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GRADO EN BIOTECNOLOGÍA

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Impacto de la evolución del nivel de tacrolimus en sangre en la supervivencia a alotransplante renal

Tacrolimus residual blood level trajectories and kidney allograft survival

TRABAJO FIN DE GRADO

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Tutor Académico

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TITULO - IMPACTO DE LA EVOLUCIÓN DEL NIVEL DE TACROLIMUS EN SANGRE EN LA SUPERVIVENCIA A ALOTRANSPLANTE RENAL

Memoria presentada por Pablo Ignacio Marcos López para la obtención del título de Graduado en Biotecnología por la Universidad Politécnica de Madrid

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Summary

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Abstract

Kidney transplantation is the best available treatment for end-stage renal disease. Transplant recipients require lifelong immunosuppressive therapy to prevent renal allograft rejection, and tacrolimus, the most common immunosuppressive drug, has a narrow therapeutic window, so its blood levels need to be carefully monitored.

In this study, we have investigated the trajectories of residual tacrolimus blood levels over time in renal transplant recipients treated at *Centre Hospitalier Universitaire Grenoble-Alpes* Using Dynamic Time Warping (DTW) as a method to compare the distance between individual trajectories, and Hierarchical Ascending Clustering to generate groups based on these distances, we managed to identify three groups of patients with similar medical histories and blood tacrolimus trajectories, although we failed to associate these groups to a better or worse death-censored graft life expectancy.

As a pilot study to investigate the feasibility of DTW-based trajectory clustering to generate clusters of interest, our work is relevant as a first approach to a novel method that, after enriching the clinical dataset or improving the clustering algorithm used, could be useful to provide relevant and innovative patient groups in both nephrological and non-nephrological settings.

CHAPTER 1: INTRODUCTION AND OBJECTIVES

1.1. Kidney transplantation

A Kidney transplantation, otherwise known as renal transplant, is the process by which a malfunctioning or defective kidney from a medical patient with end-stage kidney disease is replaced with a working organ from a donor, which can be either alive or recently deceased.[1] Kidney transplants are some of the most common types of transplants, since a human can live with just one working kidney (and thus, donate the other), and they vastly reduce the cost per patient (since complex dialysis machines no longer need to be used) while improving their living conditions, including an average life expectancy increase of 12.4 years.[2]

In France, the number of kidney transplants has augmented from 2976 per year in 2011[3] to 3643 in 2019,[4] a 22% increase, with a waiting time that can reach up to 5 years (although 80% of patients will go through surgery in less than 3 years).[2] In this conditions of high demand, it is essential to ensure that the transplanted organs can remain in optimal condition as long as possible, so patients have to be treated with immunosuppressants to ensure the receiver's body does not reject the transplant.

One of the most commonly used immunosuppressant drugs is **Tacrolimus**, a macrolide lactone discovered in 1987 by the fermentation of a broth from a Japanese soil.[5] Originally produced by *Streptomyces tsukubaensis*, it works by inhibiting calcineurin, a calcium-dependent protein phosphatase involved in the production of Interleukin-2 by T cells, which cannot proliferate without it.[6]

1.2. Therapeutic Drug Monitoring (TDM)

One of the main problems with tacrolimus is that, while at low doses its effect as an immunosuppressant is limited, at high doses it is nephrotoxic (i.e. it damages precisely the kidney that we want to protect), so there is a "narrow therapeutic index", between 4 and 10 µg/l, in which its effect is optimal.[7]

To maintain this virtuous point, Therapeutic Drug Monitoring (TDM, a branch of medicine) studies how to measure and adjust the blood levels of certain drugs, so as to avoid under or overdosing.[8]

In the case of Tacrolimus, to keep measuring simple, predose trough blood concentrations (C_0) are measured at the time of intake of the next dose. Although this does not accurately reflect total drug exposure as measured by the 12-h dose interval area under the concentration curve ($AUC_{0\text{-}12\ h}$), which has been proven to be a superior metric,[8] it permits easier follow-ups and is less time consuming for patients.[7]

1.3. History of TDM correlation with outcomes

Existing literature already points in the direction that high levels of Intra-Patient Variability can be detrimental to patient outcome. For instance, Pollock-BarZiv et al.[9] retrospectively analyzed the variability of tacrolimus trough levels by computing the standard deviation of 6 months of data, and found that patients undergoing an acute cellular rejection had a mean standard deviation almost twice as those who had none.

