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Capillary rarefaction in advanced chronic kidney disease is associated with high phosphorus and bicarbonate levels

Oanh H.D. Thang[1,2], Erik H. Serné[2,3], Muriel P.C. Grooteman[1,2], Yvo M. Smulders[2,3], Piet M. ter Wee[1,2], Geert-Jan Tangelder[2,4] and Menso J. Nubé[1,2]

[1]Department of Nephrology, VU University Medical Center, Amsterdam, The Netherlands, [2]Institute for Cardiovascular Research, VU University Medical Center, Amsterdam, The Netherlands, [3]Department of Internal medicine, VU University Medical Center, Amsterdam, The Netherlands and [4]Department of Physiology, VU University Medical Center, Amsterdam, The Netherlands

Correspondence and offprint requests to: Muriel P.C. Grooteman; E-mail: mpc.grooteman@vumc.nl

Abstract

Background. In patients with chronic kidney disease (CKD), disorders of mineral metabolism are associated with vascular calcifications and mortality. Microvascular dysfunction, by affecting flow resistance and tissue perfusion, may explain the cardiovascular sequelae of CKD-associated disorders of mineral metabolism. We investigated whether advanced CKD is associated with a decrease in the functional and structural number of capillaries in skin and subsequently whether capillary rarefaction is related to mineral metabolism.

Methods. Capillary density was measured by nailfold microscopy in 19 predialysis and 35 CKD Stage 5 (CKD5) patients and 19 controls. In CKD patients, calcium, phosphorus, parathyroid hormone, 25-hydroxyvitaminD3 (25vitD3) and 1,25-dihydroxyvitaminD3 (1,25vitD3) were analysed as well.

Results. Capillary density at baseline was 42 ± 15/mm^2 in predialysis patients, 45 ± 17/mm^2 in CKD5 patients and 56 ± 20/mm^2 in controls (patients versus controls, respectively, $P < 0.05$ and $P = 0.05$). Absolute capillary recruitment during post-occlusive reactive hyperaemia was 17 ± 7/mm^2, 14 ± 6/mm^2 and 23 ± 8/mm^2, respectively ($P < 0.05$ for both patients and controls). Capillary density during venous occlusion was 59 ± 20/mm^2, 59 ± 21/mm^2 and 77 ± 21/mm^2, respectively ($P < 0.05$ for both patients and controls). In multiple regression analysis, both serum phosphorus and bicarbonate values were independently and inversely associated with capillary density at baseline (r^2 of model = 19%) as well as during venous occlusion (r^2 of model = 28%). Furthermore, both serum phosphorus and bicarbonate were inversely and female gender positively correlated with capillary density during recruitment (r^2 of model = 37%).

Conclusion. Advanced CKD is characterized by an impaired functional and structural capillary density in skin, which is related to both high phosphorus and bicarbonate values.

Keywords: bicarbonate; capillary density; capillary rarefaction; chronic kidney disease; microcirculation; phosphorus

Introduction

Patients with chronic kidney disease (CKD) have an increased risk of cardiovascular morbidity and mortality [1]. The mechanisms by which uraemia promotes cardiovascular disease are incompletely understood. Especially non-traditional risk factors, such as the accumulation of uraemic toxins and disorders of mineral metabolism, have been correlated with an increased risk of cardiovascular disease [2, 3]. In CKD patients, hyperphosphataemia hypo-vitaminosis D and hyperparathyroidism are strongly associated with vascular calcifications and mortality [4, 5]. The term 'chronic kidney disease-mineral bone disorder' (CKD-MBD) encompasses the combined presence of bone disease, mineral imbalance and large-vessel calcifications in patients with CKD [6].

Microvascular dysfunction, by affecting flow resistance and tissue perfusion, underlies much of the organ dysfunction associated with conventional cardiovascular risk factors and appears crucial for their pathogenesis and progression [7, 8]. Correspondingly, microvascular dysfunction may explain the cardiovascular consequences of CKD-MBD [9, 10]. Microvascular dysfunction may be caused not only by impaired microvascular endothelium-dependent vasodilatation but also by microvascular rarefaction, i.e. a reduced number of arterioles and capillaries in a given volume of tissue. Abnormal microvascular endothelium-dependent vasodilatation is common in haemodialysis (HD) patients [11, 12], and the most severe microvascular abnormalities were found in individuals with overt large-vessel calcifications [9]. Impaired microvascular endothelium-dependent vasodilatation of the skin has recently been associated with congestive heart failure, coronary artery disease and cardiovascular mortality in patients with CKD Stage 5 (CKD5) [12]. Data on the association between microvascular rarefaction and CKD-MBD, however, are presently lacking. Microvascular rarefaction reduces the surface area available for exchange and increases the distance between capillaries and target cells. In experimental conditions, it was shown that a reduced vessel density was associated with an

inadequate perfusion and tissue hypoxia, especially in situations of high metabolic demand [13, 14].

