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Pancreas-Kidney Transplantation: risk factors for Peripheral Arterial Disease in Diabetes Mellitus

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Pancreas-Kidney Transplantation: risk factors for Peripheral Arterial Disease in Diabetes Mellitus

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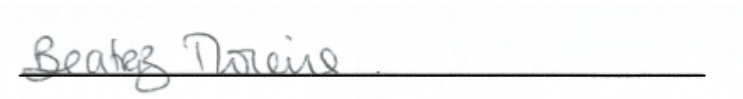

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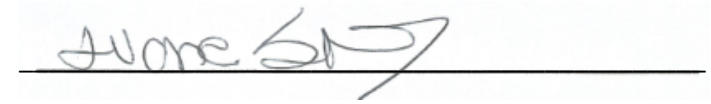

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Resumo

Introdução: A Diabetes Mellitus tipo I tem um início precoce com consequências ao longo da vida que podem ser catastróficas - uma das mais temíveis é o desenvolvimento e a progressão da Doença Arterial Periférica. Uma vez que o transplante renopancreático parece ser uma opção bastante promissora para estes doentes, avaliar o *outcome* da Doença Arterial Periférica neste grupo de doentes parece ser um assunto deveras pertinente e ainda algo inexplorado.

Objetivo: O objetivo deste estudo é identificar os possíveis fatores que influenciam o desenvolvimento e a progressão da Doença Arterial Periférica em doentes submetidos a transplante renopancreático no Centro Hospitalar Universitário do Porto.

Métodos: Trata-se de um estudo observacional retrospetivo de um grupo de 229 pacientes com Diabetes Mellitus tipo I e Doença Renal Crónica em estadio terminal submetidos a transplante renopancreático no Centro Hospitalar Universitário do Porto entre maio de 2000 e dezembro de 2019. Foi elaborada uma base de dados através da consulta dos processos clínicos eletrónicos de todos os doentes com o intuito de identificar os potenciais fatores de risco para o desenvolvimento e para a progressão de Doença Arterial Periférica.

Resultados: Os doentes com Doença Arterial Periférica apresentaram níveis mais elevados de colesterol LDL (p = 0,040) antes do transplante, sendo que LDL elevado conferiu um risco 1,011 vezes maior de desenvolver a doença. Os doentes com Doença Arterial Periférica também apresentaram HbA1c mais elevada aos 6 meses e aos 3 anos após o transplante (p = 0,033 e p = 0,022, respetivamente), associada a um risco de desenvolver a doença de 1,512 e 1,334 vezes superior, respetivamente. A presença de cistatina C elevada, 5 anos após o transplante, foi outra variável associada ao maior risco de desenvolver Doença Arterial Periférica (p = 0,015), proporcionando riscos 2,405 vezes maiores de desenvolver a doença. Além disso, a presença de cardiopatia isquémica foi mais prevalente nos doentes com Doença Arterial Periférica (p = 0,037), e foi associada a um risco 3,220 vezes superior de desenvolver a doença.

Conclusão: O transplante renopancreático é certamente a melhor opção de que dispomos atualmente para os doentes com Diabetes Mellitus tipo 1 e Doença Renal Crónica em estadio terminal. No entanto, não soluciona todos os problemas, particularmente o desenvolvimento e progressão da Doença Arterial Periférica, com uma origem multifatorial e complexa, sendo necessários mais estudos para que se atinja uma maior compreensão da sua patogénese que permita, no futuro, o desenvolvimento de uma melhor estratégia de prevenção e controlo nesta população.

Abstract

Background: Type 1 diabetes mellitus has a very early onset with life-time consequences that can be disastrous – one of the most alarming being the progression of Peripheral Arterial Disease. Since pancreas kidney transplantation seems to be a promising option for these patients, assessing the overall and PAD outcome of this group of patients is a very pertinent and yet unexplored matter.

Objective: The aim of this study is to identify possible factors that influence the development and progression of peripheral arterial disease in pancreas-kidney transplanted patients at Centro Hospitalar Universitário do Porto.

Methods: Retrospective observational study on a group of 229 patients with type I Diabetes Mellitus and End Stage Renal Disease who underwent pancreas-kidney transplantation in Centro Hospitalar Universitário do Porto between May of 2000 and December of 2019. A data base was elaborated by access to the electronic clinical records of all patients.

Results: Patients with peripheral arterial disease were characterized by higher levels of LDL-C prior to transplant (p = 0.040), which were associated with a 1.011-fold higher risk of developing the disease. Higher levels of HbA1c 6 months and 3 years after transplant were also present among peripheral arterial disease patients (p = 0.033 and p = 0.022), associated with a respectively 1.512-fold and 1.334-fold higher risk of developing the disease. Patients with peripheral arterial disease were also characterized by higher levels of Cystatin C 5 years after transplant (p = 0.015) providing a 2.405-fold higher risk of developing the disease. Besides, myocardial ischemia was also more prevalent among patients with peripheral arterial disease (p = 0.037) inducing a 3.220-fold higher risk of developing the disease.

Conclusion: Simultaneous pancreas-kidney transplantation is certainly the best option we currently have for these patients with type 1 Diabetes Mellitus and end-stage renal disease. However, it does not solve all the problems, particularly this frailty that is peripheral arterial disease in this population, with a multifactorial and complex origin, requiring more studies to reach a greater understanding of its pathogenesis that will eventually allow, in the future, the development of better prevention and control strategies for this population.

Keywords: Peripheral Arterial Disease, Diabetes Mellitus, Kidney Transplantation, Pancreas Transplantation, Risk Factors, Epidemiology, Atherosclerosis, Cardiovascular diseases, Vascular diseases.

