ORIGINAL ARTICLE

Inflammatory and related biomarkers are associated with post-transplant diabetes mellitus in kidney recipients: a retrospective study

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SUMMARY

In this study, we investigate the association between selected inflammatory-related biomarkers and post-transplant hyperglycemia in kidney transplant recipients. This retrospective analysis comprises 852 patients receiving a kidney transplant at the Norwegian national transplant center between 2007 and 2012, all having a normal oral glucose tolerance test (OGTT) before transplantation. A diagnostic OGTT was performed 10 weeks post-transplant to examine the association between inflammation-related biomarkers and two-hour plasma glucose (2HPG) by multivariable linear regression models adjusting for BMI, age, graft function, fasting insulin levels, dosage of prednisolone, and concentration of calcineurin inhibitors. Six of 20 biomarkers were significantly associated with 2HPG in multivariate analyses showing strong associations with soluble tumor necrosis factor type 1 ($P = 0.027$), Pentraxin 3 ($P = 0.019$), macrophage migration inhibitory factor ($P = 0.024$), and endothelial protein C receptor ($P = 0.001$). These associated markers reflect several distinct but also overlapping pathways including activation of tumor necrosis factor, macrophages, and endothelial cells. The multinomial logistic regression model showed a clear association between the inflammatory biomarkers and post-transplant diabetes mellitus (PTDM). The association between a range of inflammation markers and PTDM suggests that these markers may be target for future studies on pathogenesis and perhaps also treatment of PTDM.

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Key words
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Introduction

Post-transplant diabetes mellitus (PTDM) is a common complication following renal transplantation [1,2] and associated with adverse outcomes, in particular cardiovascular disease (CVD) [3–7]. PTDM displays many similarities with type 2 diabetes mellitus (T2DM). Both conditions are caused by a combination of pancreatic beta-cell dysfunction [8] and increased peripheral insulin resistance [9,10]. However, the development of PTDM is strongly associated with the use of immunosuppressive drugs [1,2,11,12], especially calcineurin inhibitors (CNI) and glucocorticoids. As confirmed by the DIRECT study, tacrolimus in high doses is more diabetogenic than cyclosporine [10,13], in particular in patients with hypertriglyceridemia and insulin resistance [14].

The association between PTDM and infectious diseases such as hepatitis C (hep-C) and cytomegalovirus (CMV) has been described [4,15,16], but if this reflects a direct effect of the virus on the pancreas (e.g., CMV) or indirect effects through induction of liver steatosis (e.g., hep-C) are not clear. Moreover, whereas low-grade systemic inflammation has been linked to T2DM, the role of inflammation in the development of PTDM is not well explored. Previously published studies have indicated that there may be a link between inflammation of the pancreatic beta-cells and PTDM [17], and also a possible association between increased innate immune system activity and development of PTDM [18] as well as involvement of tumor necrosis factor (TNF) [19]. In a recent publication, Cieniawski *et al.* [20] found increased levels of high sensitivity interleukin (IL)-6 in patients with PTDM. In addition, high levels of IL-6 and TNF receptor II were associated with increased risk of graft loss and death.

Our principal hypothesis was that an inflammatory environment would increase the risk of developing abnormal glucose metabolism in renal transplant recipients. This hypothesis was explored by examining the association between a selected panel of markers reflecting activation of different inflammatory pathways. The biomarkers were chosen due to their relevance in post-transplant complications and metabolic disturbances (see methods and Table 1). We selected pathways based on mechanisms that have been found to be involved with T2DM. These are in short macrophage activity, TNF activity, endothelial dysfunction and vascular inflammation, adipose tissue inflammation, and extracellular matrix remodeling. Macrophages and TNF activity have been seen in relation to T2DM and obesity [21]. They are associated with each other as macrophages and monocytes are the main producers of TNF, and they are prominent in the adipose tissue inflammation that is associated with insulin resistance [22]. Impaired endothelial function and vascular inflammation have been described in diabetic patients [23,24], the same is true for extracellular matrix remodeling and development of insulin resistance [25]. The biomarkers had to have expected blood levels above the lower limit of quantification of the analytical methods and be stable during storage at −80 degrees.

The DECODE study group found that an OGTT had a higher diagnostic sensitivity than fasting plasma glucose (fPG) to identify diabetic patients [26], and thus we chose two-hour plasma glucose (2HPG) as our primary outcome variable.

