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Chapter 7

Acute rejection of kidney transplants is associated with a dysbalance in angiopoietins and a sustained increase in systemic microvascular tortuosity

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Abstract

Background: Microvascular endothelial cells (ECs) are very susceptible to injury, including episodes of acute rejection (AR). Whether AR after renal transplantation is associated with sustained systemic microvascular damage is unknown.

Methods: Using SDF imaging, microvascular alterations in AR patients (n=13) were compared with transplant recipients with stable renal function (n=25). In addition, 11 patients were studied longitudinally at 1, 6 and 12 months after rejection. Angiopoietin-1 (Ang-1), Angiopoietin-2 (Ang-2), Vascular Endothelial Growth Factor-A (VEGF-A) and soluble Thrombomodulin (sTM) levels were measured.

Results: Capillary tortuosity was significantly increased in patients with AR (1.74 ± 0.5) compared with the stable group (1.41 ± 0.13, $p<0.05$). Furthermore, the Ang-2/Ang-1 ratio (0.09 ± 0.02 vs 0.05 ± 0.01), VEGF-A (567 ± 188 vs 202 ± 27 pg/ml) and sTM (19667 ± 1809 vs 9667 ± 921 pg/ml) plasma levels were significantly higher in patients with AR compared with stable patients (all $p<0.05$). Interestingly, patients with AR showed persisting increased capillary tortuosity ($p<0.05$) and a disturbed Ang-2/Ang-1 balance up to 1 year after AR. VEGF-A and sTM levels remained significantly elevated up to 1 and 6 months after AR ($p<0.05$), but returned to baseline at 12 months.

Conclusion: AR is associated with increased systemic microvascular tortuosity up to 1 year after rejection, which is associated with elevated levels of angiogenic growth factors. SDF imaging might be a useful tool to assess the degree of microvascular damage in these patients.

Introduction

Endothelial cells (ECs) line the lumina of all blood vessels and form the interface between the blood and tissue. In the context of kidney transplantation, ECs are very susceptible to injury during episodes of allograft rejection. ECs get activated in response to cytokines and growth factors that are produced as part of the alloimmune response (1-6). These activated ECs express adhesion molecules, release cytokines, chemokines and growth factors that mediate recruitment of recipient leukocytes (4;7). This will result in perpetual EC damage and promotion of angiogenesis within the allograft (1;3;4). This process is mediated by different pro-inflammatory and angiogenic growth factors, most notably Vascular Endothelial Growth Factor (VEGF) and Angiopoietin-2 (Ang-2), which play a central role in the angiogenic and inflammatory responses (1-3;6;8-10). The upregulation of Ang-2 destabilizes the endothelial cell lining and promotes the dissociation of pericytes from ECs, which results in the formation of abnormal capillary networks and abnormal blood flow. Local areas of interstitial hypoxia trigger fibrotic and inflammatory changes and ultimately lead to the development of interstitial fibrosis and tubular atrophy (IFTA) (1;2;4;9;11;12).

The endothelium is relatively inaccessible to direct examination. Therefore, investigators have concentrated on various surrogate markers of endothelial function which includes measurement of specific plasma markers including angiopoietins, VEGF and serum soluble Trombomodulin (sTM) (13-16). In allograft rejection different studies showed elevated circulating levels of VEGF and sTM in patients after solid organ transplantation, suggesting (systemic) endothelial cell activation (1;13;17-23).

An additional method for the assessment of endothelial function includes monitoring of the microvasculature. Recently, sidestream darkfield (SDF) imaging has emerged as a non-invasive tool to visualize the human microcirculation and to assess microvascular remodeling (24). We have previously validated this technique and used it to investigate the labial mucosal capillary tortuosity, as marker for systemic microvascular disease in diabetes mellitus type I (DM) patients before and after simultaneous pancreas kidney transplantation (SPK) (24;25). Diabetic patients showed increased capillary tortuosity compared to healthy non-diabetic controls and SPK resulted in reversal of microvascular damage within 1 year after transplantation (24).