Abbreviation List

ABI | Ankle-Brachial Index

BMI | Body Mass Index

CRP | C- Reactive Protein

DM | Diabetes mellitus

ESRD | End-Stage Renal Disease

HbA1c | Hemoglobin A1c

HDL-C | High-Density Lipoprotein Cholesterol

LDL-C | Low-Density Lipoprotein Cholesterol

MI | Myocardial Infarction

NLR | Neutrophil–Lymphocyte Ratio

Ox-LDL | Oxidized Low-Density Lipoprotein

PAD | Peripheral Arterial Disease

PLR | Platelet–Lymphocyte Ratio

SPKT | Simultaneous Pancreas-Kidney Transplantation

TC | Total Cholesterol

TNF | Tumor Necrosis Factor

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Introduction and Objective

Diabetes Mellitus and Simultaneous Pancreas-Kidney Transplantation

Diabetes mellitus is one of the most common chronic diseases, affecting around 463 million individuals in the world and over one million in Portugal.[1] In 2015, type 1 DM affected around 0,16% of children in Portugal.[2] Throughout the progression of the disease, many patients are afflicted by microvascular and macrovascular complications which, if unmanaged, can lead to severe long-term complications. These include microvascular events, such as retinopathy, neuropathy and nephropathy; as well as macrovascular diseases involving cerebrovascular, coronary or peripheral vascular systems.[3] One of the most significant complications of type 1 DM is end-stage renal disease, requiring dialysis or renal transplantation for long-term management.[4]

The simultaneous pancreas-kidney transplantation emerged in 1966 and has changed the course of type 1 DM patients with ESRD management. It provides a physiological mean of achieving normoglycemia with the advantage of keeping the ESRD patient free of dialysis.[3] Further diabetic complications and some occasional reversal of established diseases have been reported.[5] Pancreatic transplantation, without an associated kidney transplant, has inferior results to simultaneous pancreas-kidney transplantation.[6] The position statement of the American Diabetes Association reaffirms that in diabetics with advanced chronic kidney disease who are candidates for transplant, the best option is the simultaneous pancreas-kidney transplantation since the addition of a pancreas does not decrease patient survival and may improve kidney graft survival.[7]

Diabetes Mellitus and Peripheral Arterial Disease

Peripheral arterial disease is a chronic atherosclerotic process that causes a partial or total obstruction of the peripheral arterial vasculature's lumen, leading to reduced arterial flow and, consequently, poorer irrigation of tissues, mainly in the lower limbs. The main risk factors for developing PAD are smoking, DM, high blood pressure and dyslipidemia. Obesity, aging, and family history of PAD, heart disease and stroke are also risk factors.[8] This disease affects more than 200 million people worldwide[9] and its prevalence is expected to increase.

PAD is an independent predictor of cardiovascular and cerebrovascular ischemic events, affecting both the quality and expectancy of life. Consistent with this, patients with PAD are at almost six-fold higher risk for acute myocardial infarction, ischemic stroke and/or death compared to general population.[10,11] Therefore, studies investigating the prevalence of PAD among high-risk groups and identifying risk factors for PAD seem crucial.

DM is a major risk factor for PAD and a marker of poor prognosis. Patients with diabetes have a 4-fold increased risk of developing PAD[12], which presents earlier and progresses more quickly when compared to the non-diabetic population. In addition, the outcome after surgical revascularization is often worser and patients are 10 to 16 times more likely to undergo major amputations,[13] due to delay in diagnosis, consequent of the presence of neuropathy that masks foot ischemia pain. For these reasons, studying the risk factors for the development of PAD in this specific group of patients is of major importance to optimize the prevention and treatment strategies, improving PAD prognosis in this population.

In general, the pathophysiology of PAD in the diabetics is similar to that in the non-diabetic population.[14] However, DM is a state of chronic inflammation combined with an increase in the levels of proinflammatory cytokines and clinical evidence supports the concept that those proinflammatory cytokines, especially C- Reactive Protein and TNF-α, play an important role in the development and progression of atherosclerosis.[15,16] CRP seems to be a significant independent predictor of the development of PAD.[17,18] These findings suggest positive correlations among inflammation, DM and PAD. Fatemi et al showed that Cystatin C was also associated with incident symptomatic PAD, implying that this biomarker is a sensitive indicators of early subclinical PAD.[19] There is also evidence that the neutrophil–lymphocyte ratio and the platelet–lymphocyte ratio might have correlation with median overall limb survival in patients with critical limb ischemia.[20] Increased LDL-C and ox-LDL in DM play an important role in the development of atherosclerosis promoting PAD progression; in a study, lowering LDL-C levels in PAD patients reduced cardiovascular mortality, ischemic complications of the extremities, altered atherosclerotic plaque progression and improved exercise tolerance.[21] In a recent study, in type 2 DM patients the variation in the HbA1c value and poor glucose control indicated higher risk for PAD.[22] Another study evaluated the association between HbA1c and ABI in subjects with and without DM and concluded that, compared to the reference group, the 10-year decline in ABI was higher in bad-controlled diabetic patients.[23]

The effects of Simultaneous Pancreas-Kidney Transplantation on Peripheral Arterial Disease

A successful pancreas transplantation, by allowing the endocrine functions of the pancreas to be restored and by optimizing metabolic control, would intuitively slow the progression of diabetic micro and macrovascular complications. In fact, it has been reported that long-term normoglycemia following successful SPKT withstands significantly improvement of the microangiopathic diabetic injuries, and that some of the already established injuries even have the potential to be reversed.[24,25] In contrast, data on the evolution of macroangiopathic diabetic injuries after SPKT is

far from clear. On one hand several studies report a significant higher incidence of PAD and worse vascular outcomes, including a higher incidence of amputation, in patients with a SPKT when compared to those receiving a kidney transplant alone.[26,27] On the other hand, there is a big amount of data implying that the progression of macrovascular disease is reduced in patients with type 1 DM after more than 5 years of successful SPKT when compared to kidney transplant alone.[28,29]

A possible explanation for these conflicting findings may be that these beneficial effects of the SPKT are only visible after more than five years of normoglycemia, and only observed in longer follow-up studies. Accordingly, recent data now indicates that with a longer follow-up there is an increasing survival benefit for simultaneously transplanted patients compared to patients who received a single kidney transplant.[30] However, this plethora of conflicting reports lead to the conclusion that the effects of SPKT on macrovascular disease are far from clear and warrant additional study and more target focus.

