Levels of Angiopoietin-Like-2 Are Positively Associated With Aortic Stiffness and Mortality After Kidney Transplantation

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BACKGROUND
Angiopoietin-like-2 (ANGPTL2) is a secreted proinflammatory glycoprotein that promotes endothelial dysfunction, atherosclerosis, and cardiovascular disease (CVD). Circulating ANGPTL2 is increased in chronic kidney disease (CKD), where the risk of CVD is amplified. The objectives of the present study were to (i) examine whether kidney transplantation (KTx) reduces ANGPTL2 levels, (ii) identify the determinants of ANGPTL2 after KTx, (iii) study the association of ANGPTL2 with aortic stiffness, and (iv) assess the impact of ANGPTL2 on mortality after KTx.

METHODS
In 75 patients, serum ANGPTL2 levels were measured at baseline and 3 months after KTx. Aortic stiffness was determined by carotid-femoral pulse wave velocity, glomerular filtration rate was estimated by CKD-EPI formula, and serum cytokines and endothlin-1 levels were determined 3 months after KTx. Survival analysis was performed using Kaplan–Meier and Cox regression after a median follow-up of 90 months.

RESULTS
After 3 months of KTx, ANGPTL2 levels decreased from 71 ng/ml (53–95) to 11 ng/ml (9–15) ($P < 0.001$). In multivariate analysis, age, lower renal function, and endothelin-1 were independently associated with higher post-KTx ANGPTL2 levels. ANGPTL2 was positively associated with aortic stiffness after KTx, even when adjusted for mean blood pressure (standardized $\beta = 0.314$; $P = 0.008$). During follow-up, 13 deaths occurred. The group of patients with higher post-KTx ANGPTL2 levels had a hazard ratio for mortality of 3.9 (95% confidence interval: 1.07–14.4; $P = 0.039$).

CONCLUSION
KTx significantly reduced serum ANGPTL2 levels. The positive association between post-KTx ANGPTL2, aortic stiffness and mortality, suggests that ANGPTL2 may play a biological role in CKD-related CVD.

Keywords: angiopoietin-like-2; aortic stiffness; blood pressure; chronic kidney disease; hypertension; kidney transplantation; mortality.

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Cardiovascular disease (CVD) is the leading cause of mortality in patients with chronic kidney disease (CKD).[1–3] However, this increased cardiovascular mortality risk cannot be entirely explained by traditional risk factors.[1] Studies have shown an association between vascular stiffness, a nontraditional risk factor, and high rate mortality in CKD patients.[4–6] The accelerated progression of aortic stiffness can result from exposure to previous traditional cardiovascular risk factors, but could also come from endothelial dysfunction, inflammation, accumulation of advanced glycation end-products and vascular calcification.[7–10] Angiopoietin-like-2 (ANGPTL2), a secreted glycoprotein that belongs to the angiopoietin-like family, is a novel proinflammatory mediator. It promotes endothelial dysfunction, atherosclerosis, and inflammation through an autocrine/paracrine pathway on endothelial cells.[11–13] The clinical importance of ANGPTL2 has been underlined by several studies. First, in the elderly without CVD, serum ANGPTL2 levels were reported to be associated with the presence of both plaque and intimal-media thickness.[13] Second, in diabetic patients without CVD, a positive correlation between ANGPTL2 levels and intimal-media thickness was also reported.[14] Third, in the general population, high ANGPTL2 levels were associated with lower renal function and increased risk of developing CVD.[15,16] Finally, in diabetic patients with CKD, we reported that ANGPTL2 levels are associated with an increased risk of death related to cardiovascular events.[17] Altogether, these data suggest that high ANGPTL2 circulating levels are detrimental for renal and cardiovascular functions.