Materials and methods

Design and study population

The study was designed as a retrospective cohort study including patients receiving a renal transplant at the Norwegian national transplant center at Oslo University Hospital Rikshospitalet between 2007 and 2012. Patients with pretransplant diabetes mellitus (DM), patients who experienced graft rejection prior to the 10-week control, and patients who failed to perform an OGTT were excluded. In addition, patients with missing inflammatory parameters were also excluded, and in 2011, 147 patients were not included due to reduced laboratory accessibility. Between 2007 and 2012, a total of 1661 patients received a renal transplant at Oslo University Hospital. After the exclusion of patients according to exclusion criteria, 852 recipients were finally included in this study (Figure 1). Patients who were excluded due to reduced laboratory accessibility resembled the final study population. The OGTTs were performed as part of the diagnostic routine; accordingly, no patients were using antidiabetic medication.

Outcome data were retrieved from the Norwegian Renal Registry. The post-transplantation follow-up at our center includes regular consultations at the transplant outpatient clinic during the first three months. At 10 weeks post-transplantation, patients undergo more extensive investigations at the neurophysiological laboratory including a diagnostic OGTT, measurement of glomerular filtration rate (GFR), and bone density measurements. Blood samples taken at 10 weeks are stored in a biobank for use in future research. All analyses in

Table 1. Inflammatory and related biomarkers.

Name in use	Full name	Description
MIF	Macrophage inhibitory factor	Important regulator of innate immunity and is classified as an inflammatory cytokine. Regulated by several cytokines including TNF alpha and sustains macrophage inflammatory functions
Periostin	Periostin	Associated with renal fibrosis and inflammation
IGFBP1	Insulin-like growth factor binding protein 1	Regulates IGF-1 bioactivity, glucose homeostasis, and tissue regeneration. Increases during inflammation
IGFB3	Insulin-like growth factor binding protein 3	Regulator of IGF signaling
IGF1	Insulin-like growth factor 1	Reduced by inflammation. Insulin sensitivity and vasculoprotective factor
CatS	Cathepsin S	Associated with IMT in CKD and could be associated with several vascular and metabolic outcomes. Induces CCL2-expression
Resistin	Resistin	Adipokine could be related to vascular and metabolic outcomes
OPN	Osteopontin	Calcification and inflammatory processes. Related to several metabolic and vascular outcomes
AXL6	Receptor tyrosine kinase 6	See GAS6
GAS6	Growth arrest-specific 6	Involved in vascular inflammation and several kidney diseases. Linked to insulin resistance
GDF-15	Growth differentiation factor 15	Belonging to the TGF-beta superfamily. Associated with metabolic disturbances. Produced by macrophages and is associated with a proinflammatory environment
Granulysin	Granulysin	Expression associated with acute rejection and steroid resistance
PTX3	Pentraxin 3	Produced and released by many cell types in response to primary inflammatory signals such as IL-1 and TNF alpha
EPCR	Endothelial protein C receptor	Enhances anticoagulation by accelerating the activation of protein C to activated protein C and mediates anti-inflammatory effects
sTNFR1	Soluble tumor necrosis factor receptor 1	General marker representing TNF activity. Increased during inflammation
Syndecan	Syndecan	Involved in fibrosis processing and associated with renal function
CXCL16	CXC chemokine ligand 16	GVHD outcome marker. Mediator of the atherogenesis development. Induces a strong chemotactic response
YKL-40	Tyrosine (Y), lysine (K) leucine (L)-40 kDa	Its pattern of expression is associated with processes related to inflammation and endothelial dysfunction. Related to insulin resistance
Chemerin	Chemerin	Linked to renal function, obesity, glucose tolerance, and hyperlipidemia
NGAL	Neutrophil gelatinase-associated lipocalin	Involved in innate immunity and is used as a biomarker of kidney injury. Also related to inflammatory intestinal diseases

CCL2: Chemokine (C-C motif) ligand 2; GDF-15: Growth differentiation factor 15; GVHD: Graft-versus-host disease; IMT: Carotid intima-media thickness; TNF: tumor necrosis factor.

this study are performed on these samples stored at −80 °C.

The study was approved by the Regional Committees for Medical and Health Research Ethics in Norway and was performed in accordance with the Declaration of Helsinki.

