UNIVERSITÀ DEGLI STUDI DI GENOVA

SCUOLA DI SCIENZE MEDICHE E FARMACEUTICHE

DIPARTIMENTO DI MEDICINA INTERNA E SPECIALITA' MEDICHE

DOTTORATO DI RICERCA IN EMATO ONCOLOGIA E MEDICINA INTERNA CLINICO-TRASLAZIONALE

Ciclo XXXVI

Curriculum: Fisiopatologia e clinica delle malattie renali, cardiovascolari e dell'ipertensione arteriosa

Tesi di dottorato

Cardiovascular event prediction in kidney transplant recipients: a new risk score derivation and internal validation

Relatore: Prof.ssa Francesca Viazzi

Correlatore: Dr. Ernesto Paoletti

Dottorando: Elisabetta Bussalino

Abstract

Background: Kidney transplant recipients (KTRs) have a cardiovascular (CV) risk 3 to 5-fold higher than that of the general population. CV disease represents the main cause of death in KTRs, both due to the presence of traditional risk factors and factors strictly related to transplant. The main CV risk calculators proved to be poorly predictive in the KTR population. We, therefore, aimed to compare the performance of CV risk scores already in use and to develop and validate a CV risk score in our KTRs population.

Methods Our analysis included 371 adult KTRs in follow-up at the outpatient clinic from 1 January 2015 to 31 December 2016. The composite outcome was the occurrence of MACE. We compared the performance of two main CV risk calculators (Framingham Risk Score -FRS- and Patient Outcomes in Renal Transplantation-PORT-risk score). Thereafter, we built a predictive model selecting study variables by backward stepwise Cox regression. The risk score for each variable was weighted according to the hazard ratio (HR) of the final multivariable model. Internal and external validation of the prediction score and its discriminative capacity was assessed via area under the curve (AUC), and the calibration via the Hosmer–Lemeshow test.

Results After a mean follow-up of 68 months, CV events occurred in 71 (19%) KTRs. FRS and PORT were demonstrated to have low predictive power in our population. The accuracy did not improve after adjustment for immunological and adjuntive transplant-related variables. Therefore, we derived a model including significative variables at multivariate analysis: age, dialysis vintage, systolic blood pressure, eGFR and 24-hour proteinuria. The model discrimination was good (Harrel's c: 0.73, AIC 713). According to the HR, 3 points were attributed to age higher than 60 years and 24 h proteinuria higher than 1 g/d, 2 points to dialysis vintage longer than 5 years, systolic blood pressure higher than 140 mmHg and eGFR less than 30 ml/min/1.73m2. The new score demonstrated good discrimination (AUC 0.70) and acceptable calibration (Hosmer-Lemeshow test $\chi^2$11.34, P=0.12). The new score was internally validated by 10-fold cross-validation (mean AUC 0.70, 95% CI 0.60-0.77). For each point of the new score, the risk of the event increased by 40% and a score higher than three was associated with a 4-fold increased risk of composite endpoint.

Conclusion We confirmed the poor predictive power of the known CV risk assessment tools in our real-world KTRs population. Moreover, their discriminatory power was not improved by adjustment for the HLA compatibility and donor-specific antibody. A new score for CV event prediction including traditional risk factors, such as age and systolic blood pressure, and variables related to renal function and the effects of previous chronic kidney disease, was developed and internally validated.

Keywords: cardiovascular risk calculator, kidney transplantation, cardiovascular outcome

Introduction

Although kidney transplant recipients (KTRs) survival has significantly improved, cardiovascular (CV) disease represents the main cause of morbidity, death and allograft loss ("death with function graft").

As a matter of fact, KTRs have 3 to 5-fold higher risk of CV events than that of the general population due to traditional and non-traditional CV risk factors, such as metabolic adverse effect of immunosuppressive therapy and uremic milieu (1,2).

CV risk stratification still relies on the Framingham Risk Score (FRS), which is derived and calibrated on the general population, and several studies demonstrated its poor accuracy in KTR population (3).

Recognizing the need of an improvement in the evaluation of CV risk after kidney transplantation, we aimed to compare the performance of thoroughly validated calculators, such as FRS (4) and Patient Outcomes in Renal Transplantation (PORT) risk score (5).

Additionally, we derived and internally validated a new risk score from a retrospective, observational, monocentric analysis of 371 subjects referred to our kidney transplant outpatient clinic between 2015 and 2016.

Methods

Study sample

Our monocentric retrospective analysis included all available adult KTRs in follow-up at the outpatient Nephrology Clinic of San Martino Hospital from 1 January 2015 to 31 December 2016, who underwent kidney transplantation for more than 12 months.