To our knowledge no previous studies have studied whether inflammatory changes in the transplanted organ can impact on the systemic microcirculation. In the present study, we therefore assessed whether AR is associated with systemic microvascular damage in a cross-sectional study using SDF imaging and correlated this with markers for endothelial function. In addition, we investigated the long-term effects of AR on the systemic microvasculature in a longitudinal study up to 1 year after rejection.

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Material and Methods

Study population

All procedures were approved by the institutions Medical Ethical Committee. Informed consent was obtained from all the patients. A total of 38 patients (24 males and 14 females) were enrolled in the cross-sectional study after given informed consent. Thirteen patients were included because of a decrease in renal function and biopsy proven acute cellular rejection (AR group). Of these patients, 11 had interstitial acute rejection, and the two other patients had vascular rejection. Ten patients were treated with methylprednisolone, one patients with anti-thymocyte globulin (ATG), one patient with methylprednisolone followed by alemtuzumab treatment and one patients with methylprednisolone followed by ATG. Of the 13 AR patients, 1 had donor specific antibodies against HLA class I antigens and none of the patients had positive HLA class II antibodies. Biochemical markers for endothelial function including, Ang-1, Ang-2, sTM and VEGF-A, together with mucosal capillary density and morphology were compared to 25 kidney transplant recipients with stable renal function (eGFR of 30 ml/min or more, stable group). In addition, analyses were performed in 10 patients from the stable renal funtion group with an eGFR similar to the AR patients, to differentiate between the effects of renal function and rejection on microvascular parameters. Patients with SPK, active infection, liver failure, active auto-immune disease, epilepsy or malignancy in the last 5 years (except patients treated for basal cell carcinoma who were in full remission) were excluded from the study. The patients in the AR group were also studied prospectively in a longitudinally study at 1 (M1, n=13), 6 (M6, n=13) and 12 months (M12, n=11, two patients discontinued their participation) after rejection. All measurements were performed before patients received treatment for AR. None of the 38 patients had signs of infection at the time of the measurements.

Transplantation aspects

All patients underwent solitary kidney transplantation at the Leiden University Medical Center (LUMC) between 2003 and 2012. Kidney transplantation was performed as described previously (24).

Patients were treated with prednisone (tapered to a dose of 10 mg by 6 weeks and a dose of 7.5 mg by 3 months), cyclosporine (targeted to an area under the curve (AUC) 5400 ng.h/ml first 6 weeks then 3250 ng.h/ml) or tacrolimus (AUC 210 ng.h/ml first 6 weeks, then 125 ng.h/ml) and mycophenolate mofetil (MMF) (AUC 30-60 ng.h/ml). In case of side effects patients were converted to everolimus (AUC 120-150 ng.h/ml). All patients received induction treatment with basiliximab (40 mg at day 0 and 4) or daclizumab (100 mg/day on the day of transplantation and 10 days after transplantation). Patients were treated routinely with oral valganciclovir prophylaxis for 3 months, except for cytomegalovirus (CMV) negative recipients receiving a CMV-negative graft.

Laboratory and urinary assessments

All persons enrolled in this study underwent routine venous blood sampling that was collected before the morning intake of immunosuppression to assess creatinine, urea, HbA1c, glucose and hemoglobin. Proteinuria was measured in collected 24-hours urine. The eGFR was calculated with plasma creatinine concentration using the Modification of Diet in Renal Disease (MDRD) formula. At the same time, blood sampling for routine laboratory measurements, blood was collected for analysis of serum Ang-1, Ang-2, VEGF-A and sTM concentrations. Both Ang-1 and Ang-2 were measured by ELISA (R&D Systems, Minneapolis, MN, USA) according to the manufacturer supplied protocol. Likewise, VEGF-A and sTM (Gen-Probe Diaclone Research, Besançon, France) levels were assessed. Since the Ang-2/Ang-1 rather than the absolute levels of either cytokine has been considered to determine the functional status of the microvasculature (9;10;14), this ratio was calculated for the different groups and time points.

