Original Research Article

Serum Apolipoprotein B and A1 Concentrations Predict Late-Onset Posttransplant Diabetes Mellitus in Prevalent Adult Kidney Transplant Recipients

Rohit Malyala[1], Lindita Rapi[1], Michelle M. Nash[1], and G. V. Ramesh Prasad[1,2] iD

Abstract

Background: Glucose metabolism links closely to cholesterol metabolism. Posttransplant diabetes mellitus (PTDM) adversely affects posttransplant outcomes, but its risk factors in relation to cholesterol metabolism have not been fully delineated. The apolipoprotein B/A1 (Apo B/A1) ratio, which is associated with insulin resistance, has not been evaluated in kidney transplant recipients as a risk factor for PTDM.

Objective: The objective of this study was to determine whether serum apolipoprotein profiles predict late PTDM, defined as a new onset diabetes occurring greater than 3 months posttransplant.

Design: Retrospective chart review of a prevalent population of kidney transplant recipients.

Setting: Large transplant center in Ontario, Canada.

Patients: We identified 1104 previously nondiabetic adults who received a kidney transplant between January 1, 1998, and December 1, 2015, and were followed at 1 transplant center.

Measurements: Recipients provided testing for serum apolipoprotein B (Apo B) and apolipoprotein A1 (Apo A1) concentrations from 2010, either at 3 months posttransplant for new transplant recipients or the next clinic visit for prevalent recipients. Late PTDM defined using Canadian Diabetes Association criteria as occurring ≥3 months posttransplant was recorded until May 1, 2016.

Methods: All analyses were conducted with R, version 3.4.0 (The R Foundation for Statistical Computing). Comparisons were made using Student t test, Fisher exact test or chi-square test, Kaplan-Meier methodology with the logrank test, or Cox proportional hazards analysis as appropriate. Covariates for the multivariate Cox proportional hazards models of PTDM as the outcome variable were selected based on significance of the univariate associations and biological plausibility.

Results: There were 53 incident late PTDM cases, or 1.71 cases per 100 patient-years. Incident late PTDM differed between the highest and lowest quartiles for Apo B/A1 ratio, 2.47 per 100 patient-years vs 0.88 per 100 patient-years ($P = .005$ for difference). In multiple Cox regression analysis, first measured serum Apo B/A1 concentration better predicted subsequent PTDM than low-density lipoprotein cholesterol (LDL-C; hazard ratio [HR] = 7.80 per unit increase, $P = .039$ vs HR = 1.05 per unit increase, $P = .774$). Non-high-density lipoprotein cholesterol (HDL-C) concentrations also did not predict PTDM ($P = .136$). By contrast to Apo B, Apo A1 was protective against PTDM in statin users (HR = 0.17 per unit increase, $P = .016$).

Limitations: Posttransplant diabetes mellitus cases occurring before apolipoprotein testing was implemented were not included in the analysis.

Conclusions: Apolipoproteins B and A1 better predict late PTDM than conventional markers of cholesterol metabolism.

Abrégé

Contexte: Le métabolisme du glucose est étroitement lié à celui du cholestérol. Le diabète sucré post-transplantation (PTDM—*Post-Transplant Diabetes Mellitus*) compromet l'état de santé après la greffe, mais le risque qu'il représente sur le métabolisme du cholestérol n'est toujours pas clairement défini. Le taux d'apolipoprotéine B/A1 (Apo B/A1), associé à l'insulinorésistance, n'a toujours pas été évalué en tant que facteur de risque pour le PTDM chez les receveurs d'une greffe rénale.

Objectif: Cette étude visait à déterminer si les profils sériques de l'apolipoprotéine sont prédicteurs d'un PTDM d'apparition tardive, soit d'un diabète se déclenchant plus de trois mois post-transplantation.

Type d'étude: Une étude rétrospective des dossiers médicaux d'une population prévalente de receveurs d'une greffe rénale.

Cadre: Un important centre de transplantation de l'Ontario (Canada).

Sujets: L'étude porte sur 1 104 adultes non-diabétiques ayant subi une greffe rénale entre le 1er janvier 1998 et le 1er décembre 2015 et ayant été suivis dans un centre de transplantation.

Mesures: À partir de 2010, les sujets se sont soumis à une épreuve mesurant les concentrations sériques d'Apo B et Apo A1 trois mois post-greffe pour les nouveaux receveurs ou lors de la prochaine consultation en clinique pour les receveurs prévalents. La survenue d'un PTDM d'apparition tardive, soit au minimum trois mois post-greffe selon le critère de l'Association canadienne du diabète, a été enregistrée jusqu'au 1er mai 2016.

Méthodologie: Toutes les analyses ont été menées avec le logiciel R (*R Foundation for Statistical Computing* version 3.4.0). Selon le cas, les comparaisons ont été effectuées par le test t de Student, le test de Fisher exact, le test de chi-deux, la méthode de Kaplan-Meier avec le test de Mantel-Haenzel ou l'analyse de régression aléatoire proportionnelle de Cox. Les covariables du modèle multivarié de régression aléatoire proportionnelle de Cox avec le PTDM comme variable résultat ont été choisies en fonction de l'importance des associations univariées et de la plausibilité biologique.

Résultats: On a répertorié 53 nouveaux cas de PTDM d'apparition tardive, soit 1,71 cas par 100 années-patient. Le nombre de nouveaux cas de PTDM d'apparition tardive différait entre le quartile le plus élevé et le quartile le plus bas pour le taux d'Apo B/A1, avec 2,47 par 100 années-patient et 0,88 par 100 années-patient respectivement (*P* = 0,005 pour la différence). Selon l'analyse par régression multivariée de Cox, la première mesure de la concentration d'Apo B/A1 s'est avérée un meilleur prédicteur d'un PTDM subséquent que la mesure de LDL-C (RR à 7,80 par augmentation d'une unité pour Apo B/A1, *P* = 0,039 contre 1,05 par augmentation d'une unité pour LDL-C, *P* = 0,774). Les taux de cholestérol non HDL n'ont pas non plus prédit un PTDM (*P* = 0,136). Contrairement à Apo B, Apo A1 protégeait contre le déclenchement d'un PTDM chez les utilisateurs de statines (RR: 0,17 par augmentation d'une unité, *P* = 0,016).

Limite: Les cas de PTDM survenus avant que l'épreuve d'apolipoprotéine ne soit mise en œuvre n'ont pas été inclus dans cette analyse.

Conclusion: Les apolipoprotéines B et A1 ont mieux prédit la survenue du PTDM d'apparition tardive que les marqueurs traditionnels du métabolisme du cholestérol.

What was known before

Conventional predictors of posttransplant diabetes mellitus (PTDM) include age, ethnicity, weight gain, and immunosuppressive medications, among other factors.

What this adds

The apolipoprotein profile is superior to conventional lipid parameters in predicting PTDM. Apolipoprotein B predicted PTDM in nonstatin users while Apo A1 protected against PTDM in statin users. The benefit of statins after transplantation might mediate through Apo B reduction, but this benefit may not be captured through low-density lipoprotein cholesterol reduction.