The reduction of ANGPTL2 levels after restoration of renal function by kidney transplantation (KTx), and its association with aortic stiffness and mortality remain, however, unknown. We hypothesized that high levels of ANGPTL2 associated with severely impaired renal function contribute

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to endothelial dysfunction, vascular disease, and mortality in patients with severe CKD. Therefore, the objectives of the present study were to examine the impact of KTx on the circulating levels of ANGPTL2, to identify the determinants of ANGPTL2 after KTx, and to examine its association with vascular stiffness and mortality.

METHODS

Study design and patient population

This is an observational study involving patients with stage-5 kidney disease who were undergoing KTx. All adult patients who were offered KTx at CHU de Québec between September 2007 and October 2011 were eligible. The inclusion and exclusion criteria of these subjects have been previously reported.[10,18] In brief, all patients were adults, with end-stage kidney disease, and were on chronic dialysis with palpable brachial, femoral, and carotid pulse so as to obtain a reliable reading of hemodynamic parameters.[10,18] Furthermore, subjects with a graft failure in need of dialysis by 3 months post-transplantation were excluded for the analysis. The protocol was approved by the Institutional Review Board (Comité d'Éthique de la recherche du CHU de Québec-Université Laval) and the study was conducted in accordance to the Declaration of Helsinki. All patients provided informed written consent.

During this period, 212 patients received a renal transplantation, 124 patients were not approached to participate because of the lack of time or personnel to enroll patients before surgery. Therefore, 88 patients were approached, met the inclusion criteria and were enrolled. Among these, 13 patients were excluded for the following reasons: consent withdrawal (n = 5), graft failure that required dialysis 3 months after KTx (n = 4), and absence of blood samples (n = 4). The analysis was therefore performed on 75 patients undergoing a KTx (Figure 1). All patients were treated with a standard immunosuppressive regimen composed of tacrolimus, mycophenolate mofetil, and corticosteroids. There were 16 living-donors KTx, 59 cadaveric donors, and 15 dual KTx. All patients underwent clinical, biochemical, hemodynamic, and pharmacological evaluations 3 months post-KTx. For survival analysis, there was no loss to follow-up and the vital status was assessed up to June 2016, for a median follow-up of 90 months (79–95).

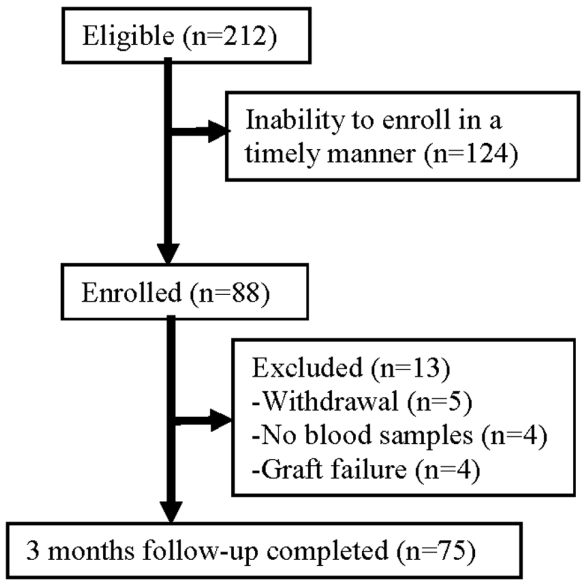

Figure 1. Study flowchart.

Hemodynamic parameters

Hemodynamic measurements were assessed after a 15-minutes rest period in a supine position. Carotid-femoral pulse wave velocity (cf-PWV) was evaluated using Complior SP (Artech Medical, Pantin, France) as previously described.[10] Brachial blood pressure (BP) was determined with an automatic oscillometric sphygmomanometer BPM-100 (BP-Tru, Coquitlam, Canada). Central BP was determined using generalized transfer function of radial artery waveform, obtained through tonometry (Sphymocor) after calibration using brachial systolic and diastolic BP. In patients with arteriovenous fistula, all measurements were performed on the contralateral side.

