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ORIGINAL ARTICLE

Predicting risk of acute rejection in patients with kidney transplants

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KEYWORDS
Kidney transplant;
Acute rejection;
Tacrolimus;
Blood concentration

Abstract
Objective: Create a model to predict the risk of acute rejection of kidney transplant considering variables related to the immunosuppressant agent used, the receiver, the donor, and the transplanted organ.
Methods: Cohort study in a population of 68 patients with kidney transplants being treated with tacrolimus triple therapy. Predicting the risk of acute rejection was carried out with a logistic regression analysis using age, sex, retransplant status, number of HLA incompatibilities, cold ischaemia time, acute tubular necrosis, induction with basiliximab or thymoglobulin, and treatment type as explanatory variables. The contribution of variables associated with determining the blood concentration of tacrolimus was also evaluated; these variables include the average blood concentration, the number of values below and included in the pre-defined therapeutic interval, and the time during which those values remained within that interval.
Results: The logistic regression analysis indicates that the risk of acute rejection depends on the acute tubular necrosis (OR=3; 95% CI, 0.7-13.2) and on the time that the blood concentrations of tacrolimus remains within the therapeutic interval (OR=0.8; 95% CI, 0.7-0.9).
The final model presents an optimal discrimination power (AUC_{ROC}=77%; 95% CI, 62-92). For the selected cut-off point (probability #>0.24) the model shows a sensitivity of 83% (95% CI, 74-90) and a specificity of 71% (95% CI, 61-80).
Conclusions: In patients with kidney transplants, the presence of acute tubular necrosis, together with the time the blood concentration of tacrolimus remained within the predetermined therapeutic interval, permitted the identification of patients with a higher probability of having an acute rejection episode during the first two weeks following the transplant.

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PALABRAS CLAVE
Trasplante renal;
Rechazo agudo;
Tacrolimus;
Concentración sanguínea

Predicción de riesgo de rechazo agudo en pacientes con trasplante renal

Resumen
Objetivo: Construir un modelo para predecir el riesgo de rechazo agudo al trasplante renal considerando variables relacionadas con el tratamiento inmunosupresor instaurado, el receptor, el donante y el órgano trasplantado.
Método: Estudio de cohortes en una población de 68 pacientes con trasplante renal en tratamiento con tacrolimus en triple terapia. La predicción del riesgo de rechazo agudo se realizó mediante un análisis de regresión logística utilizando como variables explicativas la edad, sexo, presencia de retrasplante, número de incompatibilidades HLA, tiempo de isquemia fría, necrosis tubular aguda, inducción con basiliximab o timoglobulina y tipo de tratamiento. También se evaluó la contribución de variables asociadas a la determinación de la concentración sanguínea de tacrolimus, entre ellas la media de la concentración sanguínea, el número de valores por debajo e incluidos en el intervalo terapéutico predefinido, y el tiempo que dichos valores permanecían en las condiciones referidas.
Resultados: El análisis de regresión logística indica que el riesgo de rechazo agudo depende de la necrosis tubular aguda (odds ratio [OR] = 3; intervalo de confianza [IC] del 95 %, 0,7 a 13,2) y del tiempo que las concentraciones sanguíneas de tacrolimus permanecen dentro del intervalo terapéutico (OR = 0,8; IC del 95 %, 0,7 a 0,9).
El modelo final presenta un poder de discriminación óptimo (AUC_{ROC}: 77%; IC del 95%, 62 a 92%). Para el punto de corte seleccionado (probabilidad igual o superior a 0,24) el modelo presenta una sensibilidad del 83% (IC del 95%, 74 a 90%) y una especificidad del 71% (IC del 95%, 61 a 80%).
Conclusiones: En pacientes con trasplante renal, la presencia de necrosis tubular aguda junto al tiempo de permanencia de las concentraciones sanguíneas en el intervalo terapéutico de tacrolimus predeterminado, permiten la identificación de pacientes con mayor probabilidad de aparición de un episodio de rechazo agudo durante las primeras 2 semanas postrasplante.