Patients referred to another outpatient clinic for post-transplant follow-up or who had CV events 12 months before the observation were excluded from the final analysis. Figure 1 shows the study flow-diagram (Fig.1).The clinical investigation was conducted according to principles of the Declaration of Helsinki and all participants provided informed consent.

Data collection

Data regarding demographics, transplant-related information, coexisting comorbidities as well as information on medications were obtained for all enrolled KTRs at baseline and updated at every follow-up visit by self-reports and review of medical records. Delayed graft function (DGF) was defined as the need for dialysis

within the first week after transplantation due to inadequate kidney function (6). Blood samples were obtained following 8-hour overnight fasting.

Laboratory measurements included creatinine, glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL), and 24-hour urinary protein excretion. Low density lipoprotein (LDL) concentration was calculated by Friedewald's formula (7). Office BP was measured at rest, in sitting position, three times at 5-minute intervals. Renal graft function, as expressed by estimated glomerular filtration rate (eGFR), was calculated according to the CKD-EPI formula (8). Diabetes was defined as self-reported diagnosis or use of hypoglycemic drugs, excluded SGLT2-inhibitors for protection in non-diabetic CKD. Prior CV disease was defined as a history of acute coronary syndrome (ACS) ~~myocardial infarction (MI), angina,~~ stroke or previous revascularization. HLA compatibility (number of mismatches), donor specific antibody (DSA) presence and the most recent panel reactive antibody (PRA) before transplantation were collected.

CV risk scores

The Framingham Risk Score (FRS) predicts an individual's 10-year risk of cardiovascular events based on factors such as age and gender (0-15 points), cholesterol levels (0-7 points), blood pressure (0-4 points), smoking (0-4 points), and diabetes (0-3 points). (4).

The Patient Outcomes in Renal Transplantation (PORT) Study risk calculator estimates the risk of coronary heart disease (CHD) within 3 years after a visit occurring 1–5 years posttransplant. (5). Variables include age (0-9 points), sex (0-2 points), race (0-3 points), most recent PRA (0-1 points), dialysis vintage (0-2 points), post-transplant lymphoproliferative disorder (PTLD) (0-9 points), acute rejection (0-5 points), eGFR (0-3 points), DGF (0-2 points), number of comorbid CV conditions (0-7 points) and previous cardiovascular disease (0-6 points). A higher score indicates a higher risk of an event.

Outcome

The primary outcome was fatal or nonfatal CV event, a composite of MI, unstable angina or revascularization, stroke, and hospitalization for heart failure. Events were identified by direct consultation of all hospitalized patient records. The study follow-up lasted a median time of 68 months (range 57-71

months). Cardiovascular death was defined as sudden cardiac death, death from ACS, due to heart failure or cardiogenic shock.

Statistical Analysis

Continuous variables are presented as means ± SD and compared by unpaired t-test and. Categorical variables are compared by chi-squared test. Skewed data were expressed as median with interquartile range and compared with the Kruskal-Wallis test. The normal distribution of the variables was determined by the Kolmogorov-Smirnov test.

Discrimination assessment of the considered score was performed via Area under the curve (AUC) curve comparison.

Univariate and multivariate Cox proportional hazards analysis was used to estimate hazard ratio (HR) and the corresponding 95% confidence interval (95% CI) for CV events and all-cause mortality. The risk score for each variable was weighted according to the HR of the final multivariable model.

Goodness-of-fit analysis was compared by using Akaike information criterion (AIC).

Harrell's c statistic was performed to evaluate multivariate model discrimination, and the calibration was assessed via the Hosmer–Lemeshow test. Internal validation in derivation cohort was performed via 10-fold cross-validation.

Statistical analyses were performed with Stata software (release 14.2; StataCorp, College Station, TX).

A two-sided P value <0.05 was considered statistically significant.

Results

All stable transplant patients on follow-up at our institution (Nephrology and Transplantation Clinical Unit in Genoa – IRCCS San Martino Hospital) from 1st January 2015 to 31st December 2016, were included in our analysis.

We enrolled all patients with a functioning graft transplanted 1 year before the evaluation. Patients referred to another outpatient clinic for follow-up were excluded.

After a median of 68 months, CV events occurred in 71 KTRs (19%) with an incidence rate of 51 events per 1000 patient-years. Table 1 shows baseline demographic, clinical, and laboratory parameters for the 71 KTRs with, and the 300 with no event.