Biological parameters

Serum samples were immediately centrifuged and stored at −80°C. Endothelin-1 and pentosidine were measured as previously described.[10,18] Serum cytokines profile (IFNγ, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, and TNFα) was measured by ELISA using a Ciraplex Human Cytokine 10-Plex Array (Aushon BioSystems, Billerica, MA). Serum ANGPTL2 level was measured by ELISA using a kit for human Angiopoietin-like-protein 2 (#SEB919Hu) (Cloud-Clone Corporation, Houston, TX) as previously described.[17,19]

Statistical analysis

Data are presented as mean ± SD or median (25^{th}–75^{th} percentiles). Data that were not normally distributed were log transformed where indicated. Kruskal–Wallis was used to examine the differences in the levels of ANGPTL2 with respect to the cause of renal disease. Changes of ANGPTL2 levels after KTx were tested using Wilcoxon rank test. Independent Student t, Mann–Whitney U test, or chi square was used as appropriated to examine differences between subjects according to the median level of ANGPTL2. Determinants of ANGPTL2 were further examined using forward conditional logistic regression. Survival analysis was performed using Kaplan–Meier and Cox regression analysis. Analysis was performed using SPSS 23.

RESULTS

The baseline characteristics of the patients who underwent KTx and were dialysis-independent by the third month post-KTx are presented in Table 1. Three months after KTx, the levels of ANGPTL2 decreased significantly from 71 ng/

ml (53–95) to 11 ng/ml (9–15) ($P < 0.001$), as shown in Figure 2.

Three months after KTx, the group of patients with higher ANGPTL2 levels (>median of 11 ng/ml) were older, had a lower estimated glomerular filtration rate and a higher prevalence of diabetes and CVD (Table 2). In addition, among the hemodynamic parameters, cf-PWV, and central pulse pressure were higher in the group with higher ANGPTL2 levels (Table 2). There was also a higher level of circulating endothelin-1 in the group with higher ANGPTL2 levels (Table 2). Finally, lower HDL and higher TG levels were observed in patients with higher ANGPTL2 levels (Table 2). However, no association between circulating cytokines levels and the levels of ANGPTL2 was found (Table 2). We found no differences in the levels of ANGPTL2 and the cause of kidney disease at baseline (diabetes: 71 ± 6; vascular disease: 73 ± 9; others: 77 ± 5 ($P = 0.897$)). But there was a slightly higher level of ANGPTL2 in patients with diabetic kidney disease at 3 months post-transplant (diabetes: 16 ± 2; vascular disease: 13 ± 2; others: 12 ± 1 ($P = 0.019$)).

Table 1. Baseline characteristics at the time of transplantation

Parameters	*n* = 75
Male	52 (69)
Age (year)	50 ± 14
Weight (kg)	73.8 ± 13.8
Body mass index (kg/m^2)	26.3 ± 4.5
Hypertension	72 (96)
Smoking[a]	52 (69)
Diabetes	21 (28)
CVD	18 (24)
Dialysis vintage (months)	60 [13–75]
Primary renal disease	
Diabetes	17 (23)
Vascular disease	7 (9)
Others	51 (68)
Pharmacological profile	
ACEi/ARB	46 (61)
Statins	56 (75)

Results are mean ± SD, median [25th–75th percentiles] or *n* (%). Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blockers; CVD, cardiovascular disease.

[a]Smoking (active or past history).