In the present study, we investigated whether advanced CKD is associated with a decrease in the structural and functional number of capillaries in the skin. The cutaneous microcirculation is considered a representative vascular bed to examine systemic microvascular integrity and function [15, 16]. Subsequently, we examined associations between microvascular structure and function and a number of variables, including demography, medication use and selected laboratory values concerning mineral metabolism, such as phosphorus, fibroblast growth factor-23 (FGF23), 25-hydroxyvitaminD3 (25vitD3) and 1,25-hydroxyvitaminD3 (1,25vitD3).

Materials and methods

Patients

Nineteen predialysis patients and 35 patients with CKD5 (20 HD and 15 peritoneal dialysis patients) were recruited from the outpatient clinic and the dialysis unit of our hospital. After giving informed consent, 19 apparently healthy age-matched subjects served as controls. The study protocol was approved by the local ethics committee. Exclusion criteria were as follows: patients age <18 years, life expectancy <3 months due to non-renal disease, diabetes mellitus, as defined by medical treatment for diabetes or a fasting glucose >6.0 mmol/L and active auto-immune disease.

Nailfold capillary microscopy

Microvascular measurements were performed in a temperature-controlled room (23.4 ± 0.4°C). All individuals were in the fasting state and had abstained from caffeine-containing drinks, alcohol and smoking overnight. In CKD patients, routine medication was continued. Nailfold capillaries in the dorsal skin of the third finger were visualized by a capillary microscope (Figure 1a and b), as described before [17]. The non-dominant hand was used, unless patients had a vascular access in that arm. A visual field of 1 mm^2 was recorded at baseline and after 4 min of arterial occlusion with a digital cuff inflated to 300 mmHg for 4 min (post-occlusive reactive hyperaemia) and the images were stored on a videotape. Baseline capillary density was defined as the number of continuously erythrocyte-perfused capillaries during an observation period of 15 s. Recruitment was defined as the absolute increase in erythrocyte-perfused capillaries during post-occlusive hyperaemia. Both recruitment and baseline capillary density represent the number of functional capillaries. The number of continuously erythrocyte-perfused capillaries was counted offline by an experienced investigator from a videotape. Fifteen minutes after baseline measurements, venous occlusion was applied to the same finger with the digital cuff inflated to 60 mmHg for 60 s to expose a maximal number of perfused capillaries, which is supposed to reflect structural capillary density [17]. Using the same visual fields as used during baseline measurements, capillaries were counted in the 60-s recordings. Venous occlusion at 60 mmHg for 120 instead of 60 s did not further increase the number of visible capillaries. The day-to-day coefficient of variation of the capillary density in resting state and absolute capillary recruitment were 2.3 ± 1.8% and 6.2 ± 4.3%, respectively. The day-to-day coefficient of variation of maximal capillary density during venous congestion was 9.5 ± 7.1%. Both procedures were repeated in a second visual field adjacent to the first and the mean of both measurements was used for the present analyses.

Laboratory values

Kidney function, expressed as estimated glomerular filtration rate (eGFR), was calculated by the Cockcroft & Gault formula in controls and by the mean of urine creatinine and urea clearance in CKD patients. In patients with a known venous pH value ($n = 39$), arterial pH was calculated using the regression formula: arterial pH = −0.307 + 1.05 × venous pH [18]. Besides markers of mineral metabolism, including calcium (corrected for albumin), phosphorus, intact PTH, 25vitD3 and 1,25vitD3, routine laboratory parameters, such as albumin and bicarbonate, were evaluated. All blood samples were drawn in the fasting state. PTH levels and C-terminal FGF23 were measured by commercially available immunometric assays, respectively (Luminescence Architect; Abbott Laboratories, Chicago, IL) and (Immunotopics, Inc., San Clemente, CA). 25VitD3 and 1,25vitD3 were measured by a competitive radioactive binding protein assay (Diasorin, Stillwater, MN) and radioimmunoassay after immunoextraction (IDS, Tyne and Wear, UK), respectively.