Overall, the mean age was 61±13, 34% of patients were female, 89% had hypertension and 14% diabetes.

The glycometabolic status of participants was fairly good, being the mean values of glucose and LDL-C of 103 and 96 mg/dl, respectively. The average BP was 132±14/81±4 mmHg, eGFR was 51±18 ml/min per 1.73m^2 with proteinuria 0.2 g/ 24h (Table 1). No PTLD were found in our sample. Calcineurin inhibitor (CNI) was predominantly tacrolimus (TAC 72% vs CSA 38%).

There was no statistically significant difference in transplant-related parameters and comorbidities.

Patients who experienced the composite endpoint were older and had significantly lower eGFR and higher glucose levels, SBP measurements.

Firstly, we performed the two selected scores (FRS and PORT) on our population and evaluated their performance using ROC curves. Their discrimination power was low, with ROC areas of 0.53 for FRS and 0.57 for the PORT score (Fig. 2). Moreover, the addition of immunological variables, such as the presence of DSA and HLA compatibility, did not significantly enhance FRS predictive power (Figure 3).

As shown in Table 2, in the univariate analysis, age, dialysis vintage, donor age as well as kidney function and proteinuria were significantly associated to the occurrence of the composite outcome. As for the BP profile, an increase of 10 mmHg both for SBP was associated with a 21% increased risk of developing CV events.

To derive a new calculator chosen variables were considered as categorical by identifying a cut-off via fractional polynomials method.

By multivariable Cox regression, age higher than 60 years, eGFR less than 30 ml/min/1.73 m2, proteinuria higher than 1 g/24 h, dialysis vintage longer than 5 years and SBP higher than 140 mmHg were significant predictors of composite endpoint, and according to the hazard ratio, a score is assigned as reported in Table 3.

The derived score demonstrated better accuracy than the previously tested CV risk calculators (Harrel's c new score=0.73 vs FRS = 0.55; PORT= 0.54; ROC curve 0.73 vs FRS = 0.53; PORT= 0.57, P=0.048) (Fig. 2). Calibration by Hosmer-Lemeshow test was acceptable (χ^2 11.34, P=0.12). The new score was internally validated by 10-fold cross-validation (mean AUC 0.70, 95% CI 0.60-0.77).

For each point of the new score, the risk of the event increased by 40% (HR 1.40, CI 95% 1.26 – 1.55, P=0.0001) and a score higher than three was associated with a 4-fold increased risk of composite endpoint

(HR 3.89, CI 95% 2.41-6.28, P=0.0001). The odds ratios for CV outcome onset per two points increase in prediction score are shown in Table 4.

Discussion

As demonstrated by several studies, FRS, which is the most widely used tool for estimating the 10-year risk of CV events, underestimates CV risk in KTRs (9-10). This calculator was developed and validated primarily in the general population and does not consider all the non-traditional CV risk factors peculiar to the KTR population. Although the PORT score (5) is derived from a large cohort of transplant patients and considers variables closely related to transplantation (acute rejection, DGF), its use is not widespread due to the difficulty of application in clinical practice: it requires choosing among three scores based on transplant age and includes numerous variables.

Furthermore, there is another CV risk score developed by Soveri in 2012 (2) and externally validated on the PORT cohort (12). This calculator does not include immunological variables and was derived from the Assessment of LEscol in Renal Transplantation (ALERT) study cohort (13), a multicentric trial, thus reflecting the inclusion and exclusion criteria of the study population. This score external validation on the PORT cohort showed good discriminatory ability but a lack of calibration ($\chi 2 = 19.49$, $P = 0.01$).

Our study addressed a critical gap in cardiovascular risk assessment, highlighting the lack of an easily reproducible and reliable prediction tool in the KTR population that also considers the non-traditional risk factors typical of this population. Among these, CNI and steroid use could lead to long-term adverse effects such as hypertension, obesity, dyslipidemia, and glycometabolic alterations ranging from insulin resistance to post-transplant diabetes mellitus (PTDM) development (14). Recently, several studies have demonstrated that the immunological profile can influence CV morbidity and mortality; in Opelz's study (15), cardiovascular mortality correlated with the number of mismatches in a graded manner, and the author hypothesized that a high number of HLA mismatches may lead to an increase in immunosuppressive therapy burden, guiding to the aforementioned metabolic adverse effects. Additionally, Loupy et al. (16) demonstrated that the presence of circulating DSA is associated with severe graft atherosclerosis. The risk of cardiovascular events increases by 2.5 times in immunized patients, independently of traditional risk factors .