Another metric that has been widely studied is concentration-to-dose (C/D) ratio, which can be understood as a surrogate marker of tacrolimus clearance or metabolism speed. For example, when Thölking et al.[10] divided patients into three cohorts by C/D ratio, they found that fast metabolizers, i.e., patients with lower overall C/D ratios, had a worse eGFR (estimated Glomerular Filtration Rates, a measure of kidney health) 1 year after the transplantation. However, these authors did not adjust for mean tacrolimus concentration levels, so it is possible that this difference was simply due to the lower exposure to tacrolimus over time.

In general, the golden rule of tacrolimus usage is minimization of the dosage,[11] and combination therapies with other drugs such as everolimus have been proposed to maintain a sufficient immunosuppressive effect. With regards to risk factors, a too-low median tacrolimus concentration, a high Intra-Patient Variability or a low C/D ratio remain the most important measures to take into account, as they can predict bad outcomes and rapid loss of eGFR.

1.4. Going beyond aggregated values: temporal trajectories

So far, most of the existing literature on the effect of tacrolimus on renal function after kidney transplantation is based on taking measures such as standard deviation, which necessarily reduce the richness of the data, as they summarize an entire time series in a single value. The originality of the study we propose here consists in the evaluation of the complete data series, the "trajectory" of the patients throughout their post-transplantation time, as measured by the (pre-intake) trough blood concentration.

For this purpose, we rely on a series of data from the *Centre Hospitalier Universitaire Grenoble-Alpes,* the reference medical center of the city of Grenoble and one of the most important in the *Auvergne-Rhône-Alpes* region. Each year, its *Service de néphrologie, dialyse, aphérèses et transplantation rénale* performs, on average, 140 transplants, which has enabled it to generate, since 2004, a database of about 1800 patients, offering a wealth of data that can be exploited to improve our understanding and outcomes predictions.

Following previous work by the Nephrology team of this institution, this project, code named DESTINATION (Dynamic timE tacrolimuS Trough In kidNey trAnsplanTatION) has been launched with the intention of discovering e.g tacrolimus usage patterns, such as periods of non-observance, early on, so that CHU staff can focus more attention on potential risk groups and e.g. insist on regular drug intake.

To compare the different trajectories among them, we have used Dynamic Time Warping, and we have generated data groups using Hierarchical Ascending Clustering.

CHAPTER 2: MATERIALS AND METHODS

2.1. Patients cohort

Every month, kidney transplant patients should visit the *Kidney Transplantation Unit* to have their residual tacrolimus levels measured and adjusted, trying to maintain blood levels between 5 and 8 µg/l. Thus, if a patient exceeds these levels, a lower dose is recommended, while, if they do not reach them, a higher dose is prescribed.

For this project, an initial database of N = 421 patients was selected, including those who had received a kidney transplant between 2004 and 2014 and which had more than 50 months of tracking data. Since residual tacrolimus levels above 20 µg/l are extremely rare, and probably an input error, we have removed the data for all patients with values above that number. We have also removed the data for all patients which had values equal to 0 µg/l, which are usually a prescription error (e.g., tacrolimus was noted as being taken by someone who no longer needs it), as it would be rare for a patient who has not been taking the medication to go take an analysis.