Introduction

Posttransplant diabetes mellitus (PTDM) associates with increased graft failure and mortality.[1,2] Despite numerous well-identified risk factors for PTDM, its incidence remains high.[3] Posttransplant diabetes mellitus is generally considered an early posttransplant complication occurring in the first 3 months that entails aggressive efforts such as dietary modification and a rapid reduction in corticosteroid dose. However, late PTDM is also described,[4] and management strategies targeting late PTDM will likely additionally improve long-term outcomes. Long-term prevention to reduce the overall PTDM burden mandates further efforts to understand the risk factors behind late PTDM as they occur in kidney transplant recipients (KTR).

[1]Kidney Transplant Program, St. Michael's Hospital, Toronto, ON, Canada
[2]Division of Nephrology, Department of Medicine, University of Toronto, ON, Canada

Corresponding Author:

G. V. Ramesh Prasad, Kidney Transplant Program, St. Michael's Hospital, 61 Queen Street East, 9th Floor, Toronto, ON, Canada M5C 2T2.
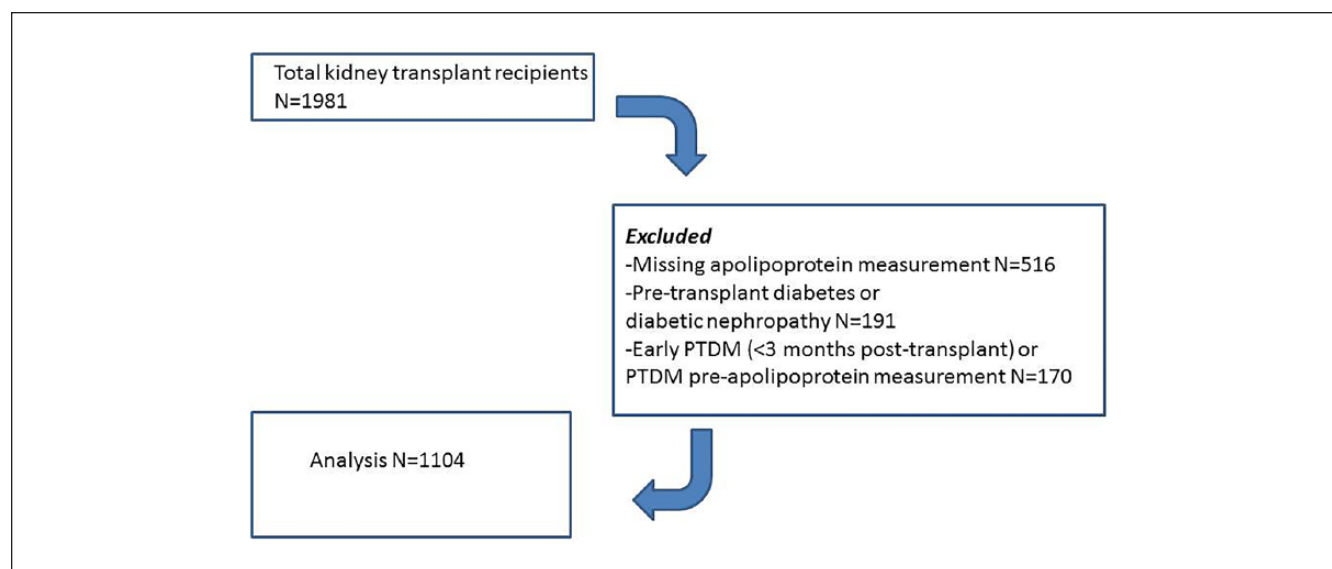Email: prasadr@smh.ca

Figure 1. Outline of inclusion and exclusion procedure.
Note. PTDM = posttransplant diabetes mellitus.

A candidate risk factor in the epidemiology of type 2 diabetes is the serum apolipoprotein profile.[5] Apolipoprotein A1 (Apo A1) and apolipoprotein B (Apo B) are the primary constituents of antiatherogenic high-density lipoprotein cholesterol (HDL-C) and atherogenic lower density lipoproteins (very low-density lipoprotein cholesterol [VLDL-C], intermediate-density lipoprotein cholesterol [IDL-C], low-density lipoprotein cholesterol [LDL-C]). To date, interest in Apo A1 and Apo B has primarily focused on their role as a independent predictors of cardiovascular disease in the general population.[6] However, the Apo B/Apo A1 ratio was also found to be correlated with insulin resistance[7] and components of the metabolic syndrome.[8] The Apolipoprotein B/A1 (Apo B/A1) ratio has also been independently associated with type II diabetes mellitus (DM).[9]

Although a shift in the serum apolipoprotein profile has been documented after kidney transplantation,[10] any impact of the posttransplant apolipoprotein profiles on posttransplant outcomes such as PTDM and cardiovascular disease has not been ascertained. The aim of this study therefore was to investigate if the serum apolipoprotein profile is independently associated with late PTDM. Our hypothesis was that a high posttransplant Apo B/A1 ratio would correlate better with PTDM incidence compared with more conventional lipid parameters, even in the presence of confounding factors such as lipid-lowering medication that are often prescribed to KTR.

Patients and Methods

Study Sample and Follow-up

St. Michael's Hospital (SMH) is an urban university-affiliated tertiary-care medical center that actively follows more than 1700 prevalent KTR and performs approximately 120 single-organ kidney transplants annually. Clinic visit frequency is typically weekly to month 1 posttransplant, biweekly to month 3, monthly to month 6, quarterly to month 12, and twice annually or annually thereafter. At each clinic visit, trained personnel record anthropometry and resting blood pressure (BP). Ethnicity is recorded based on self-report. Laboratory testing is performed as close to each clinic visit as possible, with additional testing performed between visits according to a separate, more frequent schedule. The fasting lipid profile is obtained every 6 months, and fasting or random glucose tests are performed at least once every 3 months. The estimated glomerular filtration rate (eGFR) is calculated by the Modification of Diet in Renal Disease-7 equation.[11] Starting in 2010, nonfasting Apo B and Apo A1 concentrations have been measured in all KTR at least once either at the first visit after this policy was implemented in 2010 or at 3 months posttransplant in the case of new KTR starting in 2010.

For this study, all KTR followed at SMH and transplanted between January 1, 1998, and December 31, 2015, were identified from the clinical electronic database. We performed a retrospective cohort analysis of the prevalent population of this cohort as of May 1, 2016, to ensure that all patients had a minimum of 3 months potential posttransplant follow-up. The procedure for inclusion and exclusion is outlined in Figure 1. Institutional Research Ethics Board approval (REB10-204c) was obtained for the study. As this study was a retrospective review of clinic data pertaining to a prevalent KTR population, individual informed consent was not obtained. The study was performed in accordance with the 2000 Declaration of Helsinki and the 2008 Declaration of Istanbul.