However, in our analysis, adding data regarding DSA to both FRS and PORT did not significantly increase the predictive capacity of the score, may due to the sample size.

The score we developed includes traditional risk factors such as age and hypertension, graft function-related factors such as eGFR and proteinuria, and dialysis vintage. Hypertension is a well-known CV risk factor in KTRs, and several studies have demonstrated its impact on both CV mortality (17) and graft function (14,18,19)). Renal function represents an additional factor predisposing to cardiovascular events. A post-hoc analysis from the Folic Acid for Vascular Outcome Reduction in Transplant (FAVORIT) trial demonstrated that eGFR lower than 45 ml/min/1.73 m2 is independently associated with CV outcomes, with a 69% increase in the composite outcome when eGFR is lower than 30 ml/min/1.73 m2 (20). Similarly, even moderate albuminuria at baseline (ACR > 30 mg/g) predicts CVD occurrence, regardless of renal function (21).

Similarly, dialysis vintage and subsequent uremic milieu represent factors that accelerate the atherosclerotic process, and although the CV risk after transplantation decreases drastically compared to patients remaining on hemodialysis, the time spent on hemodialysis remains an important factor influencing graft and patient survival (22, 23).

Our study has several limitations: its monocentric, observational, and retrospective nature makes it difficult to rule out the presence of confounding factors. Additionally, we recognize that the sample size and lack of external validation of the risk calculator may influence its clinical application. Moreover, PORT and FRS were derived to estimate the risk of CHD in the population under study, therefore, it is expected that their predictive power on MACE would be reduced.

However, the score was derived in a real-life population with immunosuppressive therapy management that reflects current clinical practice, with a clear prevalence of tacrolimus use over cyclosporine, unlike in the ALERT and PORT cohorts. The CV risk categories are heterogeneous, unlike what happens in the ALERT cohort, which excludes patients at high CV risk.

In conclusion, this study developed and internally validated a new cardiovascular risk calculator in kidney transplant patients. External validation of this calculator is necessary to expand the application of this score to everyday clinical practice. This tool is potentially easily usable and allows for effective stratification of CV event risk in KTRs and individualization of treatment.

References

1) Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. Am J Kidney Dis. 1998 Nov;32(5 Suppl 3):S112-9. doi: 10.1053/ajkd.1998.v32.pm9820470. PMID: 9820470.

2) Soveri I, Holme I, Holdaas H, Budde K, Jardine AG, Fellström B. A cardiovascular risk calculator for renal transplant recipients. Transplantation. 2012 Jul 15;94(1):57-62. doi: 10.1097/TP.0b013e3182516cdc. PMID: 22683851.

3) Mansell H, Stewart SA, Shoker A. Validity of cardiovascular risk prediction models in kidney transplant recipients. ScientificWorldJournal. 2014;2014:750579. doi: 10.1155/2014/750579. Epub 2014 Apr 8. PMID: 24977223; PMCID: PMC3996891.

4) Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation. 1998 May 12;97(18):1837-47. doi: 10.1161/01.cir.97.18.1837. PMID: 9603539.

5) Israni AK, Snyder JJ, Skeans MA, Peng Y, Maclean JR, Weinhandl ED, Kasiske BL; PORT Investigators. Predicting coronary heart disease after kidney transplantation: Patient Outcomes in Renal Transplantation (PORT) Study. Am J Transplant. 2010 Feb;10(2):338-53. doi: 10.1111/j.1600-6143.2009.02949.x. PMID: 20415903.

6) Siedlecki A, Irish W, Brennan DC. Delayed graft function in the kidney transplant. Am J Transplant. 2011 Nov;11(11):2279-96. doi: 10.1111/j.1600-6143.2011.03754.x. Epub 2011 Sep 19. PMID: 21929642; PMCID: PMC3280444.

7) Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972 Jun;18(6):499-502. PMID: 4337382.

8) Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J; CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration). A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009 May 5;150(9):604-12. doi: 10.7326/0003-4819-150-9-200905050-00006. Erratum in: Ann Intern Med. 2011 Sep 20;155(6):408. PMID: 19414839; PMCID: PMC2763564.

9) Ducloux D, Kazory A, Chalopin JM. Predicting coronary heart disease in renal transplant recipients: a prospective study. Kidney Int. 2004 Jul;66(1):441-7. doi: 10.1111/j.1523-1755.2004.00751.x. PMID: 15200454.

10) Kasiske BL, Chakkera HA, Roel J. Explained and unexplained ischemic heart disease risk after renal transplantation. J Am Soc Nephrol. 2000 Sep;11(9):1735-1743. doi: 10.1681/ASN.V1191735. PMID: 10966499.

