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Early posttransplant changes in circulating endothelial microparticles in patients with kidney transplantation

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ABSTRACT

Background: Endothelial microparticles (EMPs) are membrane vesicles shed from endothelial cell in response to injury, activation or apoptosis. Kidney transplantation (KTx) is the treatment of choice for patients with end stage kidney disease (ESKD). The aim of this study was to analyze changes in EMP and serum creatinine (SCr) in patients following KTx.
Methods: Blood was periodically collected from patients before (pre-KTx) and after KTx for two months. EMPs were identified as $CD31^{+}/CD42b^{-}$ microparticles and quantified by fluorescence-activated cell scanning.
Results: This study included 213 KTx, 14 kidney/pancreas (KPTx) recipients and 60 healthy donors prior to donation. The recipients were divided into 5 groups based on the cause of ESKD. No differences in the quantity of circulating EMP were seen in the pre-KPTx or KTx recipient sera and healthy donor sera. Patients with ESKD secondary to diabetes mellitus, obstructive/inherited kidney disease and autoimmune disease had a decrease in both circulating EMP and SCr by day 60 after KTx.
Conclusion: Reduction in both circulating EMP and SCr was seen after kidney KTx in patients with selective ESKD.

1. Introduction

Annually, more than 27,000 solid organ transplants are performed in the USA, which includes more than 18,000 kidney transplants, more than 6000 liver transplants and more than 2000 heart transplants [1]. Over the past few years, graft survival rates have improved due to more efficient immunosuppressive therapies and transplantation techniques. Thus, the population of patients with a functioning solid allograft has significantly increased. However, allograft rejection remains one of the main causes for allograft failure. Usually, the monitoring of renal allograft function is evaluated by serum creatinine (SCr) levels in patients. Unfortunately, SCr level elevation is a nonspecific marker of renal allograft dysfunction, as it may occur in many different conditions. Elevated SCr is seen in acute kidney injury secondary to extrarenal etiologies (such as disturbances in systemic circulation and renal blood flow, urinary outlet obstruction) or non-rejection related causes (such as infection) [2]. The "gold standard" test for the assessment of allograft rejection is renal allograft biopsy, which is an invasive, expensive and relatively risky procedure [3]. Therefore, the need for a reliable and clinically significant marker of renal allograft rejection is emerging, as early detection of graft rejection is important for efficient patient care and management.

Microparticles are submicron (0.1–1 μm) membrane vesicles released from the plasma membrane during their activation, injury and (or) apoptosis [4–6]. They express cell surface proteins and cytoplasmic components of their parent cell [7]. The formation of microparticles is a tightly regulated process and the levels of circulating microparticles are increased in patients with vascular diseases, diabetes, infection, metabolic diseases and cancer [4,8–10].

The pool of circulating microparticles is contributed to by several different cell types, including platelets, leukocytes and endothelial cells, where endothelium-derived microparticles (EMPs) represent about 10–15% of the total microparticle population [4–6]. It has been previously demonstrated that levels of circulating EMP may be used as a surrogate marker of endothelial cell dysfunction [6,8,11].

Kidney allograft rejection occurs via cellular, humoral or combined mechanisms. In many cases, the endothelium is the main target of the recipient immune system. We had previously demonstrated that circulating EMP levels decrease in patients following liver allograft implantation [12]. There is evidence that in patients with kidney allografts, EMPs also change after transplantation, but these data were obtained from a limited population of patients [13].

The aim of the current study was to investigate the changes in circulating EMP and SCr levels in a large population of patients after kidney transplantation and determine whether these changes are different in

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Table 1
Demographics of patients included into the study.

	# of patients	Age, years	Gender		Race			Allograft	
		Mean ± SD	Male	Female	Caucasian	African–American	Others	CAD	LD
Group 1	14	38.9 ± 8.5	10	4	7	6	1	14	n/a
Group 2	62	55.8 ± 10.2	35	27	38	20	4	30	32
Group 3	43	47.9 ± 12.4	25	18	40	3	0	13	30
Group 4	18	42.6 ± 14.4	9	9	14	2	2	9	9
Group 5	90	47.8 ± 12.9	51	39	66	19	5	46	44
Total	227	49.0 ± 12.8	130	97	165	50	12	112	115
Healthy donors	60	43.1 ± 11.0	11	49	51	7	2	n/a	n/a

Group 1 — patients with end stage kidney disease secondary to diabetic nephropathy because of diabetes mellitus type I, who received kidney/pancreas simultaneous allografts.
Group 2 — patients with end stage kidney disease secondary to diabetic nephropathy because of diabetes mellitus type I or type II, who received kidney allograft only.
Group 3 — patients with end stage kidney disease secondary to congenital kidney disease or acquired obstructive nephropathy.
Group 4 — patients with end stage kidney disease secondary to immune-complex mediated glomerulonephritides (IgA nephropathy, membranous glomerulonephritis, lupus nephritis).
Group 5 — patients with unknown/unclassified end stage kidney disease.
CAD — cadaveric donor renal allograft; LD — living donor renal allograft.
Data are presented as number of patients or mean ± SD, when applicable.

patients with various underlying causes of end stage kidney disease (ESKD).