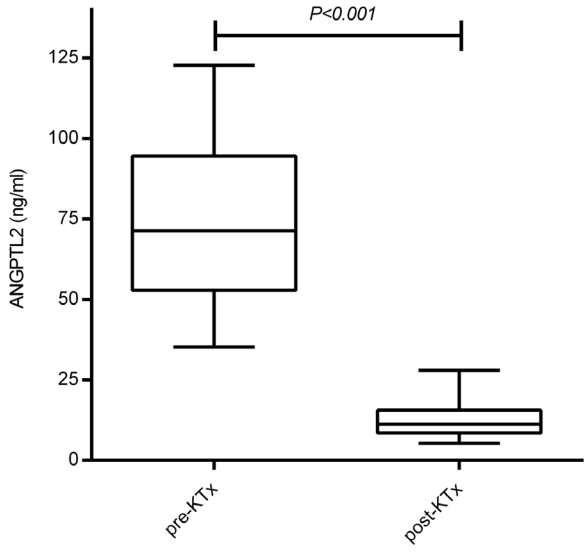

Figure 2. Levels of ANGPTL2 before and 3 months after kidney transplantation. Boxplots graph shows a significant reduction of angiopoietin-like-2 (ANGPTL2) after kidney transplantation (whiskers represent 5th and 95th percentiles).

In a multiple logistic regression analysis, using a forward conditional approach, only age, estimated glomerular filtration rate, and endothelin-1 were independently associated with higher levels of ANGPTL2, whereas prevalence of CVD and diabetes were excluded from the model based on statistical criteria (Table 3). This association remained the same even after incorporation of cause of renal diseases into the model.

Figure 3 shows a positive relationship between aortic stiffness assessed by cf-PWV and ANGPTL2 levels measured 3 months after KTx. In a linear regression analysis, ANGPTL2 was positively associated with aortic stiffness assessed by cf-PWV (standardized $\beta = 0.280$; $P = 0.015$) and with central pulse pressure (standardized $\beta = 0.269$; $P = 0.02$). Adjustment for mean BP did not affect the relationships between ANGPTL2 and aortic stiffness assessed by cf-PWV (standardized $\beta = 0.314$; $P = 0.008$) and central pulse pressure (standardized $\beta = 0.290$; $P = 0.02$). However, after adjustment for age, this relationship was no longer statistically significant ($P = 0.46$).

After a median follow-up of 90 months (79–95), 13 (17%) deaths occurred. Figure 4 shows that post-KTx ANGPTL2 levels above median were significantly associated with a higher mortality ($P = 0.0263$). In a Cox regression analysis, the hazard ratio for mortality in the group of higher ANGPTL2 was 3.9 (95% confidence interval: 1.07–14.4; $P = 0.039$). Because of low number of events, further adjustment was not possible. Table 4 shows the relative importance of each parameter and its relation to mortality using a univariate Cox regression analysis.

DISCUSSION

This study demonstrates for the first time that circulating levels of ANGPTL2 are extremely elevated in patients with stage-5 CKD, but KTx strongly reduces ANGPTL2 levels, indicating that the kidney is a source of ANGPTL2. Most importantly, it shows that, after KTx, ANGPTL2 levels are positively associated with aortic stiffness, central pulse pressure, renal function and mortality, independently of the inflammatory status of the patient, suggesting that ANGPTL2 may play a biological role in CKD-related CVD. ANGPTL2 is a proinflammatory circulating protein with structural homology to the angiopoietins: similarly to the latter, ANGPTL2 is composed of a N-terminal coiled-coil domain and a C-terminal fibrinogen-like domain, but it

Table 2. Clinical, hemodynamic, and biochemical parameters 3 months post-transplantation