Objective

The aim of this study is to identify possible factors that influence the development and progression of peripheral arterial disease in pancreas-kidney transplanted patients at Centro Hospitalar Universitário do Porto.

Methods

Study design and participants

This research consisted of a retrospective observational study by access to the electronic clinical records of 229 patients with type I Diabetes Mellitus and End Stage Renal Disease who underwent SPKT at Centro Hospitalar Universitário do Porto between May of 2000 and December of 2019. At our institution, SPKT indications include every patient with type I Diabetes Mellitus and End Stage Renal Disease requiring renal transplant.

Clinical features studied included sex, age, age at transplant, Body Mass Index (BMI), years of diabetes prior to transplant, months of dialysis prior to transplant, smoking, antihypertensive drugs intake, statins intake, cerebrovascular disease, myocardial ischemia, retinopathy, cholesterol levels (TC, LDL-C, HDL-C), triglycerides, HbA1c levels, creatinine, cystatin C, CRP, albumin and levels of neutrophils, lymphocytes, and platelets. To define the presence of dyslipidemia, we used HDL <40 mg/ dl in men and <50 mg/ dl in women, LDL > 120 mg/ dL, total cholesterol > 200 mg / dl and triglycerides ≥ 150 mg / dl, as criteria.[31] We also registered all follow-up periods, grafts' viabilities and grafts' survival time.

From the 229 patients, 13 patients had to be excluded from the study - 6 died at the time of transplant and 7 were lost during the follow-up. Therefore, our final data base consisted of 216 patients, divided into two groups: a group of 32 patients with symptomatic PAD manifested as intermittent claudication or chronic limb threatening ischemia (rest pain and ulceration) who required revascularization and a group of 184 patients without any symptoms of PAD. A detailed review of the presentation of patients with manifestations of PAD was performed according to the Rutherford scale. The revascularization procedures were classified as endovascular, surgical or hybrid. The anatomical classification of the lesions was performed similarly to Dihem et al according to the arteries submitted to treatment: aortoiliac (common, external and internal iliac arteries), femoropopliteal (common, superficial and deep femoral arteries, popliteal artery) and infrageniculate or distal (tibiofibular trunk, anterior and posterior tibial arteries, fibular artery) disease.[32]

Statistical analysis

The statistical calculations were performed using the IBM SPSS Statistics version 27.0.

The study groups stratified as patients with PAD and patients without PAD were compared on clinical characteristics using independent t-test for continuous normally distributed variables,

Mann–Whitney test for continuous non-normally distributed variables and chi-square test for categorical variables.

The odds ratios (OR) for prevalent PAD associated with the cross-classification of LDL cholesterol, myocardial ischemia, HbA1c 6 months and 3 years after transplant and Cystatin C 5 years after transplant were calculated using logistic regression models.

A Kaplan-Meier survival analysis was performed to study the influence of PAD on the patients' survival, as well as on the of the pancreas and kidney grafts survival rates.

For the statistical significance, a *p*-value < 0.05 was established. The research results are presented in the form of mean ± standard deviation or median (interquartile range) for quantitative variables if they were normally or non-normally distributed, respectively. Qualitative variables are expressed as absolute numbers and percentages. A confidence interval of 95% was used.

Ethics Statement

This research was approved by the Bioethics Committee of the Instituto de Ciências Biomédicas Abel Salazar/ Centro Hospitalar Universitário do Porto, by the Gabinete Coordenador de Investigação from Instituto de Ciências Biomédicas Abel Salazar and by the Ethics Committee from Centro Hospitalar Universitário do Porto [N/REF.ª 2020.277 (213-DEFI/222-CE)].

Results

Characteristics

Our study was conducted on a group of 216 patients – 111 (51.4%) men and 105 (48.6%) women – with mean age of 46.01 ± 0.48 years and mean age at transplant of 35.54 ± 6.02 years. 141 (65.9%) patients were non-smokers, 40 (18.7%) were former smokers and 33 (15.4%) were current smokers. The immunosuppressive therapy was almost identical in all patients and consisted of tacrolimus, mycophenolate mofetil, prednisolone and anti-thymocyte globulin for induction; and tacrolimus, mycophenolate mofetil and prednisolone for maintenance.

The mean follow-up time was 8.91 ± 0.34 years. The mean years a pancreas graft survived was 7.90 ± 5.36, while the mean survival of a kidney transplant was 8.91 ± 4.95 years. The technical success of the SPKT was achieved in all patients, although in 50 patients (23.15%) there was a need to re-intervene early because of different post-operative complications. During the follow-up period, the pancreatic graft remained functional in 174 (80.56%) patients, with graft failure being observed in the remaining 42 (19.44%). Six patients with failed pancreas graft received a second pancreas transplant and it remained functional in only one patient being the cause of rejection of the other 5 grafts unknown. Regarding the kidney transplantation, the kidney graft remained functional in 192 (88.89%) patients and graft loss during follow-up was observed in the remaining 24 (11.11%). Five of the patients with lost grafts were then re-transplanted and the rest started a hemodialysis program. Two of the retransplanted patients maintained a functioning graft whereas the 3 others lost the graft due to unknown causes and started hemodialysis. The post-operative complications as well as the causes of graft failure are detailed in Table I.

The PAD characteristics of the 32 PAD patients are described in Table II and all procedures and outcomes of the 23 patients who had chronic limb threatening ischemia and required revascularization are characterized in Table III.

The clinical characteristics compared between PAD and non-PAD patients are represented in table IV. The logistic regression performed is exhibited in Table V.

Analysis

A total of 184 patients had no symptoms of PAD, most were male (n = 98; 53.3%;), with a mean age of 45.89 ± 0.53 years and a mean age at the date of the transplant of 35.53 ± 6.04 years. Whereas PAD patients' group included a total of 32 patients, males were the minority (n = 13; 40.6%), with a mean age of 46.72 ± 1.10 years and mean age at the date of the transplant of 35.63 ± 5.99 years.