Data Collection

Occurrence of PTDM was routinely captured by a review of transcriptions from local and citywide hospital databases as part of routine care and supplemented by patient and family physician interviews where necessary. The main outcome measure was incident PTDM, diagnosed and transcribed by a physician using the 2008 Canadian Diabetes Association Guidelines[12] while metabolic syndrome status was assessed using National Cholesterol Education Program Adult Treatment Panel III guidelines.[13] At least 2 blood glucose readings above diagnostic cut-points or other valid diagnostic tests such as oral glucose tolerance testing were required for the diagnosis of PTDM, which was then validated by dietician and/or endocrinologist consult notes. Patients without measurements for either Apo A1 or Apo B, PTDM within 3 months posttransplant, or known type 2 diabetes that occurred prior to transplant including diabetic nephropathy were excluded. Transplant and pretransplant characteristics were determined at the time of transplantation. All clinical information, laboratory measurement, medication, and physical characteristics data were obtained at the time of apolipoprotein measurement or at the closest posttransplant follow-up date to the date of apolipoprotein measurement. A more restricted 2010-2015 cohort was also identified to assess patients without previous long-term posttransplant follow-up, to exclude any possible impact of subtle preventative measures against PTDM in the older patients.

Our primary outcome of interest was the development of late PTDM, diagnosed 3 months posttransplant or later. Earlier PTDM may be more proportionally attributable to unidentified preexisting disease burden, postoperative stress, and higher immunosuppression rather than traditional risk factors for diabetes, similar to the case with major adverse posttransplant cardiac events.[14] The first apolipoprotein measurement, which was used for this analysis, was typically obtained 3 months posttransplant. Temporality between the measurement of apolipoprotein concentrations and PTDM diagnosis was assured by recording PTDM events only after the apolipoprotein measurement. As routine apolipoprotein testing for postoperative KTR began in 2010, patients who developed PTDM prior to 2010 were excluded from analysis. Fasting lipid profile and other laboratory measurements were recorded through a review of the clinical chart utilizing those closest to the date of the apolipoprotein concentration measurement, again preceding any diagnosis of PTDM.

Statistical Analysis

All analyses were conducted with R, version 3.4.0 (The R Foundation for Statistical Computing). All data are reported as mean ± SD, unless otherwise stated. Two-tailed *P* values below .05 were taken to imply statistical significance. All missing values were handled by exclusion from the relevant analysis without imputation. Comparisons were made using Student *t* test, Fisher exact test or chi-square test, Kaplan-Meier methodology with the logrank test, or Cox proportional hazards analysis as appropriate. Normality and skewness were assessed visually or with skewness tests for all subgroups, supplemented with the Shapiro-Wilk normality test for small-n subgroups. The selection of covariates for the multivariate Cox proportional hazards models of PTDM as the outcome variable was based on the significance of the univariate associations as well as biological plausibility.[15,16]

Results

A total of 1981 patients were identified on initial screening. There were 516 patients excluded from further analysis due to missing apolipoprotein concentration measurements, 191 due to pretransplant diabetes or diabetic nephropathy, and 170 due to a diagnosis of PTDM either in the first 3 months posttransplant or prior to apolipoprotein concentration measurement. Therefore, 1104 patients met inclusion criteria for further analysis (Figure 1). Full primary data were available for more than 95% of all physical and laboratory parameters measured. Patients were censored if lost to follow-up (no laboratory testing or clinic visits for 12 months) on the date of their last clinic visit. Median time to first Apo B/A1 measurement was 1.73 years for the 1998-2015 cohort and 3.3 months for the 2010-2015 cohort.

Demographic and other characteristics organized by quartiles of the Apo B/A1 ratio are provided in Table 1. Each quartile provided an approximately equal 775 patient-years of follow-up. A higher Apo B/A1 ratio correlated significantly with male sex, as did a higher waist and hip circumference, and polycystic kidney disease. Other notable associations with higher Apo B/A1 ratios included greater use of cyclosporine, higher serum C-reactive protein, parathyroid hormone, and uric acid concentrations, higher urine albumin-to-creatinine ratios, and decreased kidney allograft function (Table 1). More than 98% of all patients received prednisone. All measured lipid parameters demonstrated normality of distribution. Demographics for the restricted 2010-2015 cohort are provided in Supplementary Table 1.

Posttransplant Diabetes Mellitus

There were 53 incident late PTDM cases in the total cohort over 3100 patient-years follow-up, corresponding to 1.71 cases/100 patient-years, including 29 incident PTDM cases in the 2010-2015 cohort. Median follow-up time after apolipoprotein measurement for the non-PTDM group was 3.07 years. Median time-to-incident PTDM after apolipoprotein measurement was 0.72 years or 262 days. Incidence rates by Apo B/A1 quartile demonstrate progressively increasing PTDM risk with increasing Apo B/A1 ratio (Figure 2). Late PTDM incidence differed between the highest and lowest quartiles for the Apo B/A1 ratio, 2.47 per 100 patient-years vs 0.88 per 100 patient-years (*P* = .005 for difference).

Table 1. Patient and Transplant Characteristics Across Apolipoprotein B/A1 Quartiles.