11) Kiberd B, Panek R. Cardiovascular outcomes in the outpatient kidney transplant clinic: the Framingham risk score revisited. Clin J Am Soc Nephrol. 2008 May;3(3):822-8. doi: 10.2215/CJN.00030108. Epub 2008 Mar 5. PMID: 18322053; PMCID: PMC2386708.

12) Soveri I, Snyder J, Holdaas H, Holme I, Jardine AG, L'Italien GJ, Fellström B. The external validation of the cardiovascular risk equation for renal transplant recipients: applications to BENEFIT and BENEFIT-EXT trials. Transplantation. 2013 Jan 15;95(1):142-7. doi: 10.1097/TP.0b013e31827722c9. PMID: 23192156.

13) Holdaas H, Fellström B, Jardine AG, Holme I, Nyberg G, Fauchald P, Grönhagen-Riska C, Madsen S, Neumayer HH, Cole E, Maes B, Ambühl P, Olsson AG, Hartmann A, Solbu DO, Pedersen TR; Assessment of LEscol in Renal Transplantation (ALERT) Study Investigators. Effect of fluvastatin on cardiac outcomes in renal transplant recipients: a multicentre, randomised, placebo-controlled trial. Lancet. 2003 Jun 14;361(9374):2024-31. doi: 10.1016/S0140-6736(03)13638-0. PMID: 12814712.

14) Paoletti E, Citterio F, Corsini A, Potena L, Rigotti P, Sandrini S, Bussalino E, Stallone G; ENTROPIA Project. Everolimus in kidney transplant recipients at high cardiovascular risk: a narrative review. J Nephrol. 2020 Feb;33(1):69-82. doi: 10.1007/s40620-019-00609-y. Epub 2019 Apr 27. PMID: 31028549.

15) Opelz G, Döhler B. Association of HLA mismatch with death with a functioning graft after kidney transplantation: a collaborative transplant study report. Am J Transplant. 2012 Nov;12(11):3031-8. doi: 10.1111/j.1600-6143.2012.04226.x. Epub 2012 Aug 17. PMID: 22900931.

16) Loupy A, Vernerey D, Viglietti D, Aubert O, Duong Van Huyen JP, Empana JP, Bruneval P, Glotz D, Legendre C, Jouven X, Lefaucheur C. Determinants and Outcomes of Accelerated Arteriosclerosis: Major

Impact of Circulating Antibodies. Circ Res. 2015 Aug 14;117(5):470-82. doi: 10.1161/CIRCRESAHA.117.306340. Epub 2015 Jun 8. PMID: 26056252.

17) Opelz G, Döhler B; Collaborative Transplant Study. Improved long-term outcomes after renal transplantation associated with blood pressure control. Am J Transplant. 2005 Nov;5(11):2725-31. doi: 10.1111/j.1600-6143.2005.01093.x. PMID: 16212633.

18) Mallamaci F, D'Arrigo G, Tripepi R, Leonardis D, Porto G, Testa A, Abd ElHafeez S, Mafrica A, Versace MC, Provenzano PF, Tripepi G, Mancini P, Zoccali C. Office, standardized and 24-h ambulatory blood pressure and renal function loss in renal transplant patients. J Hypertens. 2018 Jan;36(1):119-125. doi: 10.1097/HJH.0000000000001530. PMID: 28858982.

19) Kasiske BL, Anjum S, Shah R, Skogen J, Kandaswamy C, Danielson B, O'Shaughnessy EA, Dahl DC, Silkensen JR, Sahadevan M, Snyder JJ. Hypertension after kidney transplantation. Am J Kidney Dis. 2004 Jun;43(6):1071-81. doi: 10.1053/j.ajkd.2004.03.013. PMID: 15168388.

20) Weiner DE, Carpenter MA, Levey AS, Ivanova A, Cole EH, Hunsicker L, Kasiske BL, Kim SJ, Kusek JW, Bostom AG. Kidney function and risk of cardiovascular disease and mortality in kidney transplant recipients: the FAVORIT trial. Am J Transplant. 2012 Sep;12(9):2437-45. doi: 10.1111/j.1600-6143.2012.04101.x. Epub 2012 May 17. PMID: 22594581; PMCID: PMC3424309.