Diagnostics

The modified ADA criteria, as recommended for PTDM, were used to classify patients in the following categories: PTDM; fPG concentration ≥7.0 mmol/L, and/or two-hour plasma glucose concentration (2HPG) ≥ 11.1 mmol/l;

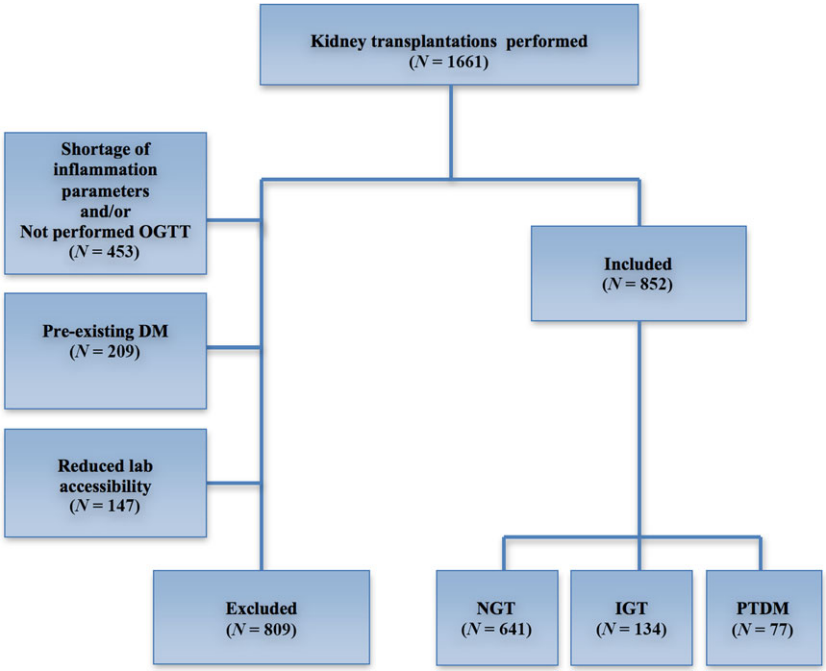

Figure 1 Flowchart – Patient disposition.

impaired glucose tolerance (IGT); fPG < 7.0 mmol/l and 2HPG 7.8–11.0 mmol/l, and normal glucose tolerance (NGT); fPG < 7.0 mmol/l and 2HPG < 7.8 mmol/l.

Immunosuppressive therapy

Standard immunosuppressive protocol at our center in the study period consisted of induction treatment with the IL-2 receptor antibody basiliximab, and maintenance therapy consisting of corticosteroids, the cell proliferation inhibitor mycophenolate, and a CNI [27]. Tacrolimus has been used in most patients, but between 2007 and 2009, cyclosporine A was preferred for older patients and for patients with increased risk of metabolic syndrome.

Registration of data

After 10 weeks, the following data were retrieved: recipient age, sex, height, weight, fPG and 2HPG, blood concentration of tacrolimus and cyclosporine and dose of prednisone. All of the data were retrieved from the National Renal Registry, hospital records, and the biobank.

Inflammatory and related biomarkers used in analyses are presented in Table 1. They were measured in plasma or serum by enzyme immunoassays (EIAs) from R&D Systems (Stillwater, MN, USA). Intra- and inter-assay coefficients of variation were <10% for all assays. The biomarkers were retrieved at the 10-week follow-up. This included markers of leukocyte activation (e.g., macrophage inhibitory factor (MIF) and granulysin), fibrosis/extracellular matrix (ECM) remodeling (e.g., GDF-15, periostin, syndecan), vascular inflammation and angiogenesis (e.g., CXCL16, Pentraxin 3 (PTX3), YKL-40), insulin signaling (insulin-like growth factors), adipose tissue inflammation (e.g., chemerin and resistin) and more specific renal inflammation (e.g., NGAL).

Glucose measurements

OGTT was performed after an overnight fast. The patients were instructed not to eat or drink and not to take any medication during the last 8 h before the test. Blood samples were drawn at 0 and 120 min after ingestion of 75 g anhydrous glucose dissolved in 250 ml water. Glucose measurements were performed in fresh venous whole blood using Hemocue AB B-glucose analyzer and presented as plasma glucose.