Microcirculatory imaging

The SDF microscan (MicroVision Medical Inc., Wallingford, PA, U.S.A) was performed as described previously (24;25). Before analysis, the video files were anonymized so that the assessor was blinded to the subject's identity. Capillary loops were assessed by two individual assessors in a randomized, blind fashion. From the forty video files obtained of the microcirculation in each subject, four technically best files were selected from each lip quadrant for analysis. Mean vessel density (capillaries/mm^2) was calculated by counting the number of vessels per screen shot. Subsequently, tortuosity of capillary loops was assessed using a validated scoring system described previously and the average of assessed capillary tortuosity was used to calculate mean tortuosity index per patient (25).

Statistical analyses

Continuous normally distributed data are presented as mean ± SEM, unless stated otherwise. Differences between two groups in the cross-sectional study were analysed using the unpaired two-sample T-test. When criteria for parametric testing were not met, median and interquartile range (IQR) are presented and tested with the Mann-Whitney test. For categorical variables cross-tables were used and analysed with the chi-square test. In addition, multivariable linear regression was used to adjust for possible confounders. Comparisons of mean differences between the different time points in the longitudinal study were performed using ANOVA analysis.

Correlations between interval variables were calculated using the Spearman rank correlation coefficient. Differences were considered statistically significant with p<0.05. Data analysis was performed using SPSS version 17.0 (SPSS Inc, Chicago, IL) and GraphPad Prism, version 5.0 (GraphPad Prism Software Inc, San Diego, CA).

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Results

Characteristics of transplant recipients with stable renal function and patients with AR
Baseline subject characteristics are presented in Table 1. Renal function of the patients with stable allograft function (47.2±13 ml/min/1.73 mm^2) was better compared to AR patients (38.1±13 ml/min/1.73 mm^2, p<0.05). In order to elucidate if renal function itself affect capillary tortuosity and levels of angiogenic growth factors, we selected a subgroup of 10 patients with stable renal allograft function with comparable allograft function (36.7±6 ml/min/1.73 mm^2) as patients with AR (38.1±13 ml/min/1.73 mm^2, p>0.05) group. Patients in this subgroup had comparable age (52.6±14 years), body mass index (BMI) (25.2±4 kg/m^2), systolic (131±12 mmHg) and diastolic (84±12 mmHg) blood pressure as AR patients (52.7±10 years, 24.3±3 kg/m^2, 142±19 and 83±13 mmHg, respectively, p>0.05). Patients with AR (8.3±3 mmol/L) had increased glucose levels compared to stable patients (6.0± 2 mmol/L) and also compared to the subset of 10 stable patients (5.6±2 mmol/L, both p<0.05). In the AR group, 2 patients were treated for type II diabetes (both with insulin) which was not significantly different from the stable group (n=4, 3 patient treated with insulin and 1 with oral anti-diabetics). In addition, 4 patients with AR and 4 stable patients stopped MMF due to side effects. In 1 patient from the stable group, tacrolimus was replaced by everolimus because of side effects.

In the longitudinal study (Table 1) patients showed significant decreased renal function during all time points after AR compared to stable patients. Glucose levels were increased during AR and showed a significant decrease at 1 and 12 (p<0.05) months following rejection. Moreover, one patient developed new-onset diabetes after treatment with methylprednisolone and was treated with oral anti-diabetics. In four patients MMF was discontinued before rejection, due to side effects. In 2 patients treatment with tacrolimus was added, because of rejection under dual therapy with prednisone and MMF. In 1 patient tacrolimus was given instead of cyclosporine because of rejection.

***Patients with AR have increased capillary tortuosity compared to stable patients***
Patients with AR (1.70 ±0.1, p<0.01) showed significantly increased capillary tortuosity compared to stable patients (mean 1.41 ±0.06) (Fig 1AB), also after adjustment for age, sex, BMI, blood pressure, glucose levels and smoking. Capillary tortuosity in the subset of 10 stable patients with eGFR of 36.7 ml/min/1.73 mm^2 (1.40 ±0.13) was comparable with capillary tortuosity of the remaining patients with a stable allograft function with eGFR of 54.6 ±13 ml/min/1.73 mm^2 (1.41 ±0.06). Capillary density showed no significant difference between the stable renal function group and patients with AR (p=NS, data not shown).