21) Weiner DE, Park M, Tighiouart H, Joseph AA, Carpenter MA, Goyal N, House AA, Hsu CY, Ix JH, Jacques PF, Kew CE, Kim SJ, Kusek JW, Pesavento TE, Pfeffer MA, Smith SR, Weir MR, Levey AS, Bostom AG. Albuminuria and Allograft Failure, Cardiovascular Disease Events, and All-Cause Death in Stable Kidney Transplant Recipients: A Cohort Analysis of the FAVORIT Trial. Am J Kidney Dis. 2019 Jan;73(1):51-61. doi: 10.1053/j.ajkd.2018.05.015. Epub 2018 Jul 20. PMID: 30037726; PMCID: PMC6309643.

22) Haller MC, Kammer M, Oberbauer R. Dialysis vintage and outcomes in renal transplantation. Nephrol Dial Transplant. 2019 Apr 1;34(4):555-560. doi: 10.1093/ndt/gfy099. PMID: 29897595.

23) Aalten J, Hoogeveen EK, Roodnat JI, Weimar W, Borm GF, de Fijter JW, Hoitsma AJ. Associations between pre-kidney-transplant risk factors and post-transplant cardiovascular events and death. Transpl Int. 2008 Oct;21(10):985-91. doi: 10.1111/j.1432-2277.2008.00717.x. Epub 2008 Jun 28. PMID: 18564985

TABLES

Table 1. Baseline characteristic from study patients stratified by cardiovascular event occurrence.

Variable	All	No CV event	CV event	P
N	371	300	71	
Age, years	**60.6 ± 13.5**	**59.1 ± 13.6**	**66.7 ± 11.4**	**0.001**
Gender = female, n (%)	125 (34)	99 (33)	26 (36)	0.28
Smoking, n (%)				
Dialysis vintage, months (IQR)	36 (19-60)	36 (19 - 56)	38 (19 - 70)	0.42
With hypertension, n (%)	330 (89)	271 (90)	62 (87)	1.00
With diabetes, n (%)	53 (14)	38 (12)	15 (21)	0.09
With previous cardiovascular disease, n (%)	49 (13)	38 (12)	11 (15)	0.56
Second kidney transplant, n (%)	37 (10)	29 (10)	8(11)	1.00
Donor Age, years	**51.1 ± 15.3**	**49.7 ± 15.6**	**56.6 ± 12.7**	**0.005**
Mismatches, n	3.2 ± 1.2	3.1±1.17	3.3±1.17	0.45
DGF, n (%)	70 (19)	59 (20)	11 (15)	0.50
With DSA, n (%)	13 (3.5)	13 (4)	0 (0)	0.14
With PRA higher than 10% at tranplantation, n (%)	30 (9)	25 (8)	5 (7)	1.00
SBP, mmHg	**132 ± 14**	**131± 14**	**136 ± 15**	**0.004**
DBP, mmHg	**81 ± 4**	**79 ± 8**	**90 ± 6**	**0.05**
Creatinine (mg/dL)	**1.5± 0.5**	**1.5 ± 0.5**	**1.6 ± 0.7**	**0.01**
eGFR, ml/min/1.73m^2 (IQR)	**51 ± 18**	**53 ± 18**	**45 ± 16**	**0.001**
Proteinuria (g/24h)	**0.2 (0.1- 0.3)**	**0.2 (0.1 - 0.3)**	**0.3 (0.1 - 0.6)**	**0.02**
Glucose, mg/dL	**103 ± 23**	**101 ± 20**	**110 ± 33**	**0.04**
Total cholesterol, mg/dL	191 ± 41	193 ± 42	182 ± 35	0.05
HDL, mg/dL	54± 16	55 ± 17	53 ± 16	0.57
Triglycerides, mg/dL (IQR)	122 (91-172)	122 (91 - 170)	116 (88 - 180)	0.81
LDL, mg/dL (IQR)	96 (76 -115)	97 (78 - 117)	89 (72 - 108)	0.14
On steroid, n (%)	348 (94)	282 (94)	66 (93)	1.00
On CNI, n (%)	359 (97)	291 (97)	68 (98)	1.00
On mTORi, n (%)	19 (5)	18 (6)	1 (1)	0.21
On azatioprine, n (%)	7 (2)	6 (2)	1 (1)	1.00
On MMF, n (%)	315 (85)	252 (84)	63 (88)	1.00

IQR= interquantile range; DGF= delayed graft function; DSA= donor specific antibodies; PRA= panel reactive antibody; SBP= systolic blood pressure; DBP = diastolic blood pressure; eGFR= estimated glomerular filtration rate; HDL= high-density lipoprotein cholesterol; LDL= low-density lipoprotein cholesterol; CNI= calcineurin inhibitors; mTORi= mammalian target of rapamycin inhibitors; MMF= mycophenolate mofetil