Parameters	Total, *n* = 75	ANGPTL2 ≤ median, *n* = 38	ANGPTL2 > median, *n* = 37	*P* value
Demographic				
Male	52 (69)	25 (66)	27 (73)	0.500
Age (year)	51 ± 14	45 ± 14	57 ± 10	**<0.001**
Clinical				
Weight (kg)	73.4 ± 13.7	71.1 ± 11.2	75.8 ± 15.7	0.148
Body mass index (kg/m^2)	26.1 ± 4.6	25.6 ± 4.6	26.6 ± 4.6	0.346
Hypertension	64 (85)	33 (87)	31 (84)	0.306
Smoking	52 (69)	24 (63)	28 (76)	0.240
Diabetes	21 (28)	6 (16)	15 (41)	**0.017**
CVD	18 (24)	2 (5)	16 (43)	**<0.001**
Dialysis vintage (months)	63 ± 64	73.3 ± 75.4	56.6 ± 49.1	0.271
eGFR (ml/min/1.73 m^2)	61 ± 20	70 ± 18	51 ± 17	**<0.001**
Primary renal disease				**0.026**
Diabetes	17 (23)	4 (11)	13 (35)	
Vascular disease	7 (9)	3 (8)	4 (11)	
Others	51 (68)	31 (82)	20 (54)	
Hemodynamic parameters				
Heart rate (bpm)	74 ± 12	75 ± 10	73 ± 14	0.391
Peripheral				
SBP (mm Hg)	123 ± 16	123 ± 12	124 ± 19	0.672
DBP (mm Hg)	73 ± 10	75 ± 9	71 ± 12	0.059
PP (mm Hg)	51 ± 14	48 ± 8	54 ± 18	0.056
Central				
SBP (mm Hg)	109 ± 15	109 ± 12	110 ± 17	0.721
DBP (mm Hg)	74 ± 10	77 ± 8	71 ± 12	**0.017**
MBP (mm Hg)	89 ± 11	91 ± 10	88 ± 13	0.155
PP (mm Hg)	35 ± 12	32 ± 8	39 ± 14	**0.010**
PP amplification	1.5 ± 0.2	1.5 ± 0.2	1.4 ± 0.2	**0.040**
Pulse wave velocity				
cf-PWV (m/s)	10.6 ± 2.0	10.1 ± 1.9	11.2 ± 2.0	**0.020**
cr-PWV (m/s)	8.9 ± 1.3	9.0 ± 1.3	8.7 ± 1.4	0.346
Biochemical				
Total cholesterol (mmol/l)	4.5 ± 1.1	4.7 ± 1.1	4.4 ± 1.0	0.277
HDL (mmol/l)	1.4 ± 0.5	1.5 ± 0.6	1.2 ± 0.4	**0.036**
LDL (mmol/l)	2.4 ± 0.8	2.5 ± 0.8	2.2 ± 0.8	0.177
TG (mmol/l)	1.7 ± 0.9	1.4 ± 0.6	2.0 ± 1.1	**0.011**
Pentosidine (ρmol/mg)	55.4 ± 35.6	50.1 ± 34.7	60.3 ± 36.3	0.185
Endothelin-1 (ρg/ml)	10.6 ± 3.1	9.5 ± 2.2	11.9 ± 3.5	**<0.001**
ANGPTL2 (ng/ml)	13.1 ± 7.2	8.3 ± 1.7	18.0 ± 7.4	**<0.001**
Inflammatory cytokines				
IL-1α (ρg/ml)	11.9 ± 17.3	12.5 ± 18.6	11.2 ± 16.1	0.754
IL-1β (ρg/ml)	1.4 ± 2.6	1.1 ± 3.0	1.3 ± 2.2	0.843
IL-2 (ρg/ml)	1.6 ± 2.8	1.5 ± 2.8	1.7 ± 2.8	0.720
IL-4 (ρg/ml)	0.93 ± 1.86	0.92 ± 1.96	0.94 ± 1.77	0.976

Table 2. *Continued*

Parameters	Total, *n* = 75	ANGPTL2 ≤ median, *n* = 38	ANGPTL2 > median, *n* = 37	*P* value
IL-6 (pg/ml)	4.6 ± 4.2	3.9 ± 4.4	5.4 ± 4.0	0.129
IL-8 (pg/ml)	86.6 ± 75.9	82.5 ± 69.0	90.9 ± 83.4	0.636
IL-10 (pg/ml)	5.5 ± 10.7	5.9 ± 14.2	5.0 ± 5.0	0.730
IL-12 (pg/ml)	23.6 ± 95.5	32.1 ± 130.7	14.6 ± 27.8	0.434
TNFα (pg/ml)	5.0 ± 10.2	5.7 ± 12.7	4.2 ± 6.8	0.547
IFNγ (pg/ml)	0.93 ± 1.41	0.92 ± 1.59	0.94 ± 1.21	0.965
Pharmacological				
ACEi/ARB	27 (36)	13 (34)	14 (38)	0.744
Statins	51 (68)	23 (61)	28 (76)	0.160
Tacrolimus levels (µg/l)	8.72 ± 2.37	8.66 ± 2.32	8.78 ± 2.46	0.503