Table 1: Patients characteristics of acute rejection patients during AR, one (M1), six (M6) and twelve (M12) months after rejection compared with stable renal transplant recipients

Patients characteristics	Stable renal transplant (N=25)		D0 (N=13)		M1 (N=13)		M6 (N=13)		M12 (N=11)	
Age (years)	53.2	± 13	51.7	± 10	51.8	± 10	52.4	± 10	52.7	± 10
Sex, male N (%)	15	(60%)	9	(69%)						
Smoking, N (%)	3	(12%)	1	(8%)	1	(8%)	1	(8%)	0	
Median time since transplantation (months) (IQR)	5	(1-19)	12	(3-22)						
Primary kidney disease, N (%)										
Glomerulonephritis	8	(32%)	0		0		0		0	
Diabetes Mellitus	3	(12%)	1	(8%)	1	(8%)	1	(8%)	1	(8%)
Pyelonephritis or interstitial nephritis	1	(4%)	2	(15%)	2	(15%)	2	(15%)	2	(15%)
Focal segmental glomerulosclerosis	3	(12%)	0		0		0		0	
Urologic	1	(4%)	1	(8%)	1	(8%)	1	(8%)	1	(8%)
Polycystic kidney disease	4	(16%)	4	(31%)	4	(31%)	4	(31%)	4	(31%)
Hypertension	2	(8%)	2	(15%)	2	(15%)	2	(15%)	2	(15%)
Unknown	2	(8%)	2	(15%)	2	(15%)	2	(15%)	2	(15%)
Other	1	(4%)	1	(8%)	1	(8%)	1	(8%)	1	(8%)
BMI (kg/m²)	25.8	± 4	24.3	± 3	24.3	± 3	23.8	± 4	25.2	± 4
Dialysis, N (%)	4	(16%)	0		0		0		0	
Systolic BP (mmHg)	137	± 20	142	± 19	132	± 10	132	± 16	127	± 19
Diastolic BP (mmHg)	82	± 9	83	± 13	77	± 9	79	± 12	72	± 10
eGFR (ml/min/1,73 m²)	47.2	± 13	38.1	± 13[#]	35.4	± 15[#]	40.2	± 16[#]	36.0	± 18[#]
Median proteinuria (g/24hr) (IQR)	0.2	(0.2-0.4)	0.4	(0.3-1.0)[#]	0.3	(0.2-0.7)	0.2	(0.2-0.3)	0.3	(0.1-0.3)
Glucose (mmol/L)	6.0	± 2	8.3	± 3[#]	5.7	± 2[*]	6.3	± 3	5.9	± 1[*]
Hemoglobin (mmol/L)	7.9	± 1	7.6	± 1	7.3	± 1	7.7	± 1	7.7	± 1
Hematocrit (L/L)	0.40	± 0.06	0.38	± 0.04	0.36	± 0.05	0.39	± 0.05	0.39	± 0.05
Anti-hypertensives, N (%)										
ACE inhibitor	5	(20%)	2	(15%)	3	(23%)	3	(23%)	2	(18%)
Diuretics	6	(24%)	3	(23%)	4	(31%)	2	(15%)	1	(9%)
β-blockers	11	(44%)	6	(46%)	7	(54%)	7	(54%)	6	(55%)
Calcium antagonists	14	(56%)	8	(62%)	9	(69%)	6	(46%)	6	(55%)
Angiotensin-II antagonists	1	(4%)	3	(23%)	3	(23%)	2	(15%)	3	(27%)
Statines, N (%)	9	(36%)	5	(39%)	6	(46%)	7	(54%)	7	(64%)
Immunosuppressive, N (%)										
Cyclosporine	3	(12%)	3	(23%)	3	(23%)	2	(15%)	0	
Tacrolimus	19	(76%)	7	(54%)	8	(62%)	10	(77%)	9	(81%)
Prednisone	24	(96%)	13	(100%)	13	(100%)	13	(100%)	11	(100%)
Everolimus	2	(8%)	1	(8%)	0		0		1	(9%)
Mycophenolate Mofetil	21	(84%)	9	(69%)	9	(69%)	10	(77%)	7	(64%)
Donor characteristics										
Age (years)	52.3	± 9	46.4	± 11						
Sex, male N (%)	8	(32%)	4	(31%)						
Donortype, deceased donors, N (%)	5	(20%)	5	(39%)						