Introduction

A kidney transplant is the treatment of choice for certain patients with terminal chronic kidney disease, as it improves their quality of life and decreases mortality. In Spain, The number of kidney transplants has increased significantly, reaching a number of 2212 transplants in the year 2007.[1] After a kidney transplant, if the donor and the recipient are not genetically identical, the immune system of the recipient recognises the transplanted organ as a foreign element, triggering an immune response of rejection against the new organ called acute rejection. The response of acute rejection, principally mediated by cellular immunity, usually takes place during the first month after the transplant[2] and it will predict whether there will be a chronic rejection or not, compromising the survival of the graft in the long term.[3] The objective of immunosuppressive therapy is to control the immune response against the transplanted organ; in this way, with the new immunosuppressant drugs, the incidence of acute rejection have decreased in the last few years, representing between 20% and 25% depending on the immunosuppressant scheme.[4] At present, the immunosuppressant regime is based on a triple therapy formed by the association of a calcineurin inhibitor, a cellular division inhibitor or an inhibitor of the mTor protein or a corticosteroid; its aim is to obtain an additive effect, that with the posterior progressive reduction of the doses, minimizes the inherent toxicity of this treatment is achieved.[5] The narrow therapeutic index of the inhibitors of calcineurin together with its complex pharmacokinetic behaviour and its elevated variability between and within individuals, define the characteristics that its pharmacokinetic monitoring requires to individualise the dose according to blood concentrations and therefore, optimise the dosage. Thus, various authors have confirmed the association between infra-therapeutic blood concentrations of cyclosporine,[6] tacrolimus[7] or mycophenolate mofetil[8] and the risk of suffering an acute rejection episode. In addition, it is necessary to know and control diverse factors than can lead to an increase in the inter and intra-individual variability of the kinetic behaviour of these drugs, among them, the presence of genetic variability that influences the bioavailability and the metabolism, pharmacological interactions, degree of kidney and liver functionality, the gender and age of the patients, among other factors. In addition to this, the risk of acute rejection not only depends on the dosage variables of the immunosuppressant therapy, but it is also influenced by different clinical variables that depend on the recipient, the donor or the transplanted organ itself, such as age,[9] gender,[9] immunological compatibility,[9,10] the presence of re-transplants,[11,12] the presence of acute tubular necrosis,[12-14] where the transplant organ comes from (live donor or cadaver)[9] or the time that it is in cold ischaemia.[9,12] The study of the influence of these variables could help to

predict the risk of acute rejection in patients with kidney transplant.

At present, tacrolimus is the most used calcineurin inhibitor as it presents a lower incidence of adverse effects and a similar or superior efficacy to cyclosporine for short term survival.[15-17] The therapeutic interval established to ensure maximum efficacy and minimal toxicity corresponds with minimum concentrations of 5 to 15 ng/mL.[18] The frequency of monitoring and the pharmacokinetic follow-up in the immediate post-transplant period will depend on the time after the transplant and the clinical situation of the patient. Thus, during the first month, monitoring of blood concentrations is recommended with a frequency of 3 weekly determinations, that is increased progressively to once per week in the third post-transplant month.[19]

In the clinical practice, the prevention and, where applicable, the early diagnosis and treatment of acute rejection are priority objectives of the interdisciplinary kidney transplant teams, which, among other reasons, continue to be the principal risk factors to develop chronic rejection, determining the kidney function and failure of the implanted organ in the medium to long term. Within this context, the goal of this current study has been to construct a model that enables the prediction of the risk of acute rejection in patients with kidney transplants during the first two weeks after the transplant, using variables related with the pharmaco-therapeutic treatment received and clinical variables related with the recipient, the donor or the transplanted organ itself. The early identification of these variables and the quantification of their influence will allow for the development of a prognostic index capable of classifying patients regarding the risk of presenting acute kidney rejection in the first 2 weeks after transplantation. As a result, the patients identified with greater risk of suffering acute rejection will be candidates for an intensification in their normal clinical follow-up and in the pharmacokinetic monitoring of tacrolimus, as well as a modification of the daily dose of tacrolimus in order to maintain blood concentrations in the superior limit of the therapeutic interval (around 15 ng/mL), avoiding infra-therapeutic values and minimising the risk of suffering an episode of acute rejection.