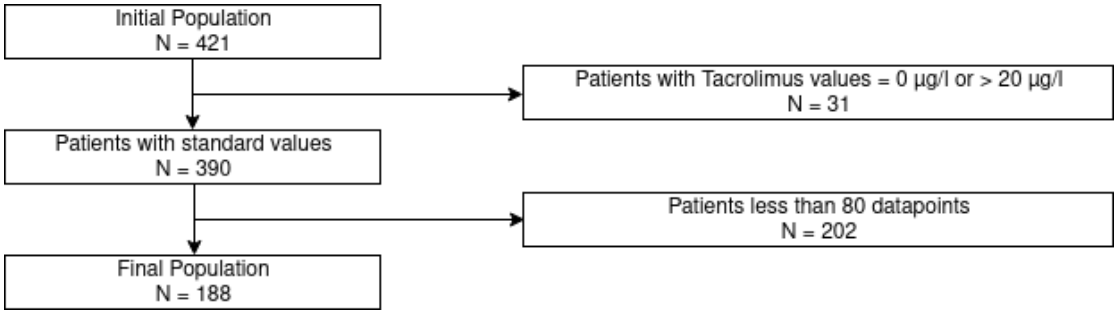

Figure 1: *Real data: subject inclusion flowchart*

2.2. Computing methods

In this work, we used mainly two programming languages: Python, to facilitate data processing, time series screening and normalization of values, and R, which allows us to generate groups of similar populations and to perform survival analysis.

Starting with the previous Final Population N=188, we removed all patients with less than 80 months of tracking data, and we trimmed the data for these patients to take only the first 80 data points, as clustering algorithms require the series to be homogeneous in length. We chose 80 as threshold to try to detect a drop in tacrolimus dose prescriptions that is usually ordered after 60 months of treatment, as part of the *Service de néphrologie*'s tacrolimus minimization strategy.

2.3. Dynamic time warping applied to tacrolimus concentration series

In order to try to understand the meaning of these data and the associations one may make, we must generate defined groups. There are many ways to do this in an unsupervised way, but the two most commonly used are model-based and shape-based clustering. One of the main advantages of shape-based clustering (the approach taken here) is that it does not rely on the assumptions of any underlying model, but rather works with the raw data. It compares the time series by generating a dissimilarity matrix, a table of the distances between all the data points in a set of time series. [12]

To generate such dissimilarity matrixes, we have used Dynamic Time Warping, a method that allows us to find the similarities between time series that are similar in shape, even if they are misaligned. A recent paper by Bottaz-Bosson et al.[12] confirmed that this is one of the most efficient shape-based algorithms, specially when combined with Hierarchical Ascending Clustering (HAC).

2.4. Clustering of tacrolimus trajectories

Hierarchic Ascending Clustering is a method for unsupervised group generation in which each individual value starts as part of a single group, with groups progressively joined (clustered) from bottom to top, from shortest to longest distance, until the specified number of clusters is reached.

In order to perform clustering, we must first define a suitable number of clusters. In our case, we have resorted to 3 methods:

- **Elbow method:** the basic idea of automatic partitioning methods is to define clusters in such a way that the total intra cluster variation is minimized. This method measures the total variation through the WSS, or Within-cluster Sum of Squares (which tends to be lower whenever the number of clusters is higher) and assumes that there will be an "elbow", or inflection point at which adding a new cluster does not substantially improve the total WSS.[13]
- **Silhouette method:** To define how well an object fits into a cluster, this method generates "silhouette values", between -1 and +1 (where a high value implies a good match and a bad one, poor match), for each object, and then adds them to generate the silhouette value for the cluster. If many clusters have a high value, then the number is appropriate.[14]
- **Dunn's index:** An internal Cluster Validity Index that, similarly to the silhouette method, evaluates how well an object fits in its cluster.

While, logically, all of these methods will produce a more optimal result with an increasing number of clusters (as clusters will increasingly be more homogeneous), the idea is to find a compromise between simplicity, e.g. an small number of clusters; and detail, e.g., good values in the three methods.

To implement our DTW strategy, we have used R's dtw library, which calculates the dissimilarity matrix for all series, and R's "hclust" function, which clusters the minimum distances between trajectories into a previously defined number of groups.