	Q1, N = 276	Q2, N = 276	Q3, N = 276	Q4, N = 276	R^2	P value
Characteristic						
Patient count, follow-up (p-y)	276, 797.4	276, 792.9	276, 741.2	276, 767.9	—	—
Apolipoprotein B/A1 ratio	0.356 ± 0.06	0.485 ± 0.03	0.603 ± 0.04	0.834 ± 0.16	—	—
Apolipoprotein A1 (g/L)	1.739 ± 0.30	1.581 ± 0.24	1.452 ± 0.23	1.318 ± 0.22	0.293	<.001
Apolipoprotein B (g/L)	0.615 ± 0.12	0.766 ± 0.12	0.874 ± 0.14	1.089 ± 0.23	0.642	<.001
Demography/allograft data						
Age at bloodwork (years)	54.98 ± 12.9	55.40 ± 13.3	54.66 ± 13.6	53.64 ± 13.4	0.000	.666
Age at transplant (years)	51.46 ± 12.9	51.42 ± 13.5	51.06 ± 13.9	49.22 ± 13.7	0.001	.261
>2 transplants	17, 6.1%	15, 5.4%	12, 4.3%	18, 6.5%	—	.977
Live donor	130, 47.1%	122, 44.2%	133, 48.2%	137, 49.6%	—	.445
Male	146, 52.9%	156, 56.5%	178, 64.5%	188, 68.1%	—	<.001
White Ethnicity	123, 44.6%	114, 41.3%	125, 45.3%	124, 44.9%	—	.144
Smoking	18, 6.52%	10, 3.62%	17, 6.16%	19, 6.88%	—	.125
ESRD cause						
Hypertension	25, 9.1%	39, 14.1%	34, 12.3%	35, 12.7%	0.44	.337
Glomerulonephritis	95, 34.4%	95, 34.4%	94, 34.1%	108, 39.1%	0.29	.365
Polycystic kidney disease	35, 12.7%	35, 12.7%	43, 15.6%	47, 17.0%	0.96	.020
Clinical characteristics						
Height (cm)	166.0 ± 22.3	168.4 ± 24.1	170.3 ± 20.4	169.5 ± 34.9	0.02	<.001
Weight (kg)	69.5 ± 16.0	73.1 ± 16.3	78.1 ± 17.1	76.9 ± 16.8	0.03	<.001
Body mass index (kg/m^2)	25.1 ± 4.5	25.7 ± 4.80	26.9 ± 5.01	26.8 ± 4.73	0.02	<.001
Systolic BP (mm Hg)	128.3 ± 15.4	128.3 ± 14.0	127.5 ± 15.9	130.0 ± 15.8	0.00	.213
Diastolic BP (mm Hg)	80.3 ± 9.5	81.2 ± 9.8	80.5 ± 9.3	83.1 ± 10.4	0.01	.003
Waist circumference (cm)	89.5 ± 16.9	91.2 ± 18.5	96.4 ± 16.7	96.5 ± 20.5	0.03	<.001
Hip circumference (cm)	95.8 ± 15.1	97.0 ± 17.0	100.2 ± 15.2	100.2 ± 20.0	0.02	<.001
Waist-to-hip ratio	0.9 ± 0.11	0.94 ± 0.14	0.96 ± 0.11	0.96 ± 0.17	0.03	<.001
Laboratory measurements						
Fasting blood glucose	5.4 ± 1.7	5.6 ± 1.6	5.8 ± 2.3	5.8 ± 1.6	0.01	.004
Random blood glucose	5.8 ± 1.9	5.9 ± 2.0	6.4 ± 2.9	6.3 ± 2.2	0.01	.002
HbA$_{1c}$ (%)	5.6 ± 0.8	5.7 ± 0.8	5.7 ± 1.1	5.9 ± 1.1	0.02	<.001
Total cholesterol	4.2 ± 1.0	4.5 ± 1.2	4.6 ± 1.1	5.2 ± 1.4	0.17	<.001
HDL-cholesterol	1.6 ± 0.5	1.4 ± 0.4	1.2 ± 0.4	1.1 ± 0.3	0.21	<.001
LDL-cholesterol	2.0 ± 0.8	2.4 ± 1.0	2.6 ± 1.1	3.1 ± 1.5	0.28	<.001
HDL/LDL ratio	1.3 ± 0.5	1.7 ± 0.7	2.2 ± 0.9	3.0 ± 1.4	0.60	<.001
Triglycerides	1.3 ± 0.9	1.6 ± 0.8	1.7 ± 0.7	2.3 ± 1.3	0.17	<.001
Non-HDL cholesterol	2.5 ± 0.8	3.0 ± 1.1	2.9 ± 7.6	4.1 ± 1.3	0.02	<.001
Total cholesterol/HDL ratio	2.7 ± 0.8	3.3 ± 1.1	3.9 ± 0.9	5.2 ± 2.9	0.36	<.001
C-reactive protein (mg/L)	3.2 ± 7.9	3.5 ± 5.3	4.9 ± 7.8	11.2 ± 29.5	0.05	<.001
Parathyroid hormone (pmol/L)	12.2 ± 14.1	12.0 ± 12.8	11.9 ± 9.9	14.5 ± 16.2	0.01	.023
Vitamin D25 (nmol/L)	56.5 ± 30.7	59.6 ± 30.6	55.3 ± 27.8	48.0 ± 27.7	0.02	<.001
Uric acid (µmol/L)	372.1 ± 105.0	376.7 ± 99.6	400.0 ± 101.0	413.5 ± 109.0	0.03	<.001
24-hour urine protein (g/d)	0.6 ± 0.7	0.5 ± 0.5	0.8 ± 1.3	0.74 ± 1.2	0.00	.184
Renal function/outcomes						
Serum creatinine (µmol/L)	117.9 ± 53.7	119.2 ± 40.6	127.4 ± 47.3	140.5 ± 78.9	0.03	<.001
Estimated GFR (mL/min/1.73 m^2)	58.3 ± 21.7	55.9 ± 18.0	53.71 ± 18.1	52.5 ± 20.1	0.02	<.001
Urine ACR (mg/mmol)	10.9 ± 32.9	10.9 ± 38.3	10.48 ± 29.9	22.9 ± 65.7	0.01	<.001
Graft failure	29, 10.5%	19, 6.9%	44, 15.9%	32, 11.6%	—	.103
Metabolic syndrome	204, 73.9%	185, 67.0%	203, 73.6%	210, 76.1%	—	.066
Acute rejection	20, 7.3%	23, 8.3%	29, 10.5%	24, 8.7%	—	.264
Delayed graft function	14, 5.07%	9, 3.26%	12, 4.35%	15, 5.43%	—	.583
Medications						
Statin	164, 59.4%	143, 51.8%	136, 49.3%	108, 39.1%	—	<.001
Cyclosporine	30, 10.9%	37, 13.4%	38, 13.8%	46, 16.7%	—	.011
Tacrolimus	230, 83.3%	217, 78.6%	220, 79.7%	208, 75.4%	—	.028
Mycophenolate mofetil	170, 61.6%	169, 61.2%	172, 62.3%	174, 63.0%	—	.939
Mycophenolic acid	60, 21.7%	63, 22.8%	73, 26.5%	58, 21.0%	—	.966

Note. R statistic not provided for binary characteristics, as logistic regression was used for these characteristics instead of linear regression. Laboratory measurements with unlisted units are mmol/L. Binary factors expressed in N (%) unless otherwise specified. p-y = patient years; ESRD = end-stage renal disease; BP = Blood pressure; HbA$_{1c}$ = glycated hemoglobin; HDL = high-density lipoprotein; LDL = low-density lipoprotein; GFR = glomerular filtration rate; ACR = albumin-to-creatinine ratio.
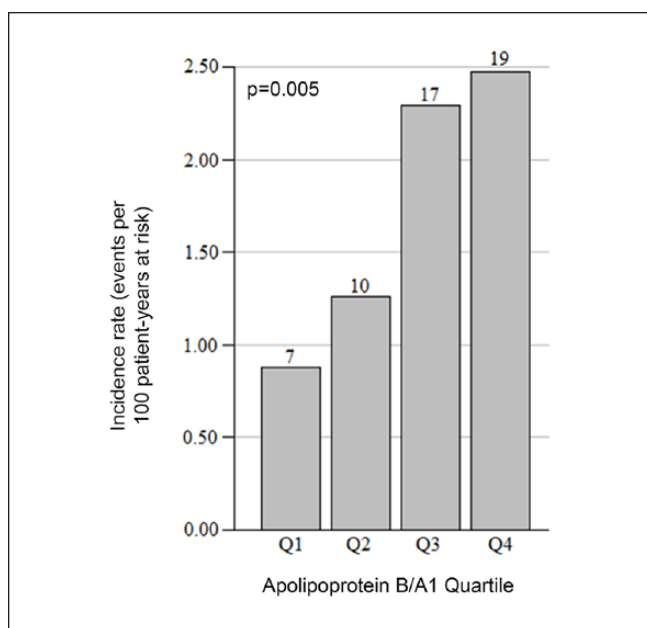
Figure 2. Incidence rate of PTDM per apolipoprotein B/A1 quartile.