Method

Patients and immunosuppressant treatment

The study population is composed of patients that have undergone a kidney transplant in a university hospital (annual coverage of 24 676 patients and 152 819 hospital stays), during 2005 and 2006, and treated with triple therapy that includes tacrolimus. The patients that received organs from elderly donors (>60-years-old) or with an elevated risk of acute tubular necrosis received induction with thymoglobulin at a ratio of 1.5 mg/kg/day intravenously, up to a maximum of 3 doses given on alternating days, or 2 doses of 25 mg of basiliximab, intravenously; in both situations, the first dose was given before the transplant. Orally administered treatment with tacrolimus was begun in the first 24 h after the transplant and the patients received between 0.15-0.30 mg/kg/day every 12 h. Subsequently, the dose was individualised depending on the results of blood concentration. When it was not possible to administer tacrolimus orally, it was given intravenously, maintaining the equivalence of the oral dose: 5:1 intravenous. Together with tacrolimus, the patients received treatment with prednisone at 20 mg daily, with a progressive reduction to 15 mg per month, 10 mg after 2 months and 5 mg after the sixth month; mycophenolate mofetil 1 initial dose of 1 g every 12 h, that was later individualised depending on the results of blood concentration, or sirolimus, 1 shock dose of 6 mg followed by 2 mg daily, that later were individualised depending on the results of blood concentration, or everolimus, 1 initial dose of 0.75 mg every 12 h, subsequently individualised depending on the results of blood concentration. The combination of tacrolimus with sirolimus or everolimus (unauthorised combinations by the Spanish Agency of Medications and Sanitary Products) was carried out in the context of clinical trials that were approved by the Research Ethics Committee of the relevant hospital. Blood samples were taken just before the morning dose, that is, in minimum blood concentration conditions (C_{min}). In our hospital, the following therapeutic interval has been established: C_{min} from 10 to 15 ng/mL for the first 6 weeks after the transplant, and from 5 to 10 mg/mL after this post-transplant period.

Type of study and data collection

Cohort study where the data corresponding to the anthropometric (age and gender), clinical (acute rejection, acute tubular necrosis, time of cold ischaemia, number of HLA incompatibilities, and presence of previous transplant), and pharmaco-therapeutic (type of immunosuppressant treatment and induction of antibodies) characteristics are registered, from the beginning of the kidney transplant. The initially established follow-up time was during the first 14 days after the transplant, as this post-transplant period corresponded with the period of maximum probability of acute rejection, or until the appearance of an episode of acute rejection when this took place before this period ended. Acute tubular necrosis was diagnosed in patients that after the kidney transplant presented sub-optimal kidney function and required dialysis, discarding causes of vascular origin or obstruction of urinary ducts; also, in patients where this situation was maintained for a week, a kidney biopsy was taken to discard the appearance of an episode of acute rejection. The electric recording of the pharmacokinetic monitoring (pKClin®) made it possible to validate and extract the information corresponding to the dose regimen of tacrolimus and the value of the C_{min}. Only the C_{min} extracted in the moment before the following dose were considered valid and never those after more than 14 h had passed after the last administration. The total determination in blood of tacrolimus was carried out using a Microparticle Enzyme Immunoassay (MEIA) for tacrolimus and its metabolites (Abbott, IMx).