2.5. Correlation of clusters with patients' characteristics and outcomes

Once we have selected the groups we wanted, and assigned the different patients to one or another group, we can proceed to associate these data with clinical parameters, such as age, sex, or previous diseases, which allow us to define risk groups. To do this, we have been fortunate enough to have a series of data collected by CHU Grenoble on their patients, which we have processed through a series of tests:

- For **categorical variables**, i.e., those that can only be True/False, 0/1, Yes/No, we performed a **chi-squared test**.
- For **continuous variables**, i.e., those that can take a wide range of values we performed a **Kruskal-Wallis test.**

Both of these two tests produce a p-value, a value that corresponds to the probability that the null hypothesis is true, and therefore represents the inverse of the possibility that a certain value is the result of chance and not of a real relationship between the data. Therefore, the lower the p-value (usually <0.05 to be statistically significant), the greater the possibility that a characteristic is related to one of our clusters.

Finally, we conducted a survival analysis, a branch of statistics that analyzes the life expectancy of kidneys (or patients) based on covariates of interest. Using R's survminer and survfit packages, we generated a series of Kaplan-Meier plots, line graphs that estimate the probability of survival of an individual over time, taking into account that some patients who lose their kidney will do so after the end of our data series (immortal time bias over the first 80 months, as required by the length of the tacrolimus time series). We focused on death-censored graft survival. Patients with a functioning kidney allograft at the end of the follow-up time are thus right-censored.

We conducted a **Log-Rank Test** on our data to check if the clusters were statistically significant predictors of death-censored kidney transplant survival.

CHAPTER 3 – RESULTS

3.1. Description of the patients' characteristics

The main characteristics of the kidney transplant patient database on which this study is based are summarized on **Figure 2**:

	Total (N=188)		Total (N=188)		Total (N=188)
Sex		Hypertension		Corticoid Treatment	
- F	76 (40.4%)	- False	47 (25.0%)	- False	39 (20.7%)
- M	112 (59.6%)	- True	141 (75.0%)	- True	149 (79.3%)
Age		[Tacrolimus]		Survival (in days)	
- Mean (SD)	53.808 (13.959)	- Mean (SD)	8.340 (2.364)	- Mean (SD)	4079.500 (769.899)
- Range	8.600 - 77.900	- Range	3.100 - 17.700	- Range	2476.000 - 5896.000
Diabetes		Initial Mass		IMC	
-	17 (9.0%)	- N-Miss	1	- N-Miss	1
- False	154 (81.9%)	- Mean (SD)	70.025 (14.521)	- Mean (SD)	24.799 (4.176)
- True	17 (9.0%)	- Range	21.700 - 112.000	- Range	14.579 - 41.864

Figure 2: *Description of the patients' characteristics*

3.2. Choice of the number of clusters, time trajectories in the clusters

We first applied the Elbow method. In **Figure 3a** we can see that, for our data, this bend is reached with a number of clusters K = 4. We then applied the Silhouette method, which, as **Figure 3b** suggests, implies that the ideal number would be K = 2. In order to find common ground between this two estimators, we have looked at the Dunn's index values in **Figure 3c**, and we have settled on an optimal number of K=3 clusters, a trade-off between complexity and parsimony.

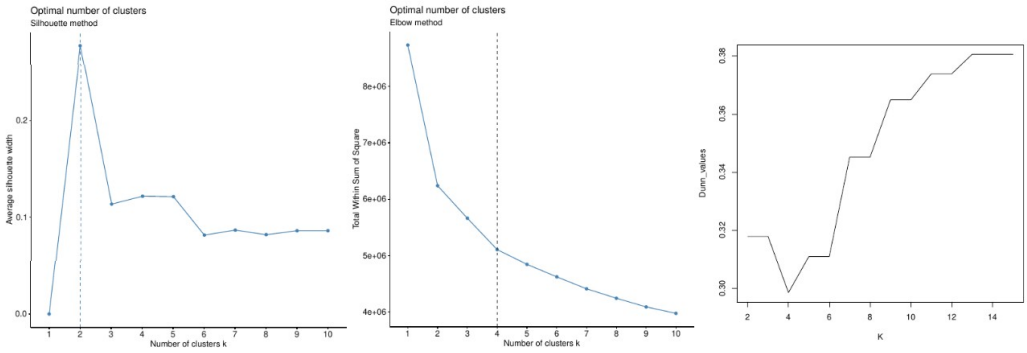

Figure 3: *Three methods for selecting the optimal number of clusters*

Then, we used R to generate **Figure 4,** which helps visualize these 3 different groups, including the upper quartile, the lower quartile, and the mean value of each one.