Note. Rate is expressed in events per 100 patient-years at risk. Case count per quartile is shown over each bar with 276 patients present in each quartile. Significance was assessed using a test-for-trend in proportions. PTDM = posttransplant diabetes mellitus.

Kaplan-Meier survival analysis of time-to-PTDM diagnosis in the entire cohort (Figure 3) indicates significance of the Apo B and the Apo B1/A ratio higher than the median concentration, but not for above-median serum HDL-C and LDL-C concentrations. Univariate Cox regression analysis of Apo B/A1 ratio on PTDM incidence limited to the 2010-2015 cohort (Table 3) indicates a similar association between Apo B/A1 and PTDM (P = .008, hazard ratio [HR] = 8.246).

A phenotype characterization of the total cohort to identify differences between late PTDM and non-PTDM subjects is shown in Table 2, and for the restricted cohort in Supplementary Table 2. No significant differences were noted in graft failure, allograft function, or immunosuppression exposure between groups. Univariate Cox proportional hazards analysis for time-to-PTDM diagnosis of the restricted cohort is provided in Table 3, with significant predictors of PTDM including the Apo B/A1 ratio and Apo B concentrations, blood glucose and HbA_{1c} concentrations, waist-to-hip ratio, and serum triglyceride concentrations. The results of multivariate Cox regression analyses in this cohort including other potential indicators of pretransplant dyslipidemia are shown in Table 4. Per-unit increases in serum Apo B (HR = 8.41, P = .007) and Apo B/A1 ratio (HR = 7.80, P = .039) outperformed other conventional lipid parameters, significantly correlating to PTDM incidence.

The selective predictive value of Apo B and Apo A1 altered when considering statin use. Table 5 provides 6 stratified survival regression analyses for time-to-PTDM accounting for potential effect modifiers of the apolipoprotein-PTDM relationship. Apolipoprotein B, an obligate protein component of LDL-C, predicted PTDM in nonstatin users while Apo A1, the major protein component of HDL-C, protected against PTDM incidence in statin users (Table 5). Kaplan-Meier analyses of PTDM-free survival (Figure 4) demonstrated a predictive value to non-HDL-C in statin users corroborating this relationship of Apo A1 to PTDM.

Discussion

This retrospective study of a large, multiethnic cohort of KTR suggests a predictive value of the posttransplant apolipoprotein profile over more conventional lipid parameters toward late PTDM. Although dyslipidemia and diabetes correlate posttransplantation,[17] HDL-C and LDL-C proved less useful than apolipoprotein measurements in this study for this purpose. Metabolic syndrome predicts PTDM[18] with the caveat that type 2 diabetes locates within the metabolic syndrome itself.[14] The association of a high Apo B/A1 ratio and Apo B concentration with late PTDM identifies a new potential risk factor for diabetes in the presence of chronic kidney disease.

Apolipoproteins provide a new potential predictive PTDM risk marker situated outside of established metabolic syndrome definitions.[14] Apolipoprotein B/A1 profile abnormalities reflect higher diabetes and cardiovascular risk or mortality in the general population[19] and prevalent dialysis patients.[20,21] Such associations were previously unclear in KTR. Besides extending these findings to KTR, this study may also provide proximal mechanistic hypotheses of PTDM genesis and ultimately help reduce not only DM but cardiovascular disease located further along the causal pathway from PTDM because PTDM predicts cardiovascular disease.[22] Conventional risk markers of lipid turnover may assist in predicting diabetes in the general population but are less useful in KTR due to the presence of multiple confounders, so the apolipoprotein profile may be of greater assistance particularly in late PTDM. This study emphasizes risk factors for late PTDM, which is not the focus of clinical trials in KTR and whose risk factors require better delineation.

Hypertriglyceridemia occurs in type 2 diabetes from increased VLDL triglycerides or defective removal of plasma triglycerides.[23] IDL-C and LDL-C are the products of VLDL and chylomicron metabolism, which themselves contain triglycerides.[24] A posttransplant state impairs triglyceride clearance.[25] Given that Apo B is an obligate component of VLDL-C, IDL-C, and LDL-C, Apo B concentrations may more accurately reflect the hypertriglyceridemia burden of diabetes, and may hence indicate the prediabetic state. Apolipoprotein B overproduction has also been demonstrated to induce insulin resistance.[26] This study extends this role of Apo B to the posttransplant milieu after the environmentally murky early posttransplant phase has passed. As dyslipidemia and diabetes cluster as risk factors causing one
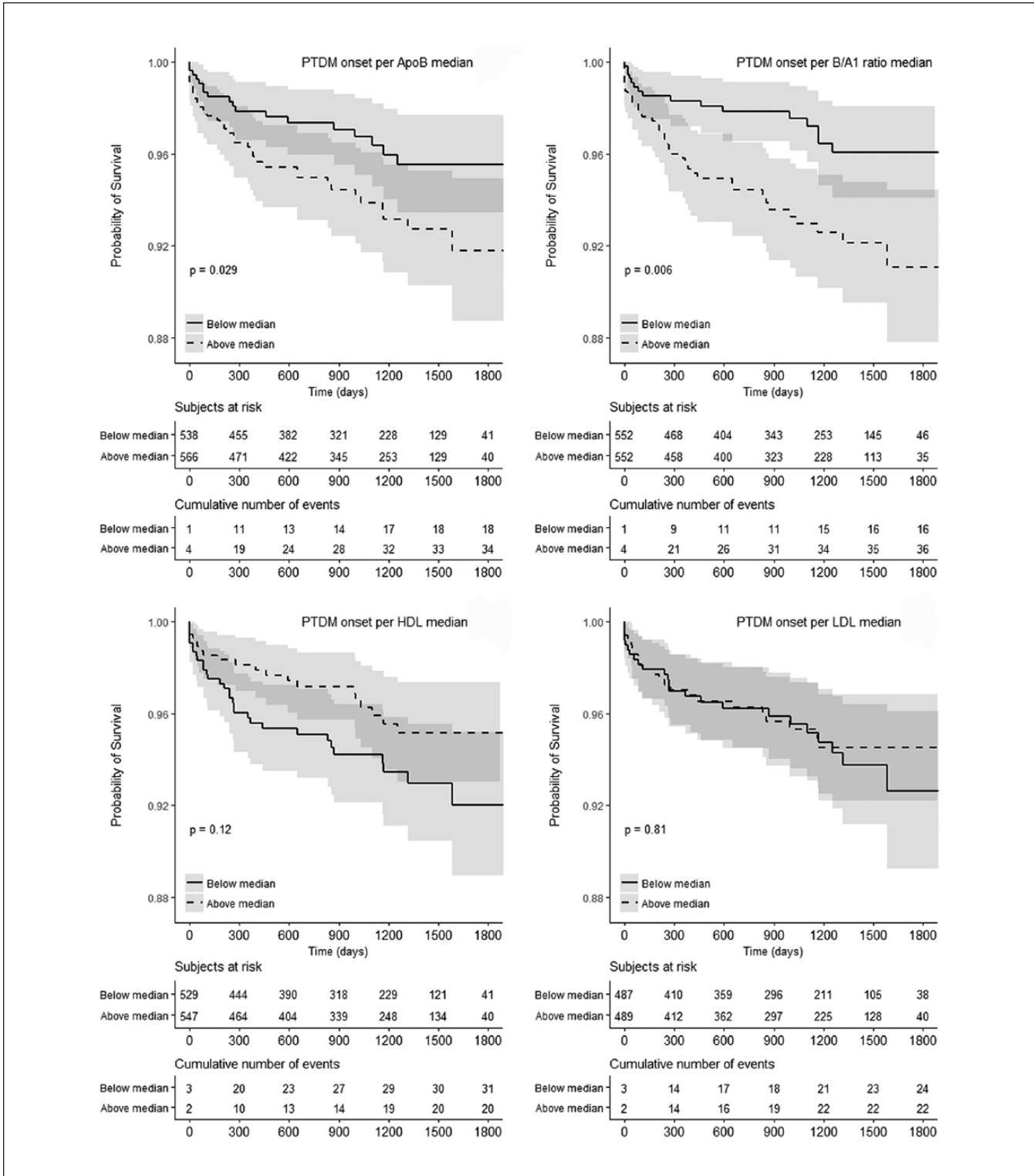
Figure 3. Time-to-PTDM incidence for Apo B, Apo B/A1, HDL-C, and LDL-C.
Note. Kaplan-Meier PTDM-free survival curves for subjects above or below the median for each lipid parameter. Log-rank test was used to assess significance. Apo A1 is not shown (*P* = .23 in Cox regression). Time is represented in days. PTDM = posttransplant diabetes mellitus; Apo B = apolipoprotein B; Apo B/A1 = apolipoprotein B/A1; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; Apo A1 = apolipoprotein A1.