Table 2. Univariate Cox regression analysis for the occurrence of fatal and non-fatal cardiovascular events

Variable	All	P
Age, years	**1.05 (1.03-1.07)**	**0.0001**
Gender = female, n (%)	1.09(0.67-1.77)	0.71
Smoking, n (%)	1.7 (0.88-2.1)	0.72
Dialysis vintage, months (IQR)	**1.01 (1.00-1.02)**	**0.03**
With hypertension, n (%)	1.02 (0.31-3.29)	0.97
With diabetes, n (%)	1.49 (0.82-2.68)	0.19
With previous cardiovascular disease, n (%)	1.19 (0.62-2.27)	0.59
Second kidney transplant, n (%)	0.99 (0.39-2.50)	0.99
Donor Age, years	**1.03 (1.01-1.06)**	**0.002**
Mismatches, n	1.15 (0.93-1.42)	0.19
DGF, n (%)	0.68(0.35-1.32)	0.26
With DSA, n (%)	1.62 (0.42-1.9)	0.60
With PRA higher than 10% at tranplantation, n (%)	1.0 (0.98-1.01)	0.70
SBP, 10 mmHg	**1.21 (1.04-1.41)**	**0.01**
DBP, 10 mmHg	1.01 (0.99-1.04)	0.19
Creatinine (mg/dL)	**1.61 (1.19-2.18)**	**0.002**
eGFR, ml/min/1.73m^2 (IQR)	**0.97 (0.95-0.98)**	**0.0001**
Proteinuria (g/24h)	**1.68 (1.27-2.23)**	**0.0001**
Glucose, mg/dL	1.01 (0.98-1.01)	0.06
Total cholesterol, mg/dL	0.99 (0.98-1.00)	0.08
HDL, mg/dL	0.99 (0.97-1.01)	0.46
Triglycerides, mg/dL (IQR)	1.00 (0.99-1.00)	0.32
LDL, mg/dL (IQR)	0.99(0.98-1.00)	0.18
On steroid, n (%)	0.88(0.31-2.44)	0.81
On CNI, n (%)	1.16(0.16-8.40)	0.88
On mTORi, n (%)	0.28 (0.03-2.04)	0.21
On azatioprine, n (%)	0.93 (0.12-6.79)	0.95
On MMF, n (%)	1.05 (0.95-2.04)	0.10

IQR= interquantile range; DGF= delayed graft function; DSA= donor specific antibodies; PRA= panel reactive antibody; SBP= systolic blood pressure; DBP = diastolic blood pressure; eGFR= estimated glomerular filtration rate; HDL= high-density lipoprotein cholesterol; LDL= low-density lipoprotein cholesterol; CNI= calcineurin inhibitors; mTORi= mammalian target of rapamycin inhibitors; MMF= mycophenolate mofetil

T**able 3. Multivariate Cox regression analyses for the occurrence of fatal and non-fatal cardiovascular**

Variable	HR (CI 95%)	P	Points
Age higher than 60 years	2.92 (1.65-5.16)	0.0001	3
Proteinuria higher than 1 g/24 h	2.60 (1.12-5.13)	0.02	3
eGFR, lower than 30 ml/min/1.73m^2	2.13 (1.04-4.34)	0.04	2
SBP higher than 140 mmHg	1.66 (1.04-1.88)	0.02	2
Dialysis vintage higher than 5 y	1.53 (1.10-1.95)	0.01	2

Table 4. Odds ratios per two-points increase in prediction score.

Total risk score	OR (CI 95%)
0-1	Reference
2-3	1.81 (0.82 - 3.98)
4-5	4.19 (1.85 - 9.50)
6-9	8.97 (3.5 - 22.88)