Age, age at the date of transplant, sex and BMI were not significantly different between the two groups.

The duration of diabetes mellitus prior to transplantation was mean 23.91 ± 5.75 years in the non-PAD group and mean 24.75 ± 5.40 years in PAD patients, difference was non-significant. In non-PAD, all patients except 8 underwent hemodialysis as a replacement therapy for renal function before transplant with a median duration of hemodialysis of 21 (19) months. While in PAD, only 1 patient did not undergo hemodialysis being the median duration of hemodialysis 29 (27) months in this group. The duration of hemodialysis prior to transplant did not show any statistically significant difference between the groups.

Regarding smoking, we found no statistically significant difference between the two groups; although former and current smoking were slightly higher in the PAD patients. No association was found between antihypertensive therapy and statins intake and the prevalence of PAD.

There was an association found between PAD and myocardial ischemia (p = 0.037) with a higher risk of developing PAD of 3.220-fold among patients with myocardial ischemia. No association between PAD and cerebrovascular disease was found. All patients in our study had diabetic retinopathy.

Patients with PAD were characterized by higher pre-transplant LDL-C levels (p = 0.040) and higher LDL-C levels were associated with a 1.011-fold higher risk of developing PAD. TC, HDL-C and triglycerides did not reveal any significant difference between the two groups. The incidence of dyslipidemia was also not significantly different between the two groups. Pre-transplant albumin levels were higher in non-PAD patients although statistical significance was not reached. Regarding inflammation associated markers, no significant difference was found in CRP, NLR and PLR between groups.

HbA1c was significantly higher at 6 months (p = 0.033) and at 3 years after transplant (p = 0.022) in PAD patients, being associated with a 1.512- and a 1.334-fold higher risk of developing PAD, respectively. Besides that, at 1 and 5 years after transplant it was also higher in PAD patients, tending towards statistical significance 1 year after transplant (p = 0.068). Cystatin C was significantly higher in PAD patients 5 years after transplant (p = 0.015) and associated with a 2.405 higher risk of developing PAD. At 3- and 10-years' time although Cystatin C was also higher in PAD patients, the difference was not statistically significant. Regarding creatinine levels no significant difference was found.

In patients with no PAD symptoms, the mean duration of the pancreatic graft was 8.07 ± 5.27 years and of the kidney graft was 8.90 ± 5.03 years while in PAD patients it was 6.92 ± 5.89 years and 9.02 ± 4.55 years, respectively; no statistically significant difference was found. Regarding the influence of PAD on pancreas and kidney grafts survival, using Kaplan Meier analysis (Figures 1 and 2), although no statistically significant difference was found, we can observe a trend to lower graft survival in PAD patients, especially regarding kidney graft survival – in Figure 2 there is clearly a trend to a diminished cumulative survival of the kidney graft in PAD patients. Accordingly, although the status of grafts' viability was not significantly different between the two groups, the survival of the kidney graft tended towards statistical significance (p = 0.083).

Through the Kaplan-Meier survival analysis performed to evaluate the effect of PAD on patients' survival, although the difference between the two groups was not statistically significant, PAD was clearly associated with lower survival time. Analyzing Figure 3, it is evident that from 10 years' time after transplant there is a much steep decline in survival among PAD patients compared to non-PAD patients.

Discussion

In this study, over the mean 9 years of follow up, the percentage of patients who developed symptomatic PAD was 14.81% which is a considerable value taking into account that the average age of our population was 46 years. In general population at age 45-49 years the prevalence of PAD is estimated to be 5.28% in women and 5.41% in men, mostly asymptomatic.[33]

The duration of diabetes and hemodialysis prior to transplant did not show a significant role in this population in the development of PAD, although there is a study demonstrating that longer diabetes duration was independently associated with PAD in type 2 DM.[34]

Regarding smoking, some studies claim that smoking is the most important modifiable risk factor for developing PAD, as a 2006 study, which reveals that among traditional risk factors, current smoking appeared to be the most powerful predictor of large vessels PAD progression[35], however, no difference between the groups was found in our population.

The presence of myocardial ischemia and PAD, associated with a 3.220-fold higher risk of developing PAD in patients with myocardial ischemia, may be explained by the idea that signs of cardiovascular disease in one vascular territory, such as PAD, carry a significantly increased risk of cardiovascular diseases in other territories, such as the myocardial ischemia.[36] On the contrary, in this study, we did not find any association between cerebrovascular disease and PAD. All patients in this study had diabetic retinopathy which is an important microvascular complication in patients with DM and has been identified as an independent predictor of significant decrease in ABI suggesting that patients with retinopathy should have ABI measurements carried out regularly for the diagnosis of peripheral arterial disease, therefore this applies to all the non-PAD patients in this study.[37]

Our findings on LDL-C are in agreement with a study from 2017, which found that LDL-C, especially ox-LDL, played an important role in the development of atherosclerosis.[8] A study from 2012 also provided evidence for the importance of high LDL-C as a major atherosclerotic risk factor and emphasized the importance of intensive LDL-C lowering in patients who are at high risk of atherosclerotic disease.[37] Regarding albuminuria, a study from 2015 observed significant association between albuminuria and ABI less than 0.9 and cardiovascular events, which agrees with our finding of a trend to higher albumin levels in non-PAD patients.[38]

Several studies suggest that clinical markers associated with inflammation, such as CRP, lymphocytes and platelets, can be associated with the development of PAD.[8,20,39-41] However, in our study, none of those connections were found.

In accordance with our findings regarding HbA1c, in the prospective Heinz Nixdorf Recall Study, previously known diabetes with poor glycemic control (indicated by HbA1c≥7.0%) was strongly associated with incident PAD over a 5-year and 10-year follow-up.[23] In the Atherosclerosis Risk in Communities (ARIC) study, the baseline HbA1c was a strong predictor for the occurrence of PAD.[42] Also, a study from 2020 showed that a higher mean of HbA1c and a higher variability indicate an increased risk for PAD.[22] So, multiple studies support our findings, indicating HbA1c as a marker for increased risk of PAD.