Statistical analysis

An analysis of logistic regression was carried out; the appearance of an episode of acute rejection was defined as the response variable. The explicative variables studies

were: age (years), sex, presence of previous transplant, number of HLA incompatibilities, time of cold ischaemia (hours), presence of acute tubular necrosis (ATN), induction of antibodies (without induction, induction with basiliximab, or induction with thymoglobulin), treatment type (tri-therapy with tacrolimus associated with corticosteroids and mycophenolate mofetil, sirolimus or everolimus). To quantify the contribution of the C_{min} of tacrolimus over the probability of acute rejection, a group of variables were defined related with this and with the time that the patient was submitted to this condition of C_{min} of tacrolimus:

1. Average blood concentration in ng/mL (C_{sm}).
2. Number of determinations within the therapeutic interval (N_benefit).
3. Time in days within the therapeutic interval (T_benefit).
4. Number of determinations under the therapeutic interval (N_inefficacy).
5. Time in days under the therapeutic interval (T_inefficacy).

The values of the quantitative variables have been presented using the respective indexes regarding the symmetry of their distribution: average (standard deviation) (symmetric distribution), and median and interquartile distance (assymetric distribution). The values of the categorical variables have been expressed as relative frequencies expressed in percentages.

For the development of the logistic regression model,a screening of the explicative variables was carried out, from the analysis of single variant regressions, to select the potential prognostic factors that would be included in the multi-variant model (values of $P<.25$). With the resulting variables, the different multi-variant models were explored with the methods of sequential inclusion and exclusion, fixing the values of P of signification for the inclusion and exclusion of variables in 0.1 and 0.2, respectively. Secondly, the interaction terms were introduced to confirm if the adjustment indexes improved (significant changes of the logarithm of verisimilitude). Finally, the indicators of sensitivity, specificity, correct classifications, and the ROC curve were calculated for the selected models (Figure 1). The selection of the final model was done regarding the area of the ROC curve and the theoretical-practical advantages and disadvantages of each model. The statistical analysis was done with the SPSS program, version 12 (SPSS Inc, Chicago, IL).

Results

A total of 68 patients was included in the study (38% women and 68% men), with an average age of 51.69-years-old (95% CI, 48.24-68.15), of which 16 patients (23%; 95% CI, 15-32) presented an episode of acute rejection. The average time of follow-up in our population was 14 days (95% CI, 13-15 days); nonetheless, in patients that presented acute rejection, the average time for the appearance of an episode of acute rejection was 9 days (95% CI, 7-12 days). Tables 1 and 2 present the measurements of the central

Results of the test	True diagnosis	
	Sick	Healthy
Positive	True positives (TP)	False positives (FP)
Negative	False negatives (FN)	True negatives (TN)

$$\text{Sensitivity} = \frac{TP}{TP + FN} \qquad \text{Specificity} = \frac{TN}{TN + FP}$$

$$PPV = \frac{TP}{TP + FP} \qquad VPN = \frac{TN}{FN + TN}$$

Figure 1 Calculation of the sensitivity, the specificity, and the positive (PPV) and negative (NPV) predictive values from the relationship between the results of a diagnostic test and the presence of an event.

Table 1 Descriptive characteristics of the quantitative variables

Variable	Mean	95% CI
Time of cold ischaemia, h	19.05	17.65-20.45
Number of incompatibilities[a]	2	0-4
ABC, ng/mL	11.47	10.68-12.26
N_benefit, no.	2.94	2.54-3.33
T_benefit, d	7.85	6.71-8.99
N_inefficacy, no.	1.97	1.61-2.31
T_inefficacy, d	5.79	4.77-6.81

ABC indicates average blood concentration; CI, confidence interval; N_benefit, number of determinations within the therapeutic interval; N_inefficacy, number of determinations under the therapeutic interval; T_benefit, time in days within the therapeutic interval; T_inefficacy, time in days under the therapeutic interval.
[a]Asymmetric distribution.

Table 2 Descriptive characteristics of the categorical variables

Variable		Relative frequency, %	95% CI
Acute tubular necrosis		40	30-50
Re-transplant		24	15-32
Induction		58	48-68
Treatment	Triple therapy_MMF	87	72-92
	Triple therapy_SIR	8	4-15
	Triple therapy_EVER	5	2-11

CI indicates confidence interval; EVER, everolimus; MMF, mycophenolate mofetil; SIR, sirolimus.