2. Material and methods

2.1. Subjects

The study population consisted of consecutive patients admitted to The Ohio State University Wexner Medical Center for kidney transplantation between October 2011 and May 2013. In addition, blood samples were collected from consecutive living donors before nephrectomy and used as healthy control. The study was approved by the Institutional Review Board and all participants were considered eligible after their written informed consent. The patient population is described in the Results section.

3. Preparation of microparticles from plasma

Blood samples were collected from the patients into EDTA-containing tubes and processed for microparticle isolation as described earlier [9,12]. Briefly, platelet-free plasma (PFP) was obtained after an initial centrifugation at 1500 *g* for 10 min followed by a second centrifugation at 1500 *g* for 15 min. Samples were aliquoted and frozen at −80 °C until further use.

3.1. Antibodies

The following antibodies were used: fluorescein isothiocyanate-Annexin V (FITC-Annexin) (Invitrogen, Carlsbad, CA), phycoerythrin-conjugated anti-CD31 (PE-CD31), anthocyanin-conjugated anti-CD45b (APC-CD45b) (BD Biosciences, San Jose, CA), and 1 μm polystyrene beads (Sigma, St. Louis, MO).

4. Immunolabeling and flow cytometry of microparticles

Endothelial-derived microparticles were labeled in 100 μL of PFP using PE-CD31 and APC-CD45b for 45 min at room temperature. In addition, these samples were labeled with FITC-Annexin according to the manufacturer's protocol [18] and analyzed on a Becton-Dickinson FACScan flow cytometer (Becton-Dickinson, Franklin Lakes, NJ). Gating parameters were defined using 1 μm standard polystyrene beads. Microparticles were defined using forward-scatter analysis. The time necessary for counting 10,000 events was determined and microparticle concentration was calculated using the formula $MP = (1000 \times Num \times 60)/(V \times t)$, where MP is the concentration of microparticles (mL/1); Num is the number of particles that passed through flow cytometer; V is the volume speed (60 μL/min); and t is the time (seconds), as we described earlier [11,12,14].

5. Statistics

Descriptive statistics was used to characterize patient's demographic data. Data are presented as mean ± standard deviation, unless specified otherwise. Mixed models were applied to the data using the EMP percent change from baseline as the outcome variable and the following as potential predictor variables: baseline EMP, day, ESKD group and SCr percent change from baseline.

6. Results

6.1. Demographics of patients and immunosuppression

During the study period, 257 recipients of kidney or simultaneous kidney/pancreas allograft were recruited, which represents 86% of the patients who received renal allografts at The Ohio State University Wexner Medical Center (OSUWMC) within the same period of time. In addition, blood samples were obtained from 60 consecutive living donors before nephrectomy. For the final analysis, only recipients of the first renal allograft were included to avoid confounding the EMP changes that may be associated with sensitization, development of donor specific antibodies or previous immunosuppression therapy. The demographic characteristics of patients included into the final study cohort (227 patients) are provided in Table 1.

The 227 patients were divided into groups based on the cause of ESKD as following: Group 1 — patients with ESKD secondary to diabetic nephropathy because of diabetes mellitus type I, who received a simultaneous kidney/pancreas transplant; Group 2 — patients with ESKD secondary to diabetic nephropathy (diabetes mellitus type I or type II), who received kidney allograft only; Group 3 — patients with ESKD secondary to congenital causes or acquired obstructive nephropathy; Group 4 — patients with ESKD secondary to immune-complex mediated glomerulonephritides (IgA nephropathy, membranous glomerulonephritis, lupus nephritis); and Group 5 — patients with unknown/unclassified ESKD.

Baseline immune suppression consisted of rabbit antithymocyte globulin (ATG) induction (1.25 mg/kg/day) with a short, 5 day course of steroid treatment. Maintenance immune suppression consisted of rapamune (sirolimus) started on post-transplant day 0 and delayed cyclosporine (Neoral) begun on day 2 or 3 following recovery of renal function. Rapamune was dosed to achieve a target serum level of 10 ng/mL and cyclosporine was dosed to achieve a C2 (concentration 2 h after the last dose) of 1000 ng/mL.

Rejection episodes occurred within the first year after the transplantation was included. Initial treatment of acute cellular rejection episodes consisted of steroids. ATG was administered for steroid resistant episodes. Antibody-mediated rejection or combined cellular and antibody-mediated rejection episodes were treated with the combinations of ATG, steroids, IVIG, and apheresis.

6.2. Endothelial microparticles and serum creatinine levels before and after kidney transplantation

EMP and SCr levels were analyzed in blood plasma before (baseline) and periodically at days 7, 14 and 21 after transplantation and monthly thereafter. Unfortunately, the number of follow-ups beyond 2 months post-transplant was low; therefore we report herein only changes in EMP and SCr up to 2 months post-transplant. Blood samples from living donors collected before nephrectomy were used as healthy controls.