Also in accordance with our findings, a recent study showed that elevated circulating levels of cystatin C were independently associated with incident PAD during long-term follow-up.[19] In our study, although there was a correlation between cystatin C elevation and the development of PAD, the same was not observed for creatinine, which suggests that this marker can have an impact on the development of PAD regardless of kidney dysfunction. Other previous studies also reported that cystatin C levels were higher in PAD patients compared to patients without PAD[43,44], and elevated cystatin C concentration was found to be associated with future PAD procedure as well (bypass surgery, angioplasty, or amputation) among persons who did not have PAD at baseline.[44] Cystatin C is known to be linked to inflammation, which may contribute to its strong association with atherosclerotic disease; and study's results highlight that reduced kidney function, even at mild to moderate stage, is a risk factor for PAD, independently of well-known traditional cardiovascular risk factors.[45] Although there are several studies that investigated the association between cystatin C and PAD in the general population, there are few studies that have done that research in patients with DM.

Although several studies found association between the viability of the pancreas and kidney grafts and the development of PAD[45,46], in our study we did not find that association, which is possibly justified by the small number of lost grafts. However, the kidney graft survival was tendency lower in PAD patients which is reinforced by the analysis of the Kaplan-Meier curve in Figure 2. Also regarding the Kaplan-Meier survival analysis - Figure 3 - it is evident that from 10 years' time after transplant there is a much steep decline in survival among PAD patients compared to non-PAD patients which comes in agreement with several studies that have already showed that PAD is a risk factor for all-cause mortality.[10,11]

Another interesting finding of our study was the high prevalence of aortoiliac arterial disease (38% of all symptomatic patients) that would not be expected as DM is described in several studies by causing more distal atherosclerosis located mainly below the knee and because DM has been reported as the most obvious risk factor for atherosclerotic involvement of infragenicular

arteries.[10,32,47] A possible explanation for this aortoiliac disease is the vascular clamp injury caused in the vessel at the time of transplant with subsequent intimal hyperplasia.[48,49] So, the anastomosis performed at time of transplant seems like a new possible reason for the development of PAD in these transplanted patients, particularly a differently distributed PAD then the one classically generated by DM. However, data in the literature are scarce with regard to the development of iliac strictures in long-term transplant patients.

Another purpose of our study was to analyze if the immunosuppressive therapy had any impact in these patients' outcome, since recent studies indicate that immunosuppression plays an important role in the overall outcome of transplanted patients and particularly modern immunosuppressive agents like tacrolimus and rapamycin have been associated with a reduction in vascular narrowing - a central feature for both chronic rejection and atherosclerosis.[50] Since the immunosuppressant regimens were very similar among all patients that was not possible.

This study has several limitations which may have compromised our results. Two limitations were that our study was a single center study, and it was retrospective. Diversely, this study also holds some strengths with a relatively large population and a mean follow of 8.91 ± 0.34 years.

Conclusion

Peripheral Arterial Disease is a devastating disease that in type 1 DM patients starts at a very young age, has a high rate of amputations associated to a premature mortality. Our study has come to agreement that the lack of metabolic control, namely the elevated levels of LDL cholesterol and HbA1c seem to contribute to the development of the disease. Elevated levels of cystatin C were also associated with PAD and this can be an independent inflammatory marker of progression of disease. Also, this study demonstrated that patients with myocardial ischemia were at higher risk of developing PAD. Besides the pathophysiology associated with diabetes mellitus and chronic kidney disease, our study suggests that vascular clamp injury may also play a role in the development of peripheral arterial disease in these patients.

This study confirms this is assuredly a peculiar population more susceptible to several diseases with distinct pathogenesis pathways that are not yet extensively explored and comprehended. SPKT is certainly the best option we currently have for these patients with type 1 Diabetes Mellitus and ESRD. However, it does not solve all the problems, particularly this frailty that is peripheral arterial disease in this population, with a multifactorial and complex origin, requiring more studies to reach a greater understanding of its pathogenesis, allowing better prevention and control strategies.

Disclosures

The authors declare no conflict of interest or financial support.

Appendix

Tables

Table I. Characterization of post-operative complications and causes of graft loss		
Post-operative complications, n		
Hemorrhage	22	
Infection	10	
Thrombosis and ischemia	8	
Intestinal Occlusion from surgical bandages	3	
Dehiscence of the surgical wound	2	
Pancreatitis	2	
Intestinal perforation	1	
Evisceration	1	
Urinary fistula	1	
Total	50	
Causes of graft failure, n	**Pancreas**	**Kidney**
Acute Rejection	4	1
Chronic Rejection	10	17
Vascular thrombosis	12	2
Hemorrhage	3	0
Infection	6	0
Non-Hodgkin lymphoma involving the graft	0	1
Unknown	7	3
Total	42	24

Table II. Characterization of the symptomatic peripheral arterial disease

Rutherford classification at presentation	**n (%)**
1-3	8 (25)
5	12 (37.5)
6	12 (37.5)
Limbs affected	**n (%)**
Bilateral	11 (34)
Unilateral	21 (66)
Right	*13 (62)*
Left	*8 (38)*
Anatomical pattern	**n (%)**
Aortoiliac	12 (38)
Femoropopliteal	14 (44)
Infrageniculate	16 (50)
Revascularization procedures	**n (%)**
Endovascular	26 (60)
Hybrid	6 (14)
Surgical	11 (26)
Total	43
Outcomes	**n (%)**
Healing	11 (38)
Minor amputation	12 (41)
Major amputation	6 (21)

Table III. Revascularization procedures in patients with chronic limb threatening ischemia