All data are mean ±SD, unless otherwise specified. [#]p<0.05 compared to stable patients. [*]P<0.05 compared to AR. BMI, body mass index;BP, blood pressure ACE,angiotensin converting enzyme; eGFR, estimated glomerular filtration rate; IQR, interquartile range.
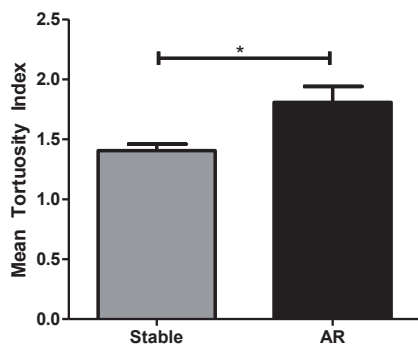
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A.
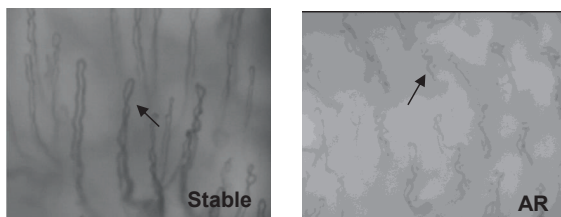
B.

Figure 1. A. Sidestream darkfield images of the oral mucosa visualizing the microvascular capillaries of a representative patient in stable and AR group. Black arrows: capillary loops. **B.** Mean tortuosity index of microvascular capillaries in the stable (n=25) and AR (n=13) group. Data shown are mean±SEM. *P<0.05 compared to stable group.

During the follow-up period after AR, capillary tortuosity remained increased at 1 month (1.72 ±0.1, p<0.01), 6 (1.71 ±0.4, p<0.05) and 12 months (1.74 ±0.1, p<0.001) following AR compared to patients with a stable renal function (1.41 ±0.06) (Fig 2AB).

Ang-2/Ang-1 ratio, VEGF-A and sTM levels are increased in AR patients

To evaluate the effects of AR on the expression of angiogenic factors, we calculated the Ang-2/Ang-1 ratio and measured serum levels of VEGF-A and sTM. The Ang-2/Ang-1 ratio was significantly increased in AR group (0.09 ±0.02) compared with stable renal transplant recipients (0.05 ±0.01, p=0.01) (Fig 3A). In line with these observations, VEGF-A serum levels were increased in AR patients (567 ±188 pg/ml, p=0.02) compared to stable recipients (202 ±27 pg/ml) (Fig 3B).

In addition, elevated sTM serum levels were detected in AR group (19667 ±1809 pg/ml) group compared with stable patients (9667±921 pg/ml, p< 0.0001) (Fig 3C). The differences remained significant after adjustment for age, sex, BMI, blood pressure, glucose levels and smoking (p<0.05).
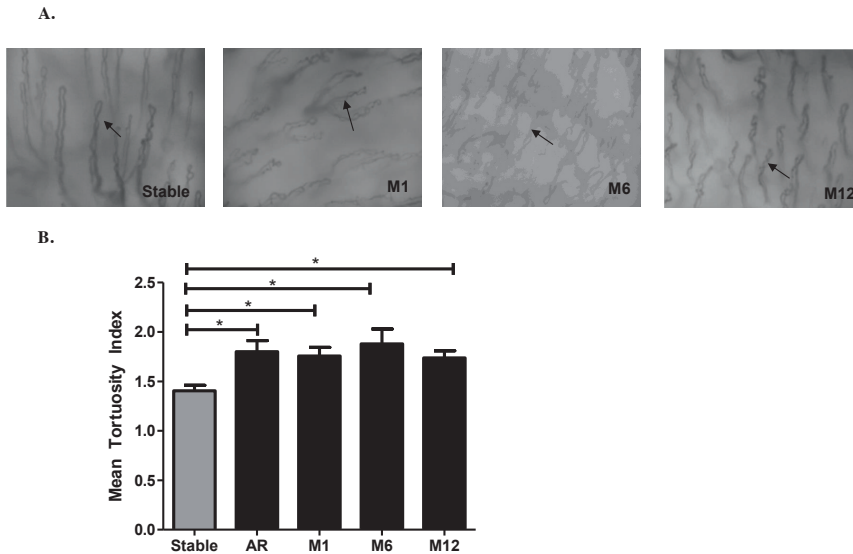
A.