Statistical analyses

The independent relationship between plasma glucose concentration and inflammation parameters was examined using linear regression models. The inflammation biomarkers were normally distributed. To decide which parameters to explore further, we used simple linear regression to identify parameters having significant correlation with the 2HPG value. These parameters were thereafter tested independently in multiple linear

Table 2. Demographics and transplant data according to glucose tolerance groups.

	All included (n = 852)	Patients with NGT (n = 641)	Patients with IGT (n = 134)	Patients with PTDM (n = 77)
Recipient age, median (years)	52.0 (14.66)	49.0 (14.56)	58.0 (13.06)	61.0 (14.22)
BMI, median (kg/m^2)	24.7 (3.98)	24.4 (3.92)	25.5 (3.99)	25.3 (4.08)
fPG, median (mmol/l)	5.3 (0.85)	5.2 (0.62)	5.6 (0.72)	7.1 (1.10)
2HPG, median (mmol/l)	6.3 (2.36)	5.8 (1.12)	8.5 (0.94)	12.5 (3.01)
Cyclosporine concentration (µg/l)	150.0 (102.8)	145.0 (52.5)	150.0 (174.7)	175.0 (169.3)
Tacrolimus concentration (µg/l)	6.6 (2.16)	6.5 (2.18)	6.6 (1.97)	6.8 (2.34)
Prednisolone dose (mg)	10.0 (4.36)	10.0 (3.56)	10.0 (5.78)	10.0 (6.43)
Fasting insulin concentration (pmol/l)	90.0 (46.83)	87.0 (44.00)	100.0 (46.72)	98.0 (64.75)
Creatinine, median (µmol/l)	115.0 (37.67)	114.0 (35.42)	118.0 (44.37)	115 (41.74)

fPG, fasting plasma glucose; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; PTDM, post-transplant diabetes mellitus; 2HPG, two-hour plasma glucose.

regression models adjusting for BMI, age, fasting insulin levels, dosage of prednisolone, trough concentration of calcineurin inhibitors and creatinine. Results were considered statistically significant for a two-sided $P < 0.05$. Furthermore, to survey the distribution and levels of inflammatory and related biomarkers among the subgroups NGT, IGT, and PTDM, we used a multinomial logistic regression model with NGT as reference category. We adjusted for the same risk factors as in the linear regression models. The statistical analyses were performed using the SPSS software version 22 and 24.

Results

At evaluation 10 weeks post-transplantation, 641 recipients (75.2%) had NGT, 134 (15.7%) had IGT, and 77 (9.0%) were diagnosed with PTDM. Patient characteristics are described in Table 2.

The analyses of potential associations between previously known risk factors and 2HPG are described in Table 3. We were not able to describe any significant relationship between the 2HPG and the concentration of neither tacrolimus nor cyclosporine. However, dosage of prednisolone ($P < 0.001$), BMI ($P < 0.001$), and age ($P < 0.001$) were significantly associated with 2HPG.

As shown in Table 4, six of the 20 analyzed biomarkers were significantly and positively correlated with 2HPG; sTNFR1, MIF, YKL-40, Endothelial protein C receptor (EPCR), and PTX3, while insulin-like growth factor binding protein 1 (IGFBP1) was negatively associated. Including these variables in a multivariable regression analysis revealed that sTNFR1 ($P = 0.027$), MIF ($P = 0.024$), YKL-40 ($P < 0.001$), EPCR ($P = 0.001$), PTX3 (0.019), and IGFBP1 (0.026) remained independently associated with 2HPG (Table 4). The total R^2 of the models was 0.201.

The multinomial logistic regression model showed a significant positive association between the inflammatory and related markers and the PTDM subgroup compared to the NGT subgroup (Table 5).

Table 3. Association between known risk factors and two-hour plasma glucose.

	Multivariable regression			
Parameter	Mean value	B-value	R^2	Significance
BMI (kg/m$^{2)}$	25.01	0.154 (0.089 to 0.220)	0.103	0.000
Age at time of transplantation (years)	50.82	0.033 (0.019 to 0.048)	0.103	0.000
Creatinine (µmol/l)	120.35	0.005 (−0.001 to 0.011)	0.103	0.098
Prednisolone dose (mg)	11.17	0.102 (0.055 to 0.149)	0.103	0.000
Cyclosporine (µg/l)	157.15	0.003 (−0.001 to 0.008)	0.128	0.150
Tacrolimus (µg/l)	6.95	−0.031 (−0.124 to 0.062)	0.103	0.513

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Table 4. Association between inflammatory and related biomarkers and two-hour plasma glucose.