Year of transplant	Year of first manifestation of DAOP	Rutherford classification to presentation	Lower limb affected	Anatomical pattern of the disease	Revascularization procedures			Follow-up time (years)	Outcome
2000	2001	6	Right	Infrageniculate;	2001 – PTA of infrageniculate arteries			1	Major amputation (below the knee)
2000	2018	6	Left	Aortoiliac;	2001 – PTA and BES of the CIA			1	Major amputation (below the knee)
2001	2013	6	Right	Femoropopliteal;	2013 – below-knee femoropopliteal bypass with saphenous vein			1	Minor amputation (toe)
	2009	6	Left	Femoropopliteal;	2009 – femoral endarterectomy + below-knee femoropopliteal bypass with saphenous vein			5	Minor amputation (toe)
2003	2008	5	Right	Aortoiliac;	2008 – ePTFE left to right femorofemoral bypass			13	Healing
2006	2010	5	Right	Aortoiliac; Femoropopliteal;	2010 – ePTFE left to right femorofemoral bypass + PTA of left SFA			5	Healing (Death -2015)
	2015	6	Left	Femoropopliteal;	2015 – below-knee femoropopliteal bypass with saphenous vein			1	Healing (Death - 2015)
2007	2010	5	Left	Femoropopliteal;	2010 – PTA of SFA			9	Healing (Death - 2019)
2007	2011	6	Right	Femoropopliteal; Infrageniculate;	2012 – PTA and stenting of SFA + PTA anterior tibial artery	2013 PTA of instent stenosis with a cutting ballon	2013 PTA of instent stenosis and PTA and stenting of SFA	3	Major amputation (below the knee) (Death - 2017)
2008	2012	5	Right	Femoropopliteal; Infrageniculate	2012 – femoroperoneal bypass with saphenous vein	2012 – bypass trombectomy and PTA of proximal anastomosis	2013 – femoral to posterior tibial artery bypass	1	Major amputation (above the knee)
2009	2017	5	Right	Femoropopliteal; Infrageniculate	2017 – PTA and stenting of SFA + PTA of popliteal and anterior tibial artery	2019 – PTA of SFA and popliteal arteries + PTA with DEB of tibioperonial trunk		4	Minor amputation (toe)
	2012	5	Left	Femoropopliteal; Infrageniculate	2014 - PTA of SFA and posterior tibial artery	2014 - PTA of infrageniculate arteries		9	Healing
2009	2011	6	Right	Infrageniculate;	2011 – PTA of peroneal artery	2011 – PTA of infrageniculate arteries		10	Minor amputation (toe)
2010	2010	5	Right	Infrageniculate;	2013 – PTA of tibioperoneal trunk			11	Healing
2011	2018	5	Right	Aortoiliac; Femoropopliteal	2018 - PTA and stenting with BES of CIA + PTA of popliteal artery			3	Healing
	2012	6	Left	Femoropopliteal; Infrageniculate;	2012 – ePTFE above-knee femoropopliteal bypass	2012 – bypass thrombectomy + PTA and stenting of distal anastomosis	2012 – bypass thrombectomy	1	Major amputation (above the knee)
2011	2009	6	Left	Infrageniculate;	2009 – PTA of infrageniculate arteries			12	Minor amputation (toe)
2012	2010	6	Left	Infrageniculate;	2010 – PTA of infrageniculate arteries			11	Minor amputation (toe)
2013	2013	5	Right	Aortoiliac;	2013 – PTA and stenting of CIA and EIA	2014 – left-to-right femorofemoral bypass		8	Healing
2015	2016	5	Left	Infrageniculate;	2017 – Popliteal to pedal bypass			4	Minor amputation (toe)
2015	2017	6	Right	Infrageniculate;	2019 – Deep vein arterialization			1	Major amputation (below the knee)
2003	2008	5	Left	Femoropopliteal;	2008 – PTA of popliteal artery			12	Healing
2009	2018	6	Left	Aortoiliac; Femoropopliteal;	2018 –PTA and stenting of CIA and EIA + below-knee femoropopliteal bypass with saphenous vein			3	Minor amputation (toe)
2011	2012	5	Right	Infrageniculate;	2012 – PTA of anterior tibial artery	2012 - PTA with DEB of anterior tibial	2012 – Popliteal to pedal bypass with	9	Healing
	2013	5	Left	Femoropopliteal;	2013 – Below-knee femoropopliteal bypass with saphenous vein	2013 – PTA of SFA	2016 – PTA and stenting of SFA	8	Healing
2013	2012	5	Right	Aortoiliac;	2016 – PTA and stenting of EIA			5	Minor amputation (toe)
2014	2015	6	Left	Aortoiliac;	2015 – PTA of EIA			5	Minor amputation (toe) (Death - 2020)
2018	2019	6	Right	Infrageniculate;	2019 – PTA of infrageniculate arteries			2	Minor amputation (foot)
	2019	6	Left	Infrageniculate.	2019 – PTA of infrageniculate arteries			2	Minor amputation (foot)

PTA – Percutaneous Transluminal Angioplasty; BES - Balloon-Expandable Stents; CIA – Common Iliac Artery; ePTFE - Expanded Polytetrafluoroethylene; SFA – Superficial Femoral Artery; DEB – Drug elucting ballon; EIA – External Iliac Artery;

Table IV. Comparison of clinical characteristics according to prevalent symptomatic Peripheral Arterial Disease