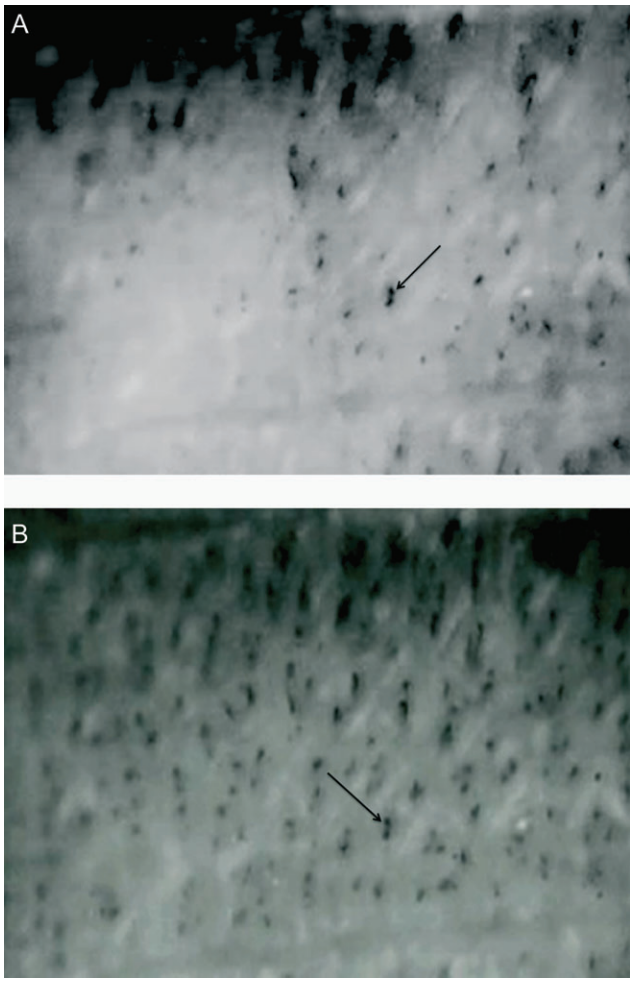

Fig. 1. Images of the capillary density measured by capillary nailfold microscopy. The black arrows show erythrocyte-perfused capillaries. (**a**) The amount of capillaries at baseline. (**b**) The amount of capillaries during post-occlusive hyperaemia.

Clinical characteristics

Clinical history and medication use were assessed by information from medical charts and patients. Body mass index (BMI) was calculated from weight and height measurements. Patients were considered to have a history of cardiovascular events if there was a previous stroke or cardiac event, including myocardial infarction, congestive heart failure and angina or clinical peripheral vascular disease.

Statistical analysis

Variables are presented as mean ± SD or as median and interquartile range. When appropriate, data with a skewed distribution were log-transformed. One-way analysis of variance was used to compare the differences between controls and CKD patients. Spearman's rank correlation was used to investigate potential relationships between markers of mineral metabolism and capillary measurements. Multiple linear regression analysis was performed to examine determinants of capillary density. Variables were selected using univariate linear regression and included in the multivariate model if $P < 0.1$, using a forward selection procedure. As age, gender and BMI were related to capillary measurements in previous studies [19, 20], the multivariate model was adjusted for these factors

afterwards, regardless of their significance levels. A P-value of <0.05 was considered statistically significant. All analyses were performed with the statistical software package SPSS version 15.0 (SPSS Inc., Chicago, IL).

Results

Demographical, clinical and laboratory characteristics

Results of the three groups are shown in Table 1. Controls were all non-smokers and did not use vasoactive medication. eGFR in predialysis patients was 12 ± 5 mL/min and in CKD5 patients 4 ± 4 mL/min. BMI was significantly higher in predialysis patients than in CKD5. Blood pressure did not differ between the three groups. Laboratory values of the patients, including markers of mineral metabolism, are shown in Table 2.

Capillary microscopy

The number of 'capillaries at baseline' was significantly lower in predialysis patients and CKD5 than in control subjects (respectively, 42 ± 15/mm^2, 45 ± 17/mm^2 and 56 ± 20/mm^2; patients versus controls, $P < 0.05$ and $P = 0.05$, respectively) (Figure 2). Moreover, peak capillary density during post-occlusive reactive hyperaemia was significantly lower in predialysis patients and CKD5 than in controls (respectively, 59 ± 19/mm^2, 58 ± 20/mm^2 and 79 ± 22/mm^2; $P < 0.05$ for both patients and controls). Capillary recruitment, defined as the absolute capillary increase during post-occlusive hyperaemia was lower in predialysis patients and CKD5 than in the controls (respectively, 17 ± 7/mm^2, 14 ± 6/mm^2 and 23 ± 8/mm^2; $P < 0.05$ for both patients and controls). The structural number of capillaries, as assessed during venous occlusion, was lower in predialysis patients and CKD5 than in controls (respectively, 59 ± 20/mm^2, 59 ± 21/mm^2 and 77 ± 21/mm^2; patients versus controls, each $P < 0.05$). As predialysis patients and CKD5 did not differ in these analyses, these groups were pooled and denoted 'advanced CKD'.

