
Three special cases were studied, namely one case of rejection (P06), one case of toxicity (P34) and one case of pregnancy (P27). During our study, only one case of irreversible acute rejection was observed 2 months after the renal transplant with patient P06. Indeed, clinically the patient had a high fever with elevated CRP and a very disturbed renal function (GFR <10, creatinine levels up to 117 mg / l) while the values of tacrolemias were normal. Therefore, it seems impossible to establish a possible link with an underdose. We also found a case of toxicity in a new transplant (P34) who had toxic residual blood concentrations of tacrolimus up to 48.2 ng / ml and this since the first assay (9 days after transplant) that corresponds to the first introduction of tacrolimus. The return to normal values was not observed despite the decrease in tacrolimus dosages from 12 mg / d to 3 mg / d. Nevertheless no disturbance of renal function was noted (normal cretininemia and GFR> 60). This toxicity of tacrolimus can be explained by pharmacogenetics (rapid absorption and slow metabolism of the patient) and thus the maintenance of high blood levels. A switch to another immunosuppressant (ciclosporin) seemed necessary. The case of pregnancy (P27) was presented as a special case since in the literature the indication of tacrolimus in pregnancy is controversial. Patient P27 was at 8 weeks of pregnancy when we started follow-up, she has normal residual blood levels of tacrolimus, normal kidney function and pregnancy is progressing well.

CONCLUSION

These results confirm the interest of the therapeutic monitoring in the individualization of the doses according to the measured concentrations.This study has provided very interesting information and has brought a significant benefit in the management of patients in renal transplantation, both in the management of drug interactions and therapeutic education of the patient. She also showed the necessity and the importance of the strong clinical-biological collaboration in this management.

[1] Netgen. Prise en charge médicale des patients greffés rénaux au-delà de la première année post-transplantation [Internet]. Revue Médicale Suisse. [2] Press RR and all. Explaining variability in tacrolimus pharmacokinetics to optimize early exposure in adult kidney transplant recipients. Ther Drug Monit. avr 2009;31(2):187-97. [3] Haufroid V and all. CYP3A5 and ABCB1 polymorphisms and tacrolimus pharmacokinetics in renal transplant candidates: guidelines from an experimental study. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg. nov 2006;6(11):2706-13.