Characteristics	Symptomatic Peripheral Arterial Disease		*p-value*
	No	Yes	
Male gender, n (%)	98 (53.3)	13 (40.6)	0.187
Age, years	45.89 ± 0.53	46.72 ± 1.10	0.537
Age at transplant, years	35.53 ± 6.04	35.63 ± 5.99	0.933
Pre-Transplant Data			
BMI, kg/m²	22.10 (3.34)	21.63 (5.27)	0.854
Duration of diabetes, years	23.91 ± 5.75	24.75 ± 5.40	0.444
Duration of dialysis, months	21 (19)	29 (27)	0.144
Smoking			0.423
Never, n (%)	122 (67.0)	19 (59.4)	
Past, n (%)	34 (18.7)	6 (18.8)	
Current, n (%)	26 (14.3)	7 (21.9)	
HbA1c, %	8.50 ± 1.62	8.57 ± 1.47	0.827
Total cholesterol, mg/ dL	166.00 (59)	179.50 (40)	0.242
LDL cholesterol, mg/ dL	**90.79 ± 33.86**	**104.31 ± 35.20**	**0.040**
HDL cholesterol, mg/ dL	48.00 (25)	51.50 (23)	0.618
Triglycerides, mg/ dL	122.50 (80)	126.00 (71)	0.463
Dyslipidemia, n (%)	122 (66.3)	21 (65.6)	0.940
Albumin, mg/ dL	3.89 ± 0.75	3.59 ± 0.50	0.077
CRP, mg/ dL	1.22 (3.40)	2.97 (13.01)	0.177
Neutrophiles, /µL	5.00 (2.21)	4.19 (2.69)	0.302
Lymphocytes, /µL	1.94 ± 0.76	1.85 ± 0.73	0.592
Platelets, /µL	262.00 (97)	279.00 (102)	0.140
Neutrophiles/ Lymphocytes Ratio	2.55 (1.60)	2.76 (2.16)	0.626
Platelets/ Lymphocytes Ratio	142.52 (74.01)	152.87 (135.41)	0.264
Antihypertensive drugs intake, n (%)	106 (91.4)	19 (95)	1.000
Statins intake, n (%)	71 (56.3)	12 (63.2)	0.576
Post-Transplant Data			
Cerebrovascular Disease, n (%)	4 (2.3)	2 (6.3)	0.233
Myocardial ischemia, n (%)	**12 (6.9)**	**6 (19.4)**	**0.037**
HbA1c at 6 months, %	**5.40 (0.70)**	**6.00 (2.70)**	**0.033**
HbA1c at 1 year, %	5.30 (0.63)	5.40 (1.70)	0.068
HbA1c at 3 years, %	**5.2 (0.70)**	**5.65 (3.20)**	**0.022**
HbA1c at 5 years, %	5.30 (0.60)	5.40 (3.20)	0.219
HbA1c at 10 years, %	5.70 (1.30)	5.50 (1.15)	0.759
Creatinine at 6 months, mg/ dL	1.11 (0.40)	1.08 (0.55)	0.943
Creatinine at 1 year, mg/ dL	1.12 (0.32)	1.10 (0.44)	0.716
Creatinine at 3 years, mg/ dL	1.11 (0.41)	1.24 (0.62)	0.313
Creatinine at 5 years, mg/ dL	1.14 (0.45)	1.41 (0.91)	0.218
Creatinine at 10 years, mg/ dL	1.22 (0.56)	1.29 (0.74)	0.246
Cystatin C at 1 year, mg/ L	1.13 (0.38)	0.97 (0.97)	0.688
Cystatin C at 3 years, mg/ L	1.18 (0.49)	1.43 (1.08)	0.199
Cystatin C at 5 years, mg/ L	**1.47 ± 0.65**	**2.01 ± 0.77**	**0.015**
Cystatin C at 10 years, mg/ L	1.58 ± 0.88	1.72 ± 0.72	0.623
Viable pancreas graft, n (%)	152 (82.6)	23 (71.9)	0.153
Duration of pancreas graft function, years	8.07 ± 5.27	6.92 ± 5.89	0.264
Viable kidney graft, n (%)	168 (91.3)	26 (81.3)	0.083
Duration of kidney graft function, years	8.90 ± 5.03	9.02 ± 4.55	0.894
Antihypertensive drugs intake, n (%)	83 (45.9)	17 (53.1)	0.448

BMI: Body Mass Index; HbA1c: Hemoglobin A1c; LDL: low-density lipoprotein; HDL: High-density lipoprotein; CRP: C-reactive Protein.
Data are presented as mean ± standard deviation, numbers (percentages) or median (interquartile range).

Table V. Logistic Regression analysis				
Cross-Classification of PAD	*n*	OR	*p-value*	C.I.
LDL-C	211	1.011	0.043	1.000 - 1.022
Myocardial Ischemia	204	3.220	0.032	1. 108 - 9.357
HbA1c 6 months	78	1.512	0.033	1.034 - 2.210
HbA1c 3 years	158	1.334	0.009	1.075 - 1.654
Cystatin C 5 years	81	2.405	0.026	1.112 - 5.203
OR: Odds-Ratio; C.I.: Confidence interval				

Figures

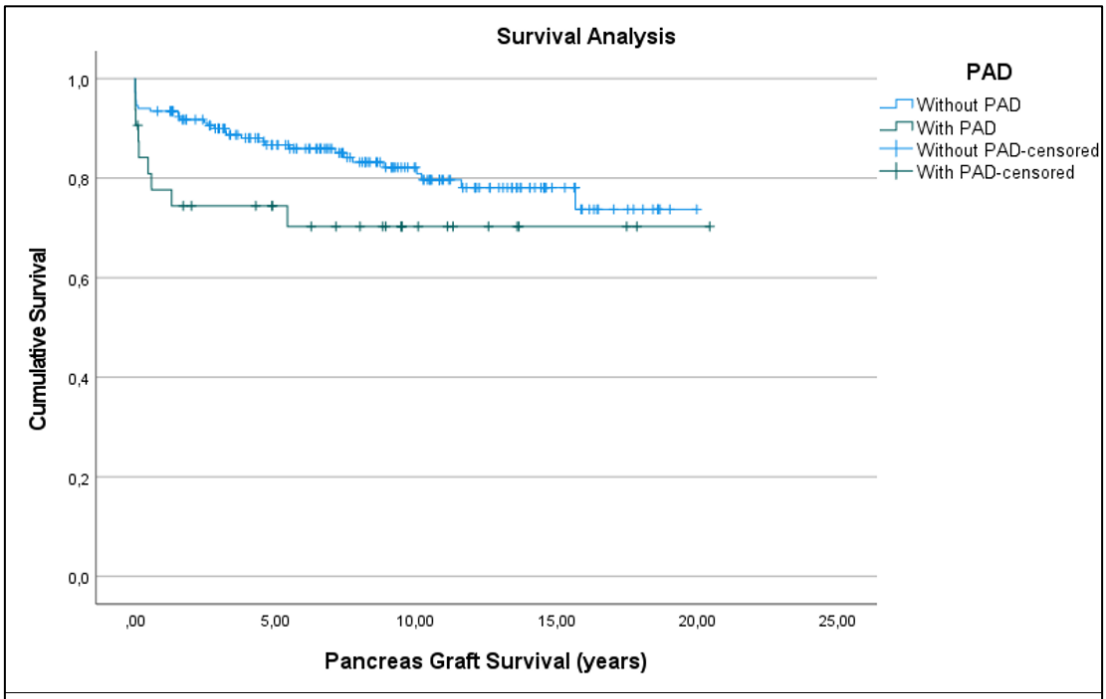

Fig. 1. Kaplan-Meier curve of pancreas graft survival by presence of PAD (p = 0.109)