Table 1. Characteristics of the study population[a]

	Controls	Predialysis	CKD5
n (male/female)	19 (10/9)	19 (11/8)	35 (25/10)
Age (years)	60.4 ± 7.3	63.6 ± 11.0	62.3 ± 13.1
BMI (kg/m^2)	24.8 ± 3.1	26.6 ± 3.8*	23.7 ± 3.2*
SBP (mmHg)	133 ± 15	147 ± 22	140 ± 25
DBP (mmHg)	82 ± 7	81 ± 15	79 ± 12
eGFR (mL/min)	85 (74–99)	12 (8–17)	3 (1–3)
History of cardiovascular event [*n* (%)]		7 (37)	13 (37)
Smoking [*n* (%)]		11 (58)	20 (57)
Diuretic use [*n* (%)]		10 (53)	19 (54)
Inhibitors of RAAS [*n* (%)]		16 (84)	23 (66)
Calcium antagonist [*n* (%)]		11 (58)*	9 (26)*
Beta-blocker [*n* (%)]		8 (42)	24 (69)
Calcium carbonate [*n* (%)]		5 (26)	16 (46)
Sevelamer HCl [*n* (%)]		5 (26)*	24 (69)*
Lanthanum carbonate [*n* (%)]		0	10 (29)*

[a]Data are presented as mean ± SD or as median (interquartile range).
*$P < 0.05$ predialysis versus CKD5.

Relationships among medication, demography, laboratory data and microvascular function in patients with advanced CKD

With respect to the use of phosphate binders and antihypertensive drugs, there were no differences between the use of drugs in each group when capillary parameters were split according to the medians (data not shown). Univariate regression analysis demonstrated that the demographic variables were not correlated with microvascular function, except for gender in recruitment (Tables 3–5). Interestingly, both phosphorus ($r = -0.35$, $P = 0.01$; $r = -0.42$, $P \leq 0.01$ and $r = -0.40$, $P < 0.01$, respectively) and bicarbonate ($r = -0.25$, $P = 0.07$; $r = -0.31$, $P = 0.02$ and $r = -0.35$, $P = 0.01$, respectively) were negatively correlated with baseline capillary density, capillary recruitment and capillary density during venous occlusion (Figure 3). In addition, a positive correlation was found between both $\log_{10}$ 25vitD3 and $\log_{10}$ 1,25vitD3 and capillary recruitment ($r = 0.29$, $P = 0.04$ and $r = 0.40$, $P = 0.003$, respectively) (Table 4).

Multiple linear regression analysis showed that both phosphorus and bicarbonate were independent and negatively associated with baseline capillary density. Together, these factors explained 19% of the observed variance (Table 3). Adjustment for age, gender, BMI or residual renal function did not change these findings. Moreover, both phosphorus and bicarbonate were independently and

Table 2. Laboratory values of predialysis patients and patients with CKD5[a]

	Reference values	Predialysis ($n = 19$)	CKD5 ($n = 35$)
Calcium (mmol/L)	2.2–2.6	2.37 ± 0.14	2.39 ± 0.15
Phosphorus (mmol/L)	0.7–1.4	1.56 ± 0.30	1.65 ± 0.42
Albumin (g/L)	35–52	39.3 ± 2.9	36.4 ± 3.9*
PTH (pmol/L)	0–11	27 (14–45)	20 (13–34)
25vitD3 (nmol/L)	25–150	49 (35–70)	41 (30–64)
1,25vitD3 (pmol/L)	50–160	29 (19–43)	20 (13–30)#
FGF23 (RefU/mL)	0–125	651 (364–1705)	2750 (1581–10 659)*
Bicarbonate (mmol/L)	22.0–26.0	22.0 ± 2.8	23.0 ± 3.3
Haemoglobin (mmol/L)	7.5–11	7.3 ± 0.7	7.4 ± 1.0
Arterial pH	7.35–7.45	7.36 ± 0.05	7.37 ± 0.07

[a]Data are presented as mean ± SD or as median (interquartile range).
*$P < 0.01$ and #$P < 0.05$ predialysis versus CKD5.