Results are means ± SD, *n* (%), or median [25th–75th percentiles]. Bold numbers refer to *P* values of <0.05. Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ANGPTL2, angiopoietin-like 2; ARB, angiotensin receptor blockers; cf-PWV, carotid-femoral pulse wave velocity; cr-PWV, carotid-radial pulse wave velocity; CVD, cardiovascular disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate using CKD-EPI formula; HDL, high-density lipoprotein; IFNγ, interferon gamma; IL-, interleukin-; LDL, low-density lipoprotein; MBP, mean blood pressure; PP, pulse pressure; SBP, systolic blood pressure; Smoking, past or active history; TG, triglyceride; TNFα, tumor necrosis factor alpha.

Table 3. Determinants of ANGPTL2 after KTx

Predictors	Exp (β)	95% CI	*P* value	Nagelkerke-R^2
Age (year)	1.10	(1.04–1.17)	0.002	0.56
Endothelin-1 (pg/ml)	1.43	(1.08–1.87)	0.011	
eGFR (ml/min/1.73 m^2)	0.95	(0.91–0.97)	0.006	

Analysis performed using a forward conditional logistic regression analysis including age, cardiovascular disease, diabetes, age, eGFR (CKD-EPI formula), and endothelin-1. Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration.

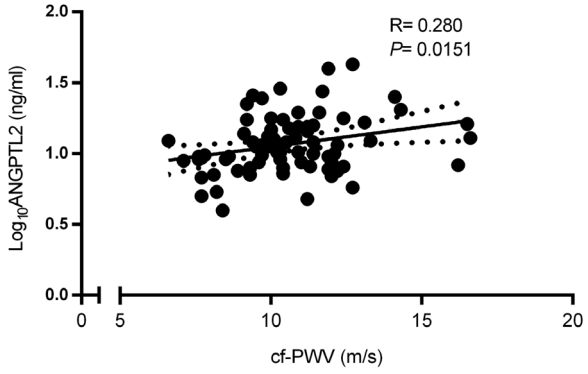

Figure 3. Association between angiopoietin-like protein 2 and aortic stiffness after kidney transplantation. The figure shows the relationship between aortic stiffness as measured by determination of carotid-femoral pulse wave velocity (cf-PWV) and circulating ANGPTL2 (log transformed) 3 month after kidney transplantation.

does not bind to angiopoietins receptors Tie1 or Tie2.[20] The nature of ANGPTL2 receptors is still under debate.[21] The first report on ANGPTL2 demonstrated that ANGPLT2 may stimulate endothelial cells through a paracrine and autocrine action.[20] More recent studies revealed high concentrations of

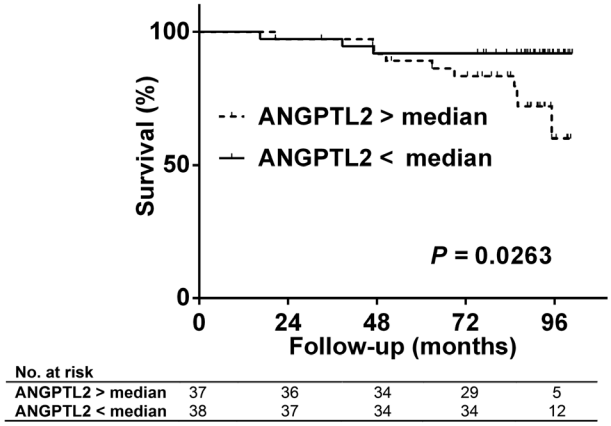

Figure 4. Mortality and levels of angiopoietin-like-2. The Kaplan–Meier analysis shows increased mortality associated with the higher ANGPTL2 level after kidney transplantation.