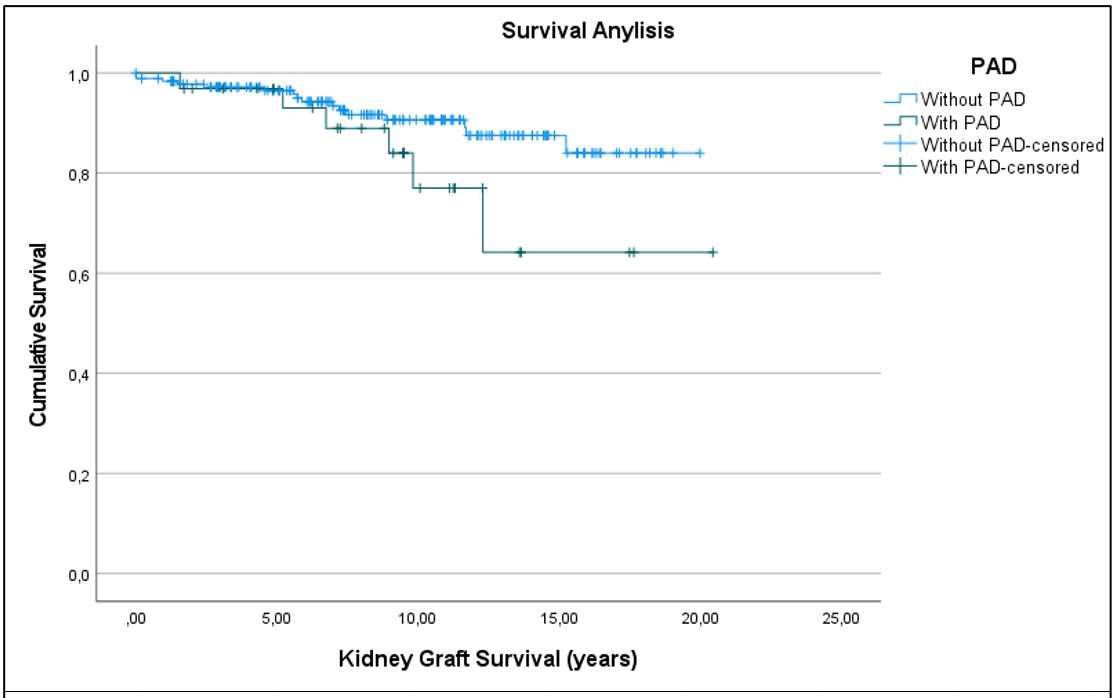

Fig. 2. Kaplan-Meier curve of kidney graft survival by presence of PAD (p = 0.100)

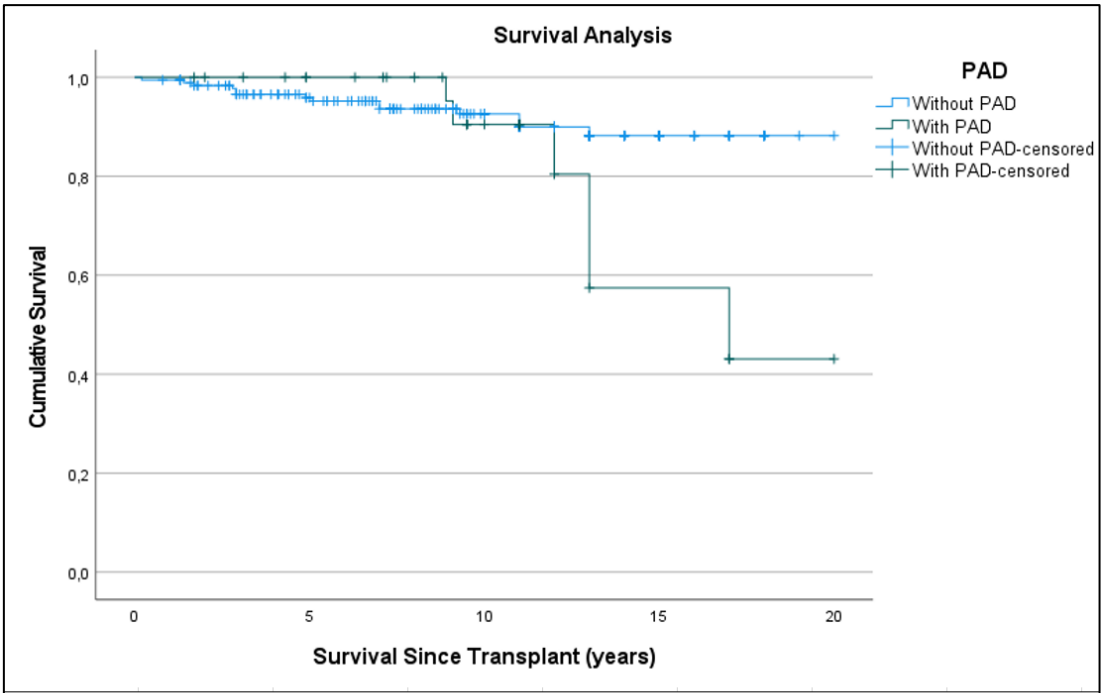

Fig. 3. Kaplan-Meier curve of survival since transplant by presence of PAD (p = 0.076)

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