Table 2. Differences in Clinical Characteristics Between Incident PTDM and Non-PTDM Cases.

Variable	PTDM (53)	No PTDM (1051)	P value
Apo A1 (g/L)	1.5 ± 0.3	1.52 ± 0.3	.328
Apo B (g/L)	0.9 ± 0.3	0.83 ± 0.2	.015
Apo B/A1 ratio	0.6 ± 0.2	0.57 ± 0.2	.026
Graft failure	6, 11.3%	118, 11.2%	.880
Serum creatinine (μmol/L)	118.3 ± 41.5	126.7 ± 58.3	.303
eGFR (mL/ min/1.73 m^2)	57.5 ± 21.9	55.0 ± 19.5	.372
FBG (mmol/L)	7.0 ± 2.2	5.57 ± 1.8	<.001
RBG (mmol/L)	8.1 ± 4.2	5.97 ± 2.1	<.001
Cyclosporine	9, 17.0%	142, 13.5%	.547
Tacrolimus	42, 79.3%	833, 79.3%	1.000
Statin	45, 84.9%	743, 70.7%	.401

Note. Significance assessed using comparison of incidence rates, Student *t* test, or Fisher exact test, as appropriate. PTDM = posttransplant diabetes mellitus; Apo A1 = apolipoprotein A1; Apo B = apolipoprotein B; Apo B/ A1 = apolipoprotein B/A1; eGFR = estimated glomerular filtration rate; FBG = fasting blood glucose; RBG = random blood glucose.

another,[27] posttransplant dyslipidemia better reflects in serum Apo B rather than LDL-C concentrations. Subclinical inflammation is also a component of the insulin resistance syndrome,[28] so Apo B may mediate posttransplant insulin resistance. Triglyceride-containing particle count, reflected through the Apo B concentration, may be more important than the triglyceride concentration itself in leading to PTDM.

Treatment of posttransplant dyslipidemia impairs the ascertainment of causal pathways toward important clinical endpoints like PTDM. Statin therapy alters the relationship between Apo B and HDL-C or LDL-C,[29] exerting a greater effect on cholesterol and triglyceride than on apolipoprotein concentrations. In this study, statin use was associated with a lower Apo B but not lower triglyceride concentration. The ineffectiveness of LDL-C to predict PTDM in multivariate analysis (Table 4) may reflect the effects of statin therapy, known to reduce posttransplant cardiac events.[30] The obligate 1:1 inclusion of Apo B in hepatic-derived VLDL-C, IDL-C, and LDL-C structure[31] allows Apo B to serve as a formal count of LDL particles, unlike LDL-C that measures the cholesterol content of LDL particles. LDL-C may predict atherosclerosis only when LDL-C and LDL particle count are concordant.[32,33] Therefore, if Apo B and LDL-C are not concordant in KTR, then Apo B and Apo A1 may provide greater predictive value toward posttransplant metabolic outcomes such as PTDM.

Measured HDL-C is a summation of HDL2 and HDL3 concentrations.[34] HDL3 less effectively transports apolipoprotein C-II[35] while HDL2 more effectively reflects decreases in total HDL-C. Total HDL-C may therefore poorly indicate PTDM risk if HDL structure shifts characterize the posttransplant state. Apolipoprotein A1, representing total HDL-particle

Table 3. Univariate Cox Proportional Hazards Regressions for Late PTDM Incidence, 2010-2015.