B.

Figure 2. A. Sidestream dark field images of the oral mucosa visualizing the microvascular capillaries of a representative stable kidney transplant patient, one (M1), six (M6) and twelve (M12) months after rejection in the longitudinal rejection study. Black arrows: capillary loops.
B. Longitudinal course of the mean tortuosity index of microvascular capillaries in the stable group (n=25) and at one (M1, n=13), six (M6, n=13) and twelve (M12, n=11) months after rejection. Data shown are mean±SEM. * $P<0.05$ compared to stable group.
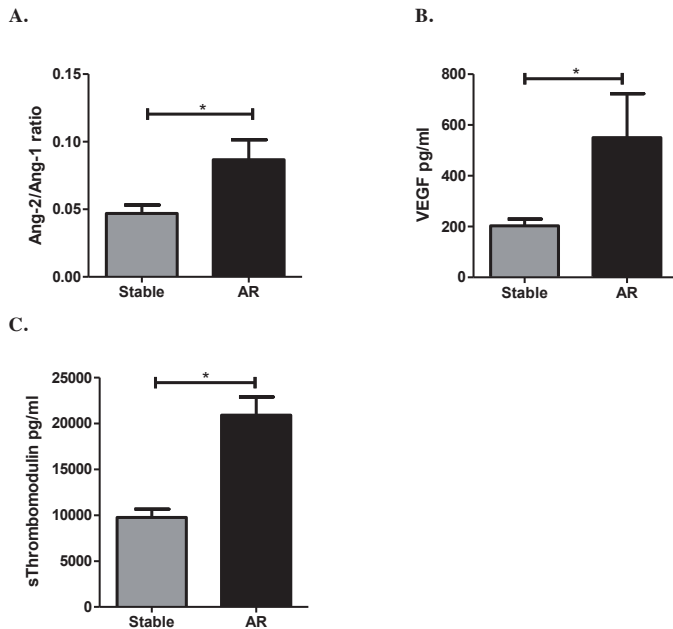
A.

B.

C.

Figure 3: Ang-2/Ang-1 ratio **(A)**, VEGF-A **(B)** and soluble thrombomodulin **(C)** serum levels (pg/ml) in stable (n=25) and AR (n=13) group. Data shown are mean±SEM. *$P<0.05$.

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Ang-2/Ang-1 ratio remained increased and VEGF-A and sTM serum levels decreased in the first year after acute rejection