Table 3 Single variant regressions on the clinical variables and those dependent on treatment

Variable	*P*	Exp (B)	95% CI of Exp (B)
Sex	.174	0.495	0.179-1.364
Age, y	.876	0.997	0.962-1.034
Time of cold ischaemia, h	.270	1.125	0.951-1.333
ATN	.150	2.415	0.727-8.020
Number of incompatibilities	.121	0.701	0.447-1.099
Re-transplant	.791	1.316	0.173-10.01
Induction with antibodies	.419	0.776	0.419-1.436
Type of treatment	.620	0.746	0.234-2.375
ABC, ng/mL	.846	0.984	0.833-1.162
N_inefficacy, no.	.056	0.665	0.438-1.010
N_benefit, no.	.030	0.693	0.497-0.965
T_inefficacy, d	.053	0.869	0.754-1.002
T_benefit, d	.011	0.848	0.746-0.903

ABC indicates average blood concentration; CI, confidence interval; N_benefit, number of determinations within the therapeutic interval; N_inefficacy, number of determinations under the therapeutic interval; ATN, acute tubular necrosis; T_benefit, time in days within the therapeutic interval; T_inefficacy, time in days under the therapeutic interval.

Table 4 Final logistic regression model

Variable	B	*P*	Exp (B)	95% CI of Exp (B)
ATN	1.141	.121	3.130	0.740-13.235
T_benefit	−0.196	.030	0.822	0.688-0.98
Constant	−0.468	.536	0.626	

ATN indicates acute tubular necrosis; CI, confidence interval; T_benefit, time within the therapeutic interval.

$$\mathbf{Pr(Y=1/X)} = \frac{1}{1+e^{-(-0-47-0.2\times T\_benefit+1.14\times ATN)}}$$

Figure 2 Equation of the predictive model for acute rejection.

tendencies (mean and median) for the quantitative variables and the relative frequency for the predictive categorical variables evaluated, together with their 95% CI.

In the construction of the predictive model, the single variant selection of the variables fell to sex, ATN, N_benefit and T_benefit as they presented a $P<.25$ and a correct value in the sign of the coefficient. The results of the single variant regressions are shown in Table 3 regarding the clinical variables and the variables that depended on the treatment. With the sequential inclusion and exclusion methods, 2 models were obtained: model A (were the ATN and T_benefit variables were maintained) and model B (where the T_benefit variable was maintained). The exploratory process of both models showed that model A presented greater predictive validity (greater ROC curve) (Table 4). The predictive equation of the selected model (Figure 2) is represented using the logistic regression model and it provides the probability of the appearance of an episode of acute rejection in the first 2 weeks after the transplant (Figure 3) depending on the explicative variables: time within the therapeutic interval, that reduces the risk of the appearance of an episode of acute rejection by presenting a coefficient inferior to the unit, and acute tubular necrosis (ATN), included in the final model as it improves its predictive power.