Variable	HR (95% CI)	P value
Apolipoprotein parameters		
Apolipoprotein B/A1	8.25 (1.72-39.5)	.008
Apolipoprotein A1 (g/L)	1.18 (0.37-3.74)	.777
Apolipoprotein B (g/L)	12.2 (3.13-47.5)	<.001
Clinical characteristics		
Body mass index (kg/m^2)	1.02 (0.95-1.11)	.543
Waist circumference (cm)	1.02 (0.99-1.05)	.131
Hip circumference (cm)	1.01 (0.98-1.04)	.711
Waist to hip ratio (cm/cm × 100)	1.06 (1.01-1.10)	.021
Weight (kg)	1.00 (0.98-1.03)	.826
Height (cm)	0.98 (0.95-1.02)	.397
Systolic BP (mm Hg)	1.00 (0.98-1.03)	.732
Diastolic BP (mm Hg)	1.01 (0.98-1.05)	.427
Age at transplant (years)	1.01 (0.98-1.03)	.681
Transplant number	1.00 (0.24-4.20)	.996
Live donor	0.54 (0.23-1.27)	.158
Male	0.95 (0.45-1.99)	.893
White ethnicity	0.43 (0.18-1.06)	.066
Metabolic Syndrome	4.42 (1.34-14.6)	.015
Laboratory measurements		
Serum creatinine (μmol/L)	1.00 (0.99-1.01)	.476
Fasting blood glucose (mmol/L)	1.17 (1.09-1.25)	<.001
Random blood glucose (mmol/L)	1.15 (1.08-1.23)	<.001
HbA$_{1c}$ (%)	1.59 (1.24-2.02)	<.001
eGFR (MDRD, mL/min/1.73 m^2)	1.01 (0.99-1.02)	.590
Total cholesterol (mmol/L)	1.50 (1.11-2.02)	.009
HDL cholesterol (mmol/L)	1.60 (0.74-3.47)	.232
LDL cholesterol (mmol/L)	1.43 (0.96-2.13)	.075
HDL/LDL cholesterol ratio	1.23 (0.83-1.82)	.299
Triglycerides (mmol/L)	1.21 (0.96-1.52)	.115
Non-HDL cholesterol (mmol/L)	1.42 (1.03-1.97)	.034
Total cholesterol/HDL-C ratio	1.02 (0.91-1.14)	.724
C-reactive protein (mg/L)	1.01 (1.00-1.02)	.150
Urine ACR (mg/mmol)	1.00 (0.98-1.02)	.959
Uric Acid (μmol/L)	1.00 (0.99-1.00)	.133
24-hour urine protein (g/d)	0.30 (0.06-1.42)	.129
Medications		
Statin	0.86 (0.41-1.83)	.696
Prednisone	1.18 (0.28-4.98)	.821
Cyclosporine	1.57 (0.54-4.51)	.405
Tacrolimus	0.66 (0.25-1.72)	.392
Mycophenolic acid	1.05 (0.451-2.44)	.911

Note. PTDM = posttransplant diabetes mellitus; HR = hazard ratio; CI = confidence interval; BP = blood pressure; HbA$_{1c}$ = glycated hemoglobin; eGFR = estimated glomerular filtration rate; MDRD = Modification of Diet in Renal Disease; HDL = high-density lipoprotein; LDL = low-density lipoprotein; ACR = albumin-to-creatinine ratio.

count, may consequently also poorly reflect PTDM risk except in the presence of statins, which affects LDL-C more. Clinically, the benefit of statins in metabolic syndrome[36] might mediate through Apo B reduction, but this benefit may not be captured through LDL-C reduction.

Table 4. Multivariate Cox Proportional Hazards Regressions for Late PTDM Incidence, Using the 2010-2015 Cohort.

Variable	HR (95% CI)	P	Variable	HR (95% CI)	P
Apo B/A1	7.80 (1.1-55)	.039	Apo B (g/L)	8.41 (1.7-40)	.007
BMI (kg/m^2)	0.99 (0.9-1.1)	.859	BMI (kg/m^2)	0.99 (0.9-1.1)	.841
SBP (mm Hg)	0.99 (1.0-1.0)	.464	SBP (mm Hg)	0.99 (1.0-1.0)	.347
Age (kg/m^2)	1.00 (1.0-1.0)	.981	Age (kg/m^2)	1.00 (1.0-1.0)	.974
Live donor	0.47 (0.2-1.4)	.176	Live donor	0.43 (0.1-1.3)	.136
White	0.44 (0.2-1.1)	.083	White	0.46 (0.2-1.2)	.097
Tac (vs CsA)	1.11 (0.3-3.9)	.869	Tac (vs CsA)	1.16 (0.3-4.2)	.815
Waist-to-hip	1.04 (1.0-1.1)	.222	Waist-to-hip	1.04 (1.0-1.1)	.142
Metabolic syndrome	5.34 (1.2-23.0)	.027	Metabolic syndrome	5.37 (1.2-24.0)	.026
Non-HDL-C	1.21 (0.9-1.6)	.136	LDL-C	1.05 (0.7-1.5)	.774
BMI (kg/m^2)	0.97 (0.9-1.0)	.392	BMI (kg/m^2)	0.99 (0.9-1.1)	.717
SBP (mm Hg)	0.99 (1.0-1.0)	.599	SBP (mm Hg)	1.00 (1.0-1.0)	.773
Age (kg/m^2)	1.00 (1.0-1.0)	.893	Age (kg/m^2)	1.00 (1.0-1.0)	.928
Live donor	0.70 (0.3-1.3)	.227	Live donor	0.61 (0.3-1.2)	.160
White	0.50 (0.3-1.0)	.036	White	0.52 (0.3-1.0)	.054
Tac (vs CsA)	1.96 (0.8-5.0)	.162	Tac (vs CsA)	1.74 (0.7-4.5)	.250
Waist-to-hip	1.05 (1.0-1.1)	.016	Waist-to-hip	1.05 (1.0-1.1)	.265
Metabolic syndrome	7.46 (1.8-31)	.006	Metabolic syndrome	6.60 (1.6-28)	.010

Note. Four separate models are shown. Age refers to the age at time of measurement. Binary variables coded as 0 = no, 1 = yes. Multiple regression with Apo A1 not shown, HR (95% CI) = 0.51 (0.2-1.6), *P* = .248. Metabolic syndrome diagnoses use National Cholesterol Education Program Adult Treatment Panel III criteria. Non-HDL-cholesterol and LDL cholesterol expressed in mmol/L. PTDM = posttransplant diabetes mellitus; HR = hazard ratio; CI = confidence interval; Apo B/A1 = apolipoprotein B/A1; Apo B = apolipoprotein B; Apo A1 = apolipoprotein A1; BMI = body mass index; SBP = systolic blood pressure; Tac = tacrolimus; CsA = cyclosporine A; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol.

Table 5. Cox Regression Analyses for Apo B and Apo A1, Stratified by Statin Use, High Fasting Blood Glucose and White Ethnicity in the 1998-2015 Cohort.

Variable	Cox regression, Apo B		Cox regression, Apo A1	
Total n (case count)	Yes	No	Yes	No
Statin use	1.55 (0.34-7.05)	7.19 (2.22-23.3)	0.17 (0.04-0.71)	1.58 (0.45-5.62)
Yes: 551 (26), no: 553 (21)	*P* = .571	*P* = .001	*P* = .016	*P* = .476
FBG > 7.0 mmol/L	11.3 (1.59-79.5)	1.39 (0.35-5.55)	1.64 (0.35-7.80)	0.26 (0.07-0.89)
Yes: 73 (14), no: 936 (34)	*P* = .015	*P* = .639	*P* = .53	*P* = .032
White	0.11 (0.01-1.80)	10.3 (2.78-38.5)	0.2 (0.03-1.16)	0.5 (0.13-1.96)
Yes: 441 (13), no: 395 (28)	*P* = .122	*P* < .001	*P* = .124	*P* = .318

Note. The stratification is dichotomized. Each group is tested in univariate Cox regression with Apo B and Apo A1, respectively. Apo B = apolipoprotein B; Apo A1 = apolipoprotein A1; FBG = fasting blood glucose.