At first sight, one can see that patients in Cluster 1 had the greatest variability, with patients that started with high levels of tacrolimus but stabilized around 6 µg/l; patients

in Cluster 2, meanwhile, had a smaller variability and a value that stabilized just below 6 µg/l, while patients in Cluster 3 did not stabilize at all and had a continuous decrease of their tacrolimus trough levels to almost 4 µg/l after 80 months.

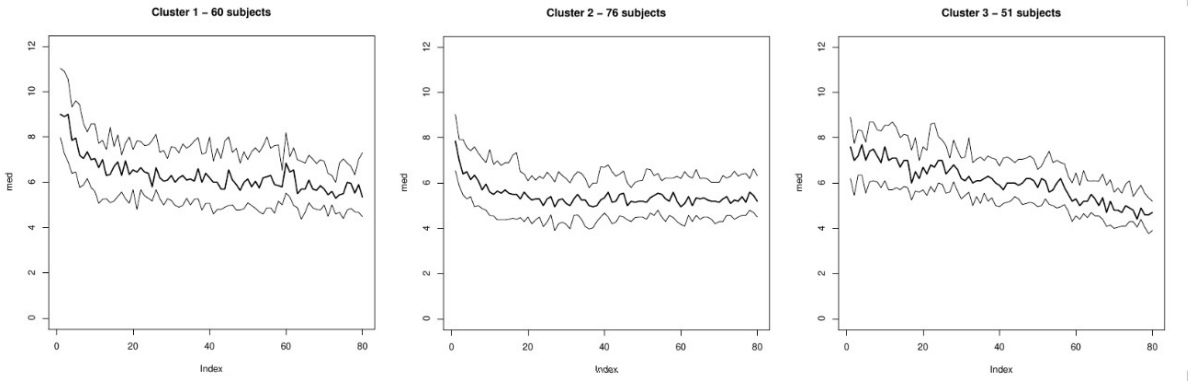

Figure 4: Median tacrolimus trajectories. Patients clustered in three groups.

To account for patient selection bias, we performed a sensitivity analysis by repeating our work with 60 points, and found similar results, reinforcing the study's robustness.

3.3. Correlation of clusters with clinical characteristics

After analyzing these three clusters, and performing the tests described in section 2.4, we get the results summarized in **Figure 5**:

	1 (N=89)	2 (N=46)	3 (N=53)	p-value
Sex				0.063
- F	39 (43.8%)	18 (39.1%)	19 (35.8%)	
- M	50 (56.2%)	28 (60.9%)	34 (64.2%)	
Age				0.075
- Mean (SD)	51.621 (14.199)	57.300 (13.840)	54.449 (13.205)	
- Range	8.600 - 75.100	21.100 - 77.900	20.100 - 74.700	
History of Hypertension				**0.007**
- False	31 (34.8%)	5 (10.9%)	11 (20.8%)	
- True	58 (65.2%)	41 (89.1%)	42 (79.2%)	
History of Diabetes				0.345
-	12 (13.5%)	3 (6.5%)	2 (3.8%)	
- False	70 (78.7%)	38 (82.6%)	46 (86.8%)	
- True	7 (7.9%)	5 (10.9%)	5 (9.4%)	
[Tacrolimus]				**0.020**
- Mean (SD)	8.401 (2.524)	7.580 (1.960)	8.898 (2.271)	
- Range	3.100 - 17.400	3.400 - 11.900	3.500 - 17.700	
Survival (in days)				0.111
- Mean (SD)	4174.472 (789.902)	3882.239 (751.560)	4091.226 (732.726)	
- Range	2476.000 - 5855.000	2509.000 - 5896.000	2541.000 - 5848.000	
Initial Mass				0.707
- N-Miss	0	1	0	
- Mean (SD)	69.513 (14.056)	69.378 (12.944)	71.434 (16.592)	
- Range	21.700 - 109.000	42.000 - 97.000	44.000 - 112.000	
IMC				0.898
- N-Miss	0	1	0	
- Mean (SD)	24.680 (4.327)	24.778 (3.641)	25.017 (4.405)	
- Range	14.579 - 41.864	17.710 - 33.058	17.625 - 35.750	
Corticoid Treatment				**0.022**
- False	13 (14.6%)	16 (34.8%)	10 (18.9%)	
- True	76 (85.4%)	30 (65.2%)	43 (81.1%)	