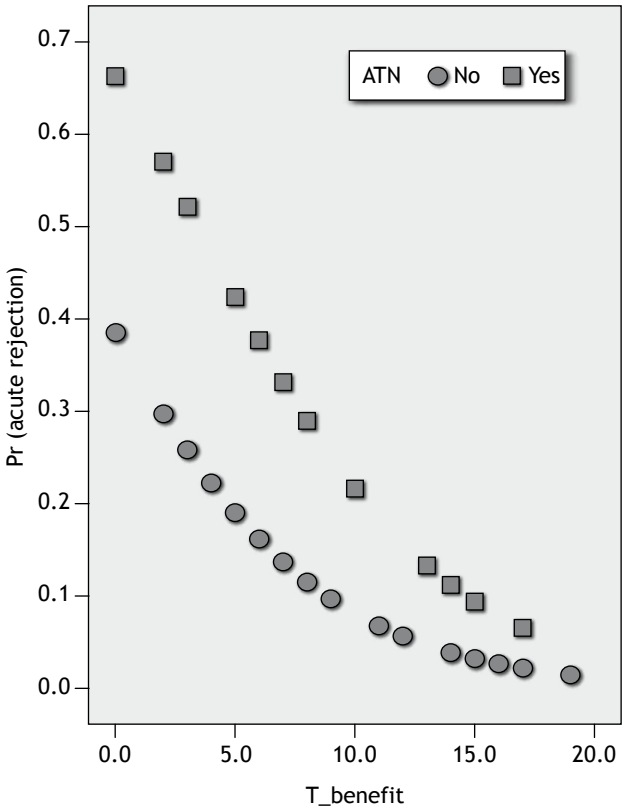

Figure 3 Probability of suffering an episode of acute rejection regarding the time stayed within the therapeutic interval (T_benefit) and the presence or absence of acute tubular necrosis (ATN) (in the abscissas, the ATN and T_benefit variables are represented and in the ordinates, the probability of suffering an episode of acute rejection)..

The area below the diagnostic performance curve (AUCROC) is of 77% (95% CI, 62-92) and the optimal cut-off point in the ROC curve (Figure 4), or point that offers the best ratio between sensitivity and specificity, corresponds with a probability value of acute rejection ≥0.24; a value that is used to classify patients in 2 risk groups regarding acute rejection:

1. Patients with a probability of acute rejection equal to or greater than the cut-off point selected with the acute rejection diagnosis according to the logistic regression model.

2. Patients with a probability of acute rejection lower than the cut-off point selected, not diagnosed with acute rejection according to the logistic regression model.

In Figure 5, the contingency table is represented that results from applying the prediction model for the appearance of an episode of acute rejection in our study population.

For the selected cut-off point (0.24), the proposed model presents a sensitivity of 83% (95% CI, 74-90) and a specificity of 71% (95% CI, 61-80), with a positive predictive value of 46% (95% CI, 36-56) and a negative predictive value of 94% (95% CI, 87-98).

Discussion

The current immunosuppressant regimens have made it possible to reduce the incidence of acute rejections in the population with kidney transplants to 20%-25%[4]; acute rejection is the principal predictor of chronic rejection and of losses of the kidney transplanted.[3] In addition to this, it must be kept in mind that the incidence of acute rejection depends on factors related with the immunosuppressant therapy as well as on clinical factors. Eighty-three percent (95% CI, 74-90) of our study population presented a triple immunosuppressant regimen based on tacrolimus and mycophenolate mofetil. Contrary to that published in the references consulted,[22,23] in our study, no statistically significant relationship has been observed between the type of immunosuppressant regimen and acute rejection, possibly because we had a reduced population of patients for the subgroups being treated with sirolimus and everolimus.

Fifty-eight percent (95% CI, 48-68) of our patients received induction therapy with basiliximab or thymoglobulin; no statistically significant relationship was observed with the acute rejection, contrary to what happened in the study published by Nashan et al,[24] where they studied the incidence of acute rejection in 376 patients and observed that the patients that received basiliximab presented a reduction of 32% of acute rejections compared with the placebo (difference of 14.2%; 95% CI, 3-24; *P*=.012).

The monitoring of the blood concentrations of the immunosuppressant drugs is a useful method to establish relationships between the pharmacokinetic response and acute rejection in patients with kidney transplants.[6-8] Thus, for cyclosporine, Perico et al[6] found statistically significant differences between the blood concentrations of the second day after the transplant and the appearance of acute rejection during the first 6 months after the transplant. For mycophenolate mofetil, Borrows et al[25] observed statistically significant differences between the average concentrations and the appearance of acute rejection in the first month after the transplant. In a recent study, Le Meur et al[8] confirmed that this drug is more effective and safe when its dose is individualised according to the plasma concentration value instead of using fixed doses of mycophenolate mofetil. In this case, lower AUC's are reached than in the group of individualised doses; they found statistically significant differences in the appearance of acute rejection in the first year after the transplant (20% compared to 8%, respectively). For tacrolimus, various authors[7,26,27] have studied the relationship between the infra-therapeutic concentrations of tacrolimus and the risk of acute rejection. Bottinger et al[26] studied the relationship between the value of the blood concentration of tacrolimus the same day of the rejection and the acute rejection; no statistically significant relationship was observed. Other authors have studied the relationship of the blood concentration of tacrolimus with the acute rejection from the average value of blood concentrations of tacrolimus in the first month after the transplant.[7,27] Thus, in a study conducted by Silva et al,[27] the relationship with acute rejection has not been confirmed; on the contrary, in the study conducted by Staatz et al,[7] a statistically significant difference was found (average concentration of tacrolimus in the group with acute rejection of 5.09 ng/mL compared with 9.02 ng/mL in the group that did not present acute rejection; *P*=.04).