Although our study presents an association between the posttransplant apolipoprotein profile and PTDM, it is limited by its retrospective, observational nature. The association between apolipoprotein concentrations and PTDM does not imply causality or the direction of the cause-effect relationship. Measurement of apolipoprotein profiles only since 2010 in a 1998-2015 cohort allowed for 3-month posttransplant standardized measurement in only a subgroup of patients, even though incident PTDM was recorded in all patients only after apolipoprotein profiles were measured. PTDM was ascertained through recorded transcription, possibly biasing against detecting more events based on blood glucose measurements particularly

when mortality occurs or grafts fail early posttransplant. The small number of late PTDM cases, excluding a much larger number of earlier PTDM cases (170 vs 53), precluded adjusting for more potential confounders in regression analyses. We did not possess sufficiently granular information of immunosuppressive medication or their serum concentration as well as statin dosage for producing meaningful correlations. Greater patient numbers and longer follow-up will be required to better assess these and other relationships among lipid particle components in chronic kidney disease.

In conclusion, the apolipoprotein profile in our cohort was a superior predictor of late PTDM compared with
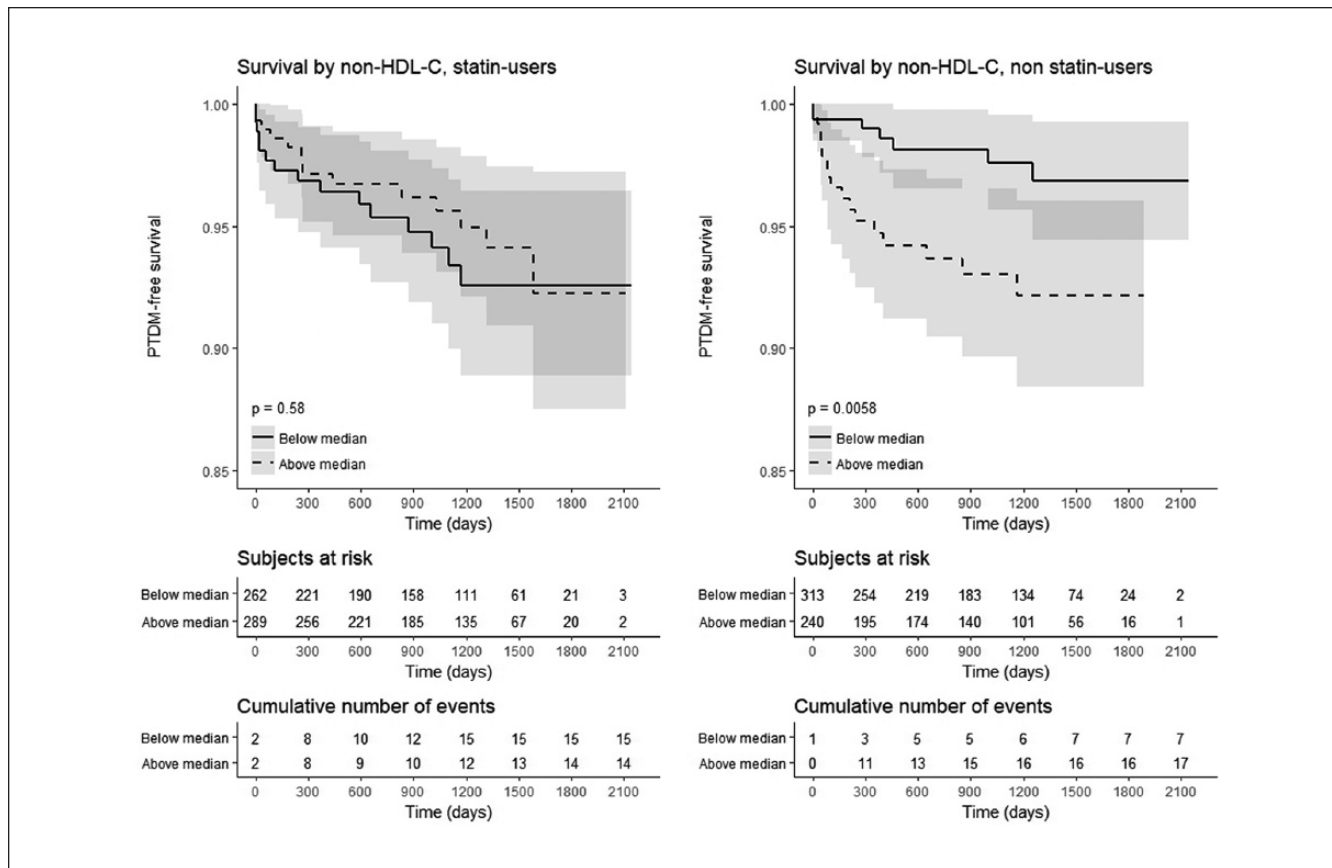
Figure 4. Kaplan-Meier analysis of PTDM-free survival by non HDL-C median based on statin use.
Note. PTDM = posttransplant diabetes mellitus; HDL-C = high-density lipoprotein cholesterol.

traditional lipid profile parameters. Routine apolipoprotein measurement post kidney transplantation, if validated in other cohorts, may facilitate investigating mechanisms for PTDM, and might ultimately contribute to better monitoring and reducing long-term posttransplant cardiovascular risk.

List of Abbreviations

Apo A1, apolipoprotein A1; Apo B, apolipoprotein B; BMI, body mass index; BP, blood pressure; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; FBG, fasting blood glucose; HbA_{1c}, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; IDL-C, intermediate-density lipoprotein cholesterol; KTR, kidney transplant recipient(s); LDL-C, low-density lipoprotein cholesterol; MDRD, modification of diet in renal diseases; PTDM, posttransplant diabetes mellitus; p-y, patient-years; RBG, random blood glucose; SBP, systolic blood pressure; SD, standard deviation; SMH, St. Michael's Hospital; TG, triglycerides; VLDL-C, very low-density lipoprotein cholesterol.

Ethics Approval and Consent to Participate

Ethics approval was obtained from the Research Ethics Board at St. Michael's Hospital. Since the study was a population-based retrospective review, individual consent to participate was not obtained.

Consent for Publication

All authors have reviewed a final version of the manuscript and have consented to publication.

Availability of Data and Materials

Data and materials may be made available to qualified investigators upon written request to the corresponding author. Reasonable requests for data access will be assessed in consultation with the appropriate Research Ethics Board.

Author Contributions

R.M. participated in research design, data collection, data analysis, and writing. L.R. participated in research design and writing. M.M.N. participated in research design and writing. G.V.R.P. coordinated research design, funding, performance of the research, data analysis, and writing.

Declaration of Conflicting Interests

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ORCID iD

G. V. Ramesh Prasad [iD] https://orcid.org/0000-0003-1576-7696

Supplemental Material

Supplemental material for this article is available online.

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