	Univariable			Multivariable			
Parameter	Mean value	Significance	R^2	B-value	Significance	R^2	Conf.-interval
YKL-40	112.84 (74.08)	0.000	0.050	0.006	0.000	0.141	0.003 to 0.010
EPCR	20.20 (9.30)	0.022	0.006	0.034	0.001	0.142	0.015 to 0.054
PTX3	4.79 (2.64)	0.004	0.010	0.111	0.019	0.126	0.018 to 0.203
MIF	8.44 (4.81)	0.021	0.060	0.047	0.024	0.123	0.006 to 0.088
IGFBP1	117.18 (74.82)	0.008	0.009	−0.003	0.026	0.117	−0.006 to −0.001
sTNFR1	1.84 (1.85)	0.000	0.037	0.427	0.027	0.129	0.048 to 0.805
GDF15	2.95 (1.31)	0.000	0.025	0.143	0.116	0.120	−0.035 to 0.321
NGAL	249.93 (95.85)	0.010	0.008	0.002	0.125	0.120	−0.001 to 0.004
Cat S	24.54 (6.25)	0.005	0.010	0.020	0.209	0.118	−0.011 to 0.050
IGF1	300.55 (171.44)	0.096	0.003	0.000	0.369	0.110	−0.001 to 0.001
Granulysin	3.64 (1.72)	0.052	0.004	−0.020	0.756	0.115	−0.144 to 0.104
Chemerin	247.41 (56.11)	0.885	0.000				
Cxcl16	1.50 (0.25)	0.413	0.001				
Syndecan1	4.96 (4.14)	0.249	0.002				
GAS6	3.79 (2.87)	0.500	0.001				
AXL6	10.54 (2.76)	0.483	0.001				
OPN	62.38 (15.72)	0.989	0.000				
Resistin	29.89 (11.56)	0.384	0.001				
IGFBP3	3.73 (0.83)	0.417	0.001				
Perisotin	242.63 (101.05)	0.499	0.001				

Total R^2 = 0.201.

AXL6: Receptor tyrosine kinase 6; EPCR: Endothelial protein C receptor; GAS6: Growth arrest-specific 6; IGF1: Insulin-like growth factor 1; IGFBP1: Insulin-like growth factor binding protein 1; IGFBP3: Insulin-like growth factor binding protein 3; MIF: macrophage inhibitory factor; NGAL: Neutrophil gelatinase-associated lipocalin; OPN: Osteopontin; PTX3: Pentraxin 3; sTNFR1: Soluble tumor necrosis factor receptor 1; YKL-40: Tyrosine (Y), lysine (K) leucine (L)-40 kDa.

Table 5. Correlation between inflammatory and related biomarkers and post-transplant diabetes mellitus and impaired glucose tolerance with normal glucose tolerance as reference value.

	IGT			PTDM		
Biomarker	aOR	Confidence interval	Significance	aOR	Confidence interval	Significance
YKL-40	1.004	0.998 to 1.007	0.092	1.008	1.003 to 1.013	0.001
EPCR	1.018	0.991 to 1.045	0.189	1.031	1.005 to 1.059	0.019
PTX3	1.020	0.876 to 1.150	0.769	1.159	1.002 to 1.311	0.028
MIF	1.005	0.956 to 1.053	0.853	1.051	1.005 to 1.099	0.030
IGFBP1	0.999	0.994 to 1.003	0.524	0.999	0.993 to 1.004	0.692
sTNFR1	1.720	1.059 to 2.726	0.006	1.496	0.679 to 2.608	0.147
GDF 15	1.141	0.841 to 1.374	0.237	1.400	1.054 to 1.792	0.008
NGAL	1.004	1.000 to 1.006	0.067	1.003	0.998 to 1.007	0.265
Cat S	1.004	0.958 to 1.047	0.857	1.050	0.996 to 1.103	0.060
IGF1	0.997	0.996 to 0.999	0.006	1.000	0.998 to 1.002	0.776
Granulysin	1.083	0.925 to 1.255	0.304	0.952	0.731 to 1.225	0.709

aOR: adjusted odds ratio; EPCR: Endothelial protein C receptor; IGF1: Insulin-like growth factor 1; IGFBP1: Insulin-like growth factor binding protein 1; IGT: impaired glucose tolerance; MIF: macrophage inhibitory factor; NGAL: Neutrophil gelatinase-associated lipocalin; PTDM: post-transplant diabetes mellitus; PTX3": Pentraxin 3; sTNFR1: Soluble tumor necrosis factor receptor 1; YKL-40: Tyrosine (Y), lysine (K) leucine (L)-40 kDa.