The principal limitation of these studies is the use of average concentrations of tacrolimus as the principle variable related with the risk of rejection, as it masks the minimal concentration values related with a greater risk of acute rejection. In this respect, in the single variant analysis, the average value of the concentration in blood in our population of patients did not present a statistically significant relationship of this variable with acute rejection.

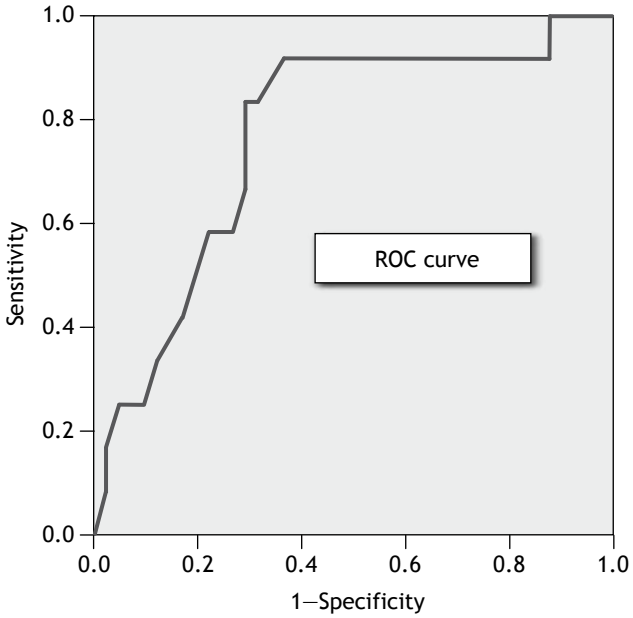

Figure 4 Area below the recipient-operating characteristic curve of the logistic regression model.

		AR observed	
		Yes	No
Diagnosis of AR with the model	**Yes**	10	12
	No	2	29

Figure 5. Contingency table (2×2) that represents the results of the application of the regression model in the prediction of acute rejection (AR).

Therefore, another step in this direction is to consider the time of exposure, as, theoretically, the longer the exposure time to infratherapeutic values, the lower the AUC of the medication, and, potentially, the higher the risk of acute rejection in the patient.

Another factor that has been considered is the frequency of infra-therapeutic episodes, that is, the number of episodes where the patient presents blood concentrations below the therapeutic interval; theoretically, the greater the number of episodes under the therapeutic interval during the study period, the greater the risk of acute rejection presented by the patient. As a result, in the single variant analyses, statistically significant relationships have been found between the variables of frequency N_benefit and acute rejection. The follow-up time of our population for the study of the incidence of acute rejection was 14 days, as more than 90% of the patients that present acute rejection, present it during the first 2 weeks after the transplant. As a result, the population studied presents an acute rejection incidence of 23% in this period (95% CI, 15-32), similar to that found in a study conducted by Mayer et al,[4] where they found an incidence of acute rejection of 25.9% in a group of 303 patients treated with tacrolimus.

Thirty-eight percent of our study population are women, which is a distribution that is equivalent to the populations observed in other studies.[9,19] In our population there is no statistically significant relationship between gender and acute rejection, a result that coincides with that observed by Bum et al.[10] By contrast, in the study conducted by Herzenberg et al,[9] when dividing the population in 2 groups depending on the presence or no of acute rejection, in the group of patients with acute rejection a greater percentage of women was observed (59%) compared to the group of patients that did not present rejection (27%), with statistically significant differences (χ^2=9.14; *P*=.003).