Table 4. Comparative impact of age, CVD, diabetes, levels of ANGPTL2, aortic stiffness, and renal function on overall mortality

	HR[a]	95% CI	*P* value
Age (year)	1.08	(1.02–1.15)	**0.008**
Diabetes	6.65	(2.04–21.72)	**0.002**
CVD	4.80	(1.60–14.43)	**0.005**
ANGPTL2 > median (ng/ml)	3.90	(1.07–14.30)	**0.039**
cf-PWV > median (m/s)	14.1	(1.80–109.0)	**0.011**
eGFR (ml/min/1.73 m^2)	0.99	(0.96–1.02)	0.408

Bold numbers refer to *P* values of <0.05. Abbreviations: cf-PWV, carotid-femoral pulse wave velocity; CI, confidence interval; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate using CKD-EPI formula.

[a]Univariate Cox regression analysis.

ANGPTL2 in endothelial cells and macrophages infiltrating atheromatous plaques and inducing vascular inflammation,

endothelial dysfunction, and atherosclerosis.[11,13,22] In case–control studies, circulating ANGPTL2 was higher in patients with acute coronary syndrome and heart failure.[23–25] In diabetes, circulating ANGPTL2 is associated with increased intima-media thickness suggesting its clinical relevance for atherosclerosis.[14] This is further supported by the findings of The Hisayama Study in a general Japanese community, where circulating ANGPTL2 is associated with the risk of developing CVD and type 2 diabetes.[15,26] Circulating levels of ANGPTL2 increase in patients with CKD 1–4 with or without type 2 diabetes.[16,17,26] In these studies, ANGPTL2 levels were positively correlated with albuminuria and negatively correlated with renal function estimated by estimated glomerular filtration rate. To the best of our knowledge, our study is the first to report ANGPTL2 levels in CDK-5 patients; these circulating levels (71 ng/ml, [53–95]) are extremely high when compared to those measured in patients with coronary heart disease,[19] acute coronary syndrome,[23,25] or heart failure.[24] It is important to note that the upper threshold for normal serum ANGPTL2 concentrations has not been defined. In healthy volunteers, circulating ANGPTL2 levels range from 1 to 3 ng/ml.[19,21,25] In a Japanese community, serum ANGPTL2 concentrations of 2.0–3.7 ng/ml were reported, and the risk of developing CKD was significantly higher in the highest ANGPTL2 quartile (≥3.66 ng/ml).[16] In contrast to the small changes in serum ANGPTL2 reported in the literature, we previously reported high concentrations of ANGPTL2 in diabetic patients with established CKD 1–4 (ranging from 11.2 to >19.5 ng/ml).[17] It is in accordance with a Chinese group[26] that also reported high ANGPTL2 levels in diabetic patients with established CKD 1–4, ranging from 2.1 to 72.3 ng/ml. It is therefore not surprising that higher levels of ANGPTL2 were measured in patients with severe CKD 5. It has indeed been proposed that reduced kidney clearance associated with CKD could cause an increase in ANGPTL2 levels,[16] explaining the discrepancies with other diseases and previous studies. The mechanism of ANGPTL2 clearance is, however, unknown and this hypothesis remains to be validated. In addition, the patients included in the present study were on dialysis before KTx, which could be another source to increase ANGPTL2 levels: dialysis promotes inflammation, high levels of cytokines and oxidative stress,[27] all known to stimulate the expression of ANGPTL2.[28,29] Furthermore, ANGPTL2 is a protein of ≈64 kDa, which potentially forms multimers in the circulation, making its glomerular excretion or its clearance during the dialysis likely impossible. This has never been confirmed for ANGPTL2, but angiopoeitin2, a glycoprotein with similar structure and molecular weight, is not excreted, not detectable in urine and is not eliminated by dialysis.[30,31] Therefore, the severity of the disease and the proinflammatory environment promoting ANGPTL2 could explain the very high levels of ANGPTL2 observed in patients with CKD5 on dialysis before transplantation. Given the biological role of ANGPTL2 in atherosclerosis,[11,13] the increased levels of ANGPTL2 in CKD could play a significant role in CKD-related endothelial dysfunction, inflammation, and CKD-related CVD.