Discussion

In this study, we explored the association between a variety of inflammation-related parameters and plasma glucose levels 2 h after an OGTT in 852 patients 10 weeks after kidney transplantation. Six parameters showed a statistically significant association with 2HPG in the multiple regression models with the strongest

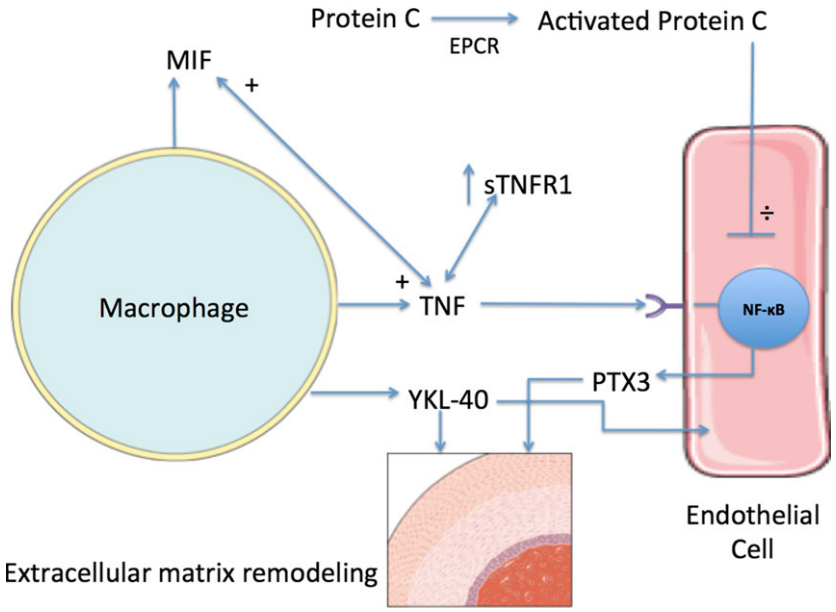

Figure 2 Interplay between the relevant inflammatory biomarkers investigated in the present analysis. Macrophages and monocytes produce acute-phase proteins after stimulation of TLR (toll-like receptor), including tumor necrosis factor (TNF). TNF binds to TNF receptors on the surface of target cells (illustrated by an endothelial cell) and stimulates the intracellular NF-kB pathway. The macrophages also secrete macrophage inhibitory factor (MIF), which may regulate macrophage activity by enhancing TLR4 expression. TNF and MIF promote the production and secretion of each other. The NF-kB pathway in endothelial cells and in other tissues is important for the production of Pentraxin 3 (PTX3), which is involved in innate immunity and in ECM remodeling. Endothelial protein C receptor (EPCR) is essential for the production of activated protein C, and activated protein C has an anti-inflammatory effect, for instance through inhibition of NF-kB. YKL-40, Tyrosine (Y), lysine (K) leucine (L)-40 kDa (YKL-40) is also produced by macrophages and plays a part in ECM remodeling and in endothelial dysfunction [37,50–52]. This presentation of the inflammatory interplay is only partial.

association with sTNFR1, EPCR, PTX3, and MIF. These findings suggest a link between inflammation and development of PTDM, but to distinguish statistical significance from clinical relevance is not feasible. Complementary, the categorical analyses suggested a clear association between inflammatory and related biomarkers and PTDM. It is also worth noticing that it appears to be a weaker correlation between the levels of the biomarkers in the IGT subgroup compared to the NGT subgroup. The association between the biomarkers and PTDM remained significant in multivariable analyses when adjusted for age, BMI, graft function, insulin levels, calcineurin inhibitor, and prednisolone dose. These factors were the main covariates previously shown to be associated with PTDM in our transplant population [1].