During follow up, the Ang-2/Ang-1 ratio remained elevated at 1 month (0.07 ±0.02, p=0.35), 6 months (0.06 ±0.02, p=0.44) and 12 months (0.07 ±0.02, p=0.36) after AR compared with stable renal transplant recipients (0.05 ±0.01) however, statistical significance was not reached (Fig 4A). Furthermore, VEGF-A serum levels remained significantly increased up to 1 month after rejection (465 ±109 pg/ml) compared with stable renal transplant recipients (202 ±27 pg/ml, p=0.03) and started to show a decrease at 6 (368 ±150 pg/ml, p=0.23) and 12 months (257 ±95 pg/ml, p=0.42) compared with period during AR (Fig 4B). Mean sTM serum levels were significantly higher at 1 (20083 ±3468 pg/ml, p=0.02) and 6 months (14927 ±2330 pg/ml, p=0.02) after rejection compared to stable patients. At 12 months after rejection, sTM serum levels showed a significant decrease (10875 ±1549 pg/ml, p<0.05) compared to during AR (19667 ±1809 pg/ml) (Fig 4C). The differences remained significant after correction for age, sex, BMI, blood pressure, glucose levels and smoking.
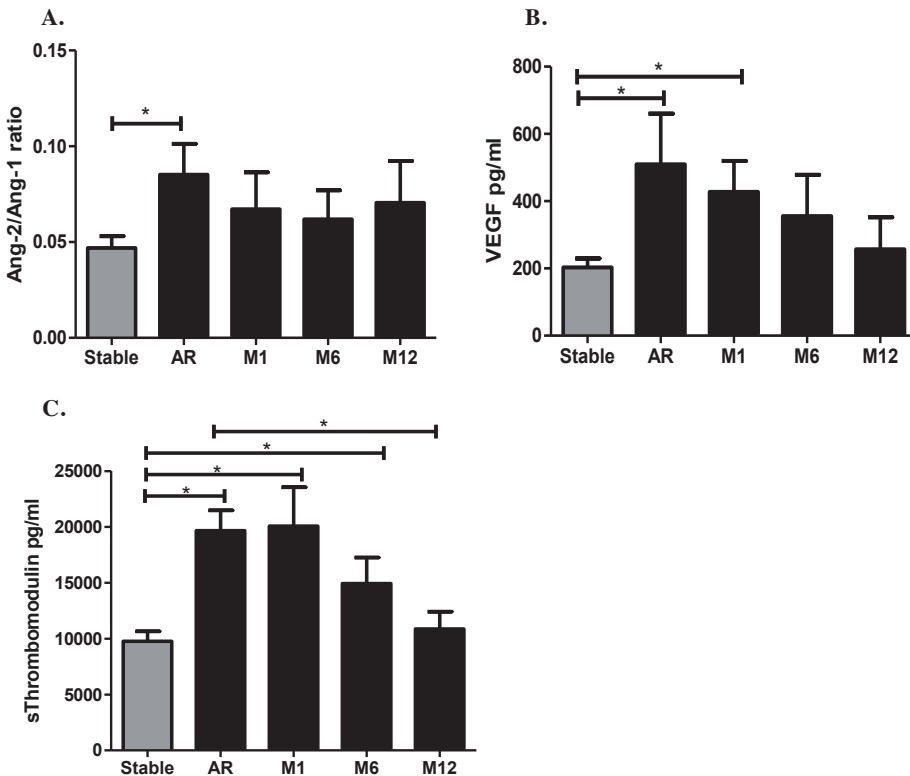

Figure 4: Longitudinal course of Ang-2/Ang-1 ratio **(A),** serum VEGF-A **(B)** and soluble thrombomodulin (pg/ml) **(C)** levels at one (M1, n=13), six (M6, n=13) and twelve (M12, n=11) months after rejection compared with stable group. Data shown are mean±SEM.
*P<0.05.

Serum levels of VEGF-A and sTM correlate with capillary tortuosity, eGFR and proteinuria

Next, the correlation between Ang-2/Ang-1 ratio, VEGF-A, sTM serum levels and capillary tortuosity, time since transplantation, eGFR, glucose levels, calcineurin inhibitors use (CNI) and proteinuria was assessed. sTM levels correlated positively with capillary tortuosity (r=0.43, p<0.0010), proteinuria (r=0.58, p<0.001) and glucose levels (r=0.44, p<0.0001). Moreover, there was a negative correlation with Ang-2/Ang-1 ratio, VEGF-A, sTM and eGFR (r= -0.43, p<0.001, r=-0.38, p=0.01, r=-0.48, p<0.001, respectively).

Discussion

In the present study, we demonstrate that the systemic microvasculature, assessed by SDF imaging, is markedly disturbed in patients with AR compared with stable renal transplant recipients. This coincides with an increased Ang-2/Ang-1 ratio as well as increased VEGF-A and sTM serum levels in the circulation. Interestingly, capillary tortuosity remained increased up to 1 year after rejection in patients in the longitudinal study. The current findings suggest that AR alter the systemic microvasculature chronically, and that SDF imaging might be a useful tool to assess the degree of microvascular damage in these patients.