The fact that ANGPTL2 levels are significantly reduced and almost normalized after KTx suggests that the diseased kidney is a significant source of ANGPTL2. This is the first report of a beneficial effect of KTx on ANGPTL2 levels. The molecular mechanisms involved in the reduction of ANGPTL2 after KTx remain to be elucidated. In our study, in patients with ANGPTL2 >median 3 months after KTx, there was a higher level of serum endothelin-1, suggesting that ANGPTL2 is associated with endothelial dysfunction; this is in accordance with our previous report showing that ANGPTL2 promotes endothelial dysfunction in mice.[12] In addition, higher post-KTx ANGPTL2 levels were also associated with an increase in aortic stiffness as assessed by cf-PWV and by an increase in central pulse pressure; accordingly, higher post-KTx ANGPTL2 levels were associated with lower renal function. While ANGPTL2 levels have previously been associated with vascular remodelling in patients with CVD,[13,14] our study is the first report of an association between circulating ANGPTL2 and aortic stiffness, a process contributing to the development of structural changes associated with aging and cardiovascular events including renal failure.[32] Our current data linking ANGPTL2 to aortic stiffness and central pulse pressure is in agreement with our recent demonstration that levels of ANGPTL2 are positively associated with resting systolic BP.[19] Altogether, these data suggest that ANGPTL2 plays a biological role in CKD-related CVD. Unexpectedly, there was no significant association between ANGPTL2 and inflammation as measured by the extensive circulating cytokines levels, suggesting that the deleterious effects of ANGPTL2 are independent of these inflammatory mediators. Beside its proinflammatory properties, ANGPTL2 is also recognized for its profibrotic activity, supported by a recent study showing that ANGPTL2 combined with TGFβ contribute to renal fibrosis in mouse models, and are coexpressed in renal tubule and glomeruli of patients with fibrotic kidney diseases compared to less fibrotic kidneys.[33] Altogether, these data support the concept that ANGPTL2 is profibrotic in addition of its proinflammatory activity, which could contribute to renal tubule fibrosis[33] and possibly to large artery fibrosis and stiffening.

Finally, in a multivariable model, age, renal function, and endothelin-1 levels predicted post-KTx ANGPTL2 levels independently of diabetes and CVD in our patient population. ANGPTL2 levels were also associated with increased risk of overall mortality. This association between ANGPTL2 and mortality is in accordance with our previous report showing that in diabetic CKD 1–4 patients, ANGPTL2 is associated with an increased risk of cardiovascular events and death.[17]

This study has several strengths as it evaluates the impact of ANGPTL2 in a well-characterized population in terms of comorbidities, hemodynamic parameters, circulating cytokine profile, and mortality. However, there are some limitations in the present study that are mainly related to a relatively small sample size. As such, the number of deaths were small and did not allow to properly study the impact of ANGPTL2 on mortality while taking into account potential confounders.

In conclusion, this is the first study to show that circulating ANGPTL2 levels improve rapidly and significantly after successful KTx. Nonetheless, post-KTx ANGPTL2 levels are associated with higher endothelin-1 levels, aortic stiffness, renal function, and mortality, suggesting that ANGPTL2 plays an active role in CVD associated with kidney disease.

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DISCLOSURES

The authors declared no conflict of interest.

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