The average age of our study population is 51.69 years old (95% CI, 48.24-68.15), slightly greater than the population included in other studies[9,13]; however, in our study no statistically significant association was found between the age and the appearance of acute rejection, the same as that found in the evaluated bibliography. The variables for the presence of re-transplant and number of HLA incompatibilities have presented a tight relationship with acute rejection in various studies[9,10,11]; however, in our study no statistically significant association was found between these co-variables and the appearance of acute rejection.

Regarding cold ischaemia time or time of conserving the organ transplanted outside of the organism, by increasing said time, and consequently the secondary damage, rejection is favoured.[13] Thus, the organs transplanted in our study population have undergone an average time of cold ischaemia of 19 h (95% CI, 17.65-20.45); no statistically significant relationship was observed regarding acute rejection.

In addition, acute tubular necrosis is the principal factor involved in the initial dysfunction of the new organ,[14] as an increase in the expression of the HLA molecules is produced by the tubular damage, and with it, a greater state of inflammation. In our study, 40% of the patients presented an episode of acute tubular necrosis, although no statistically significant relationship has been confirmed regarding acute rejection (OR=2.41; 95% CI, 0.73-8.02; *P*=.15), as seen in the studies conducted by Mauiyyedi et al[13] and Moreso et al.[14]

The statistical analysis of logistic regression indicates that the risk of acute rejection in our patients is related with 2 response variables: ATN (pharmacodynamic variable) and T_benefit (pharmacokinetic variable). Just as that which occurs in the single variant model, the test of the ratio of verisimilitude confirmed that the T_benefit variable was the variable that provided greater explication to the model (*P*=.03). In addition, the introduction of the ATN variable improves the predictive value of the model, as it presents a greater ROC area. Thus, from the regression equation, we can deduce that the probability value of the appearance of acute rejection in our patients is three times greater (95% CI, 0.7-13.2) for the presence of an episode of ATN and, on the contrary, it is multiplied by 0.8 (95% CI, 0.69-0.98) by increasing a unit of time, expressed in days, the value of the T_benefit variable. Similarly, by representing the probability of the appearance of an episode of acute rejection depending on the predicitive variables T_benefit and the presence of an ATN episode, we observe that the patients with ATN will be more sensitive to the T_benefit variable (Figure 3). In order to evaluate the validity of the logistic regression model, the receiver-operating characteristic curves (ROC curves) have been used. The AUCROC of 77% (95% CI, 62-92) indicates to us that the model presents an optimal discrimination power. From the interpretation of the ROC curve, an optimal cut-off point has been selected as the probability value of 0.24. As a result, when the probability of the appearance of acute rejection estimated with the logistic regression model is equal or greater than 0.24, the patient will be diagnosed with a high risk of presenting an episode of acute rejection. The values of sensitivity and specificity found for the selected cut-off point indicate that the ATN and the T_benefit act as useful tools for the pre-selection of patients with risk of the appearance of acute rejection, by presenting a specificity close to 75% according to the validity criteria of Doménech.[28] The positive predictive value is low, that is, our model would have little use when the result is positive; however, in our case, false positives can be accepted as the suspicion of acute rejection will not provoke severe psychological damage in the patient, although their follow-up will entail an added cost that should be evaluated in subsequent studies. In addition, the negative predictive value is high, and therefore, we can be surer that when excluding a patient from our follow-up, they will not present an episode of acute rejection.

This study allows us to conclude that the ATN together with the T_benefit variables are useful tools in the identification of patients with high risks of suffering an episode of acute rejection. As a result, the appearance of acute rejection in a patient will depend not only on clinical, non-modifiable or preventive variables, but also on the variables related with the pharmaco-therapeutic treatment that are potentially preventable with adequate monitoring or application of pharmacokinetic knowledge to the individualisation of doses and follow-up of the immunosuppressant treatment in these patients. However, an external validation of our model is needed with another group of patients with similar characteristics, to evaluate its applicability in this population.

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