Figure 5: Correlation of clusters with clinical characteristics

There, we see that only three clinical variables, which are marked in blue, reach our

threshold of p-value < 0.05: Median tacrolimus exposure, a medical history of high blood pressure, and the use of steroids at the time of hospital discharge (about 1 week post-transplantation). For Median Tacrolimus exposure, we already saw in section 3.2 that it did seem to relate to the group these patients were assigned to; for the other two covariates, they seem to point to drug-drug interactions, as both blood pressure lowering agents and steroids might interact with Tacrolimus concentrations.

In general, groups 1 and 3 seem to be more similar, with bigger differences appearing between them and group 2; for example, in **Figure 6,** we can see Group 2 has patients the smallest standard deviation with regards to tacrolimus, while groups 1 and 3 have an slightly larger one. In **Figure 5**, we could already see that there is a significantly larger percentage of patients with High Blood Tension in group 2 than in group 3, and a slightly larger number in group 3 than in group 1, and that a significantly smaller proportion of patients in group 2 were taking corticoid medication at the start of their tacrolimus treatment than patients in group 3 and 1. This makes sense, as Groups 1 and 3 are indeed more closely related; if we look at the HAC dendogram, we can see that they would cluster together into a bigger group if we were to choose 2, instead of 3, as the ideal number of clusters for our study.

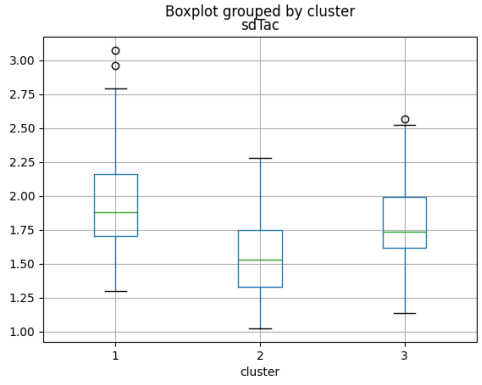

Figure 6: *Box plot for Tacrolimus' standard deviation, grouped by cluster*

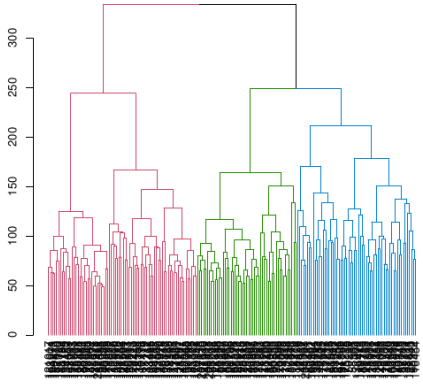

Figure 7: HAC Dendogram

3.4. Correlation of clusters with survival

The results of our analyses seem to indicate the presence of hitherto unknown patient groups. To maximize their usefulness, we want to see if any of them are particularly associated with a greater risk of early kidney loss, so we have performed a log-rank test; unfortunately, the p-value obtained for that hypothesis is just 0.56 (**see Figure 8**), so we can conclude that is not the case.