FIGURE

Figure 1. Study flow diagram

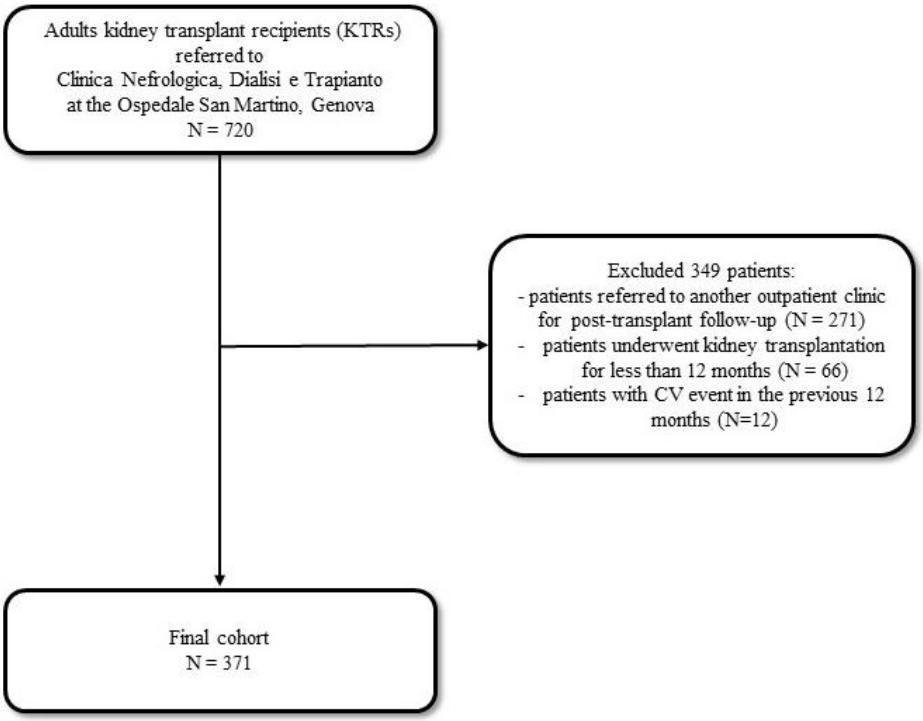

Figure 2. Area under the curve for FRS, PORT and new score (P= 0.04)

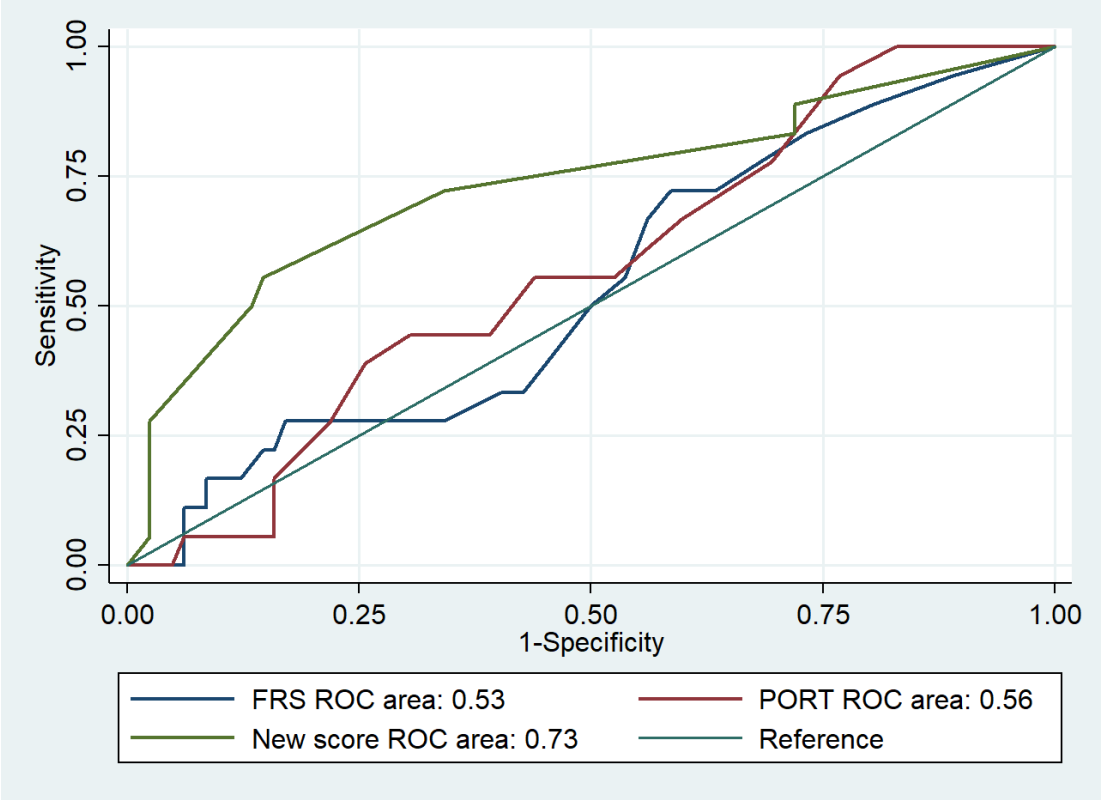

Figure 3. Area under the curve for FRS , FRS and DSA, FRS and number of mismatches (MM), FRA, DSA and number of mismatches (MM) (P=0.94)