After kidney transplantation, the levels of proinflammatory markers normalize within two months [28]. Porrini *et al.* [29] showed that the development of PTDM is bimodal: early PTDM (<3 months) and late PTDM, which developed from post-transplant prediabetes. In this study, our primary focus was on the early type. However, we also explored the association between IGT and the inflammatory biomarkers, as IGT is considered one of the primordial versions of late PTDM. Cieniawski *et al.* [20] found no association between proinflammatory markers and development of PTDM in the late phase after kidney transplantation; however, both IL-6 and TNF receptor II were associated with increased mortality or rejection. The prognostic performance of these biomarkers is of great interest and should be investigated in forthcoming studies.

The pathophysiology of PTDM is characterized by reduced insulin secretion from the pancreatic islets [8] and increased peripheral insulin resistance [9,10]. The peripheral insulin resistance in T2DM is often explained by low-grade inflammation in peripheral tissue [30,31]. In the present study, we found that certain markers of inflammation (i.e., sTNFR1, PTX3 MIF, YKL-40, and EPCR) were associated with impaired 2HPG, suggesting that similar mechanisms as seen in T2DM could be operating in PTDM. Thus, it is tempting to hypothesize that an inflammatory microenvironment in subgroups of renal transplant recipients could result in the development of peripheral insulin resistance and possibly also to impaired insulin secretion in pancreatic beta-

cells [16]. This coincides with the already published studies on the subject [17–19].

The six biomarkers that showed a significant correlation with 2HPG reflect several distinct but also overlapping pathways including activation of TNF and macrophages (e.g., MIF, YKL-40, and sTNFR1) [32–36], and endothelial cells (e.g., PTX3 and sTNFR1) [32,37,38]. Some of these are related to extracellular matrix regulation such as YKL-40 and PTX3 [34,39,40]. These findings illustrate the complex interplay of different inflammatory pathways that are activated during the development of PTDM with inflammation as a common link. As they work in concert (Figure 2), it is not possible to dissect the relevance of each individual biomarker with the current study design. Notably, of the markers that were associated with 2HPG, EPCR is the only marker that is related to anti-inflammatory pathways. EPCR impairs the TNF signaling through activation of protein C (APC) [41,42], and this could indicate that EPCR operates as a regulator of the TNF activity. If the upregulation of this molecule reflects, a counteracting mechanism will have to be further explored. In patients with hep-c, studies have shown that the presence of both hep-c and diabetes is correlated with increased TNF activity compared to patients with hep-c infection and no diabetes [43]. In line with this observation, a large retrospective cohort study among patients with rheumatic arthritis and psoriasis showed a reduced incidence of DM in those who were using TNF inhibitors [44]. Our findings also support the idea of TNF involvement and thus, this pathway is of particular interest.

Importantly, several of the markers that were independently associated with 2HPG have also been related to the development of cardiovascular events in both apparently healthy controls and in patients with various forms of established CVD. PTX-3 and sTNFR1 are established CVD risk markers, and with relevance to the present study population, these markers are also associated with graft function and the cardiorenal syndrome, and they are increased after renal transplantation [34,45–50].

Strengths of our study are the comprehensive set of analyses from a big center cohort of renal transplant recipients analyzed at the same time after transplantation and a complete set of OGTT testing. The consistent association with a large series of different inflammation-related parameters might also be considered a robustness of the study. Limitations are the cross-sectional and retrospective design, and thus, the associations are strictly observational. Also, the study has been performed in a Caucasian population and is not necessarily valid in other patient populations.

Although we have not proven the role of inflammation in the development of PTDM, our findings of an independent association between certain inflammatory molecules, reflecting activation of different but also overlapping inflammatory pathways, and 2HPG, suggest a role for inflammation in the pathogenesis of this disorder. This hypothesis should be further investigated in both clinical and experimental studies, trying to develop risk markers and targets for therapy in relation to PTDM.

Authorship

TFH, TJ, AH and AÅ: designed the study. TFH, TJ, AH, AVR and AÅ: collected the data. AM and TU: performed the biomarker analyses and TFH: the statistical analyses. TFH, TJ, AH and AÅ: wrote the manuscript, whereas all authors have been involved in the discussion of results and have contributed to, read, and approved the final version.

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Conflicts of interest

The authors have declared no conflicts of interest.

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