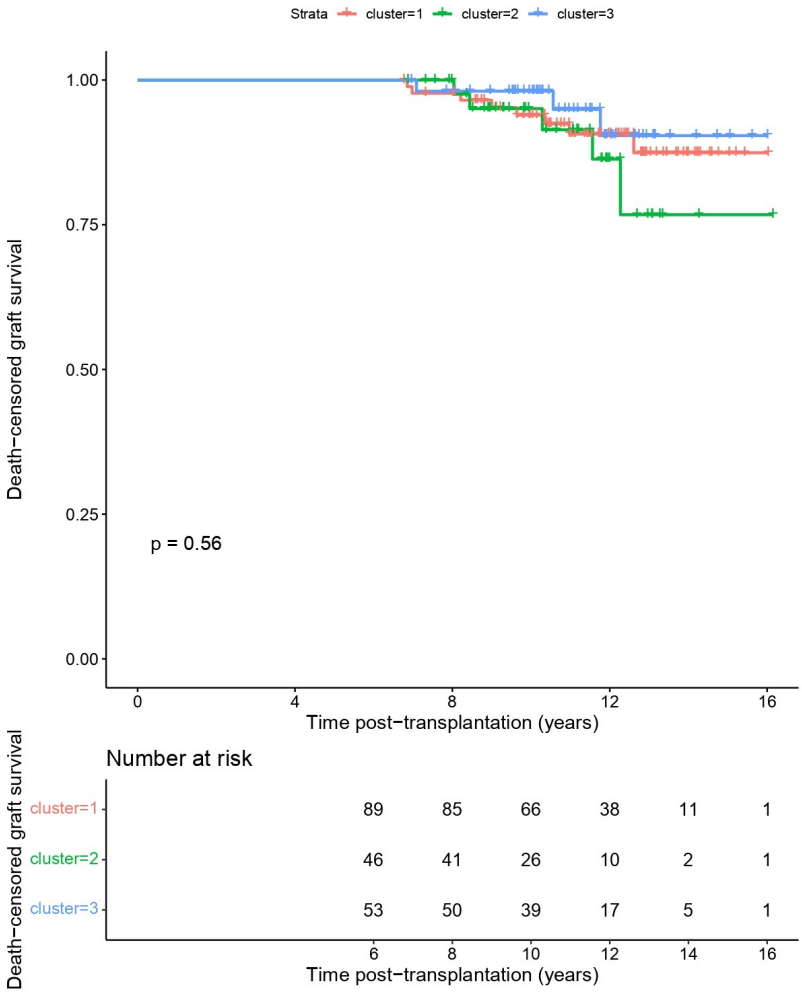

Figure 8: *Kaplan Meier plot with log rank test*

CHAPTER 4 — DISCUSSION

4.1. Main messages

This is a pilot study investigating the possibility of generating patient clusters in an unsupervised manner, using only the trajectories of their tacrolimus blood levels. Once the groups were obtained, we were able to relate them to interesting clinical characteristics. Although we were not able to correlate these groups with (death censored) graft survival, our results show a first approach to analyzing the complete tacrolimus concentration data set, something that has never been done before.

4.2. Strength of this study

One of the main strengths of this study is precisely the use of the full tacrolimus concentrations data to analyze the impact of tacrolimus blood concentrations, rather than a single summary value, either the C/D ratio or the Standard Deviation. This has allowed us to perform an exploratory analysis and to identify previously unsuspected patients' clusters, that might differ in characteristics amenable to medical intervention.

4.3. Methodological issues and limits

Some of the main limitations of this study have to do with the nature of the data collected: on the one hand, it is a retrospective collection, so we have not been able to design different types of treatments, or to work with data other than those present in the hospital database. The limited number of samples is also a problem: although 180 is a fairly low number, and we have performed the same analysis with more patients to increase the robustness of the analysis, we do not know if it is a sufficient number to be representative of the hospital, and, in any case, the data belong exclusively to the hospital of the city of Grenoble, so it is possible that the population dynamics of this hospital are not valid for the whole kidney transplanted patients cohort.

Finally, the clustering algorithm, although the most appropriate to date, could be modified, either to better detect variations in the dataset, or to increase efficiency.

As a first theoretical approach, the objective of this project has been met, and we have succeeded in generating patient groups based on trajectories alone; it remains for future studies to demonstrate how far this methodology can be taken, perhaps by using different algorithms and a larger patients' cohort.

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