To date, there are only a few studies that have used SDF for assessment of microvascular alterations after solid organ transplantation. In our previous study, it was demonstrated that capillary tortuosity is reversed in the first year after SPK in DM patients, which was also associated with a normalization of the Ang-2/Ang-1 balance and sTM levels. Interestingly, in this study DM patients who received a solitary kidney transplantation did not demonstrate reversibility of increased capillary tortuosity (24). These findings support the hypothesis that with SPK, i.e. beta cell function and renal failure reversal and not renal function alone is required to restore systemic microvascular tortuosity (24). In the current study, an increased systemic microvascular tortuosity was observed in patients after AR using SDF imaging. Interestingly, in the longitudinal rejection study this increased capillary tortuosity remained for up to 1 year after the rejection episode, suggesting that rejection can induce a sustained injury of the endothelium. Graft biopsies performed in patients after treatment of allograft rejection episodes revealed persistence of infiltrates in the graft (5;26;27). Interestingly the presence of monocytes/ macrophages and CD3^{+}T cell infiltrates was strongly associated with the induced expression of VEGF in rejecting human cardiac allografts and VEGF is likely a key intermediary between sustained cell-mediated immune inflammation and the associated angiogenesis reaction (5;6). Alternatively, loss of kidney function after rejection may be responsible for the observed sustained sysmic microvascular damage. Indeed, Edwards et al showed a significant association between retinal microvascular abnormalities and renal

function deterioration (28). To differentiate between AR and loss of renal function as a cause for the sustained change in microvascular parameters we did a case-control analysis in subjects with stable renal function that was matched for the renal function after 1 year in the AR group. Capillary tortuosity of this subset of patients was similar to the other patients with a stable renal function, making loss of renal function as an explanation less likely.

An interesting observation in our study is that the observed increased microvascular tortuosity coincided with a disturbed Ang-2/Ang-1 ratio and with increased levels of sTM and VEGF-A. This is in line with previous studies that have demonstrated a positive correlation between microvascular destabilization and such markers of endothelial dysfunction (14-16;18;24). Ang-1 is a competitive ligand for the same Tie-2 receptor as for Ang-2, with competing, antagonistic effects on angiogenesis and microvascular remodeling (10;14). It has been reported that Ang-2 displays VEGF dependent modulation of the microvascular structure, suggesting that these factors may act in concert to induce the observed microvascular damage (29). A disturbed Ang-2/Ang-1 balance and increased sTM levels, have been reported in patients with chronic kidney disease, with normalization after renal transplantation (14;16). Similarly, sTM levels have been reported to be higher during rejection after liver transplantation (23). Moreover, during chronic cardiac allograft rejection, Ang-1 expression was decreased, while Ang-2 was upregulated in the microvascular ECs of rejecting cardiac allografts (30). In our study a disturbed Ang-2/Ang-1 balance in AR patients was observed compared with stable renal transplant recipients and remained increased up to 1 year following rejection.

Several studies have reported on the pivotal role of VEGF-A in the development of endothelial dysfunction in acute and chronic rejection (4;31). High serum and urine levels of VEGF-A were found in patients with cardiac and renal allograft rejection, which return to baseline levels after successful treatment of the rejection (22;32). Increased serum VEGF-A levels were also found in our study in patients with AR, which remained significantly increased up to 1 month after rejection and had almost normalized at 1 year following rejection.

In conclusion, the current study demonstrates systemic microvascular damage in AR patients. Simultanous increase of angiogenic growth factors and capillary tortuosity suggests that these factors might participate in the pathogenesis of microvascular damage during rejection. Since EC activation results in impaired survival of allografts, therapies aimed at maintaining microvascular integrity may have beneficial effects on long-term graft survival after rejection episodes. Monitoring of the microcirculation by SDF imaging may be a novel non-invasive approach for the detection of early microvascular damage during allograft rejection.

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