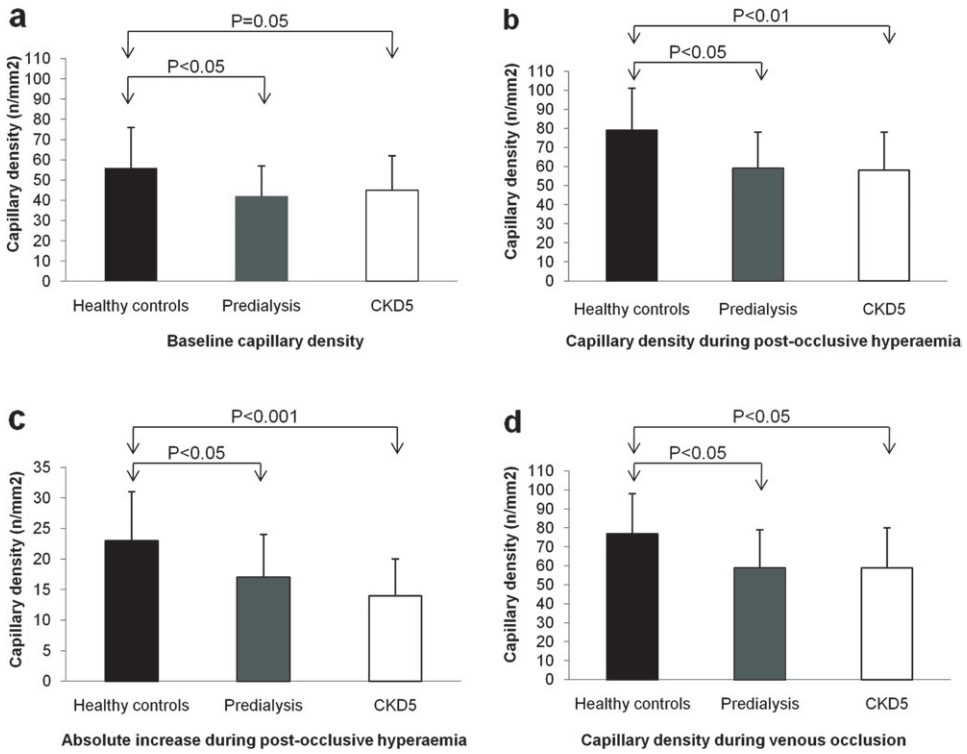

Fig. 2. Capillary measurements in healthy controls, predialysis patients and patients with CKD5. For description see text.

Table 3. Associations between baseline capillary density, demographic variables and laboratory investigations in CKD patients[a,b,c]

	Univariate model				Multivariate model			
Determinant	*B*	95% CI	P	*r*	*B*	β	95% CI	P
Age (years)	0.04	−0.17 to 0.25	0.71	0.05				
Gender (female)	0.001	−0.01 to 0.01	0.87	0.02				
BMI (kg/m^2)	0.01	−0.06 to 0.07	0.82	0.03				
SBP (mmHg)	0.16	−0.24 to 0.57	0.43	0.11				
DBP (mmHg)	0.12	−0.10 to 0.34	0.27	0.15				
eGFR (mL/min)	−0.002	−0.10 to 0.10	0.98	0.00				
Calcium (mmol/L)	0.00	−0.003 to 0.00	0.93	−0.01				
Phosphorus (mmol/L)	−0.01	−0.01 to −0.002	0.01	−0.35	−14.99	−0.35	−25.72 to −4.25	0.007
Log_{10} 25vitD3 (nmol/L)	0.001	−0.003 to 0.01	0.61	0.07				
Log_{10} 1,25vitD3 (pmol/L)	0.00	−0.01 to 0.01	0.91	0.02				
Log_{10} PTH (pmol/L)	0.001	−0.01 to 0.01	0.72	0.05				
Log_{10} FGF23 (RefU/mL)	−0.01	−0.02 to 0.003	0.16	−0.19				
Bicarbonate (mmol/L)	−0.05	−0.10 to 0.004	0.07	−0.25	−1.31	−0.26	−2.62 to −0.01	0.049

[a]CI, confidence interval.
[b]Univariate and multivariate linear regression analysis.
[c]R^2 of the multivariate model = 0.19. The *B* reflects the change of baseline capillary density (n/mm^2) related with 1 unit increment of the determinant.

inversely associated with capillary recruitment, while female gender was positively correlated. Together, these factors explained 37% of the variance (Table 4). Adjustment for age, BMI or residual renal function afterwards did not change these findings. Finally, both phosphorus and bicarbonate were independent and inversely associated with capillary density during venous occlusion. Together, these factors explained 28% of the variance (Table 5). Adjustment for age, gender, BMI or residual renal function afterwards did not change these findings.

Interaction of phosphorus and bicarbonate levels on capillary density

As phosphorus and bicarbonate were the only variables significantly associated with capillary density in this model, patients with advanced CKD were divided into tertiles of both parameters. As illustrated in Figure 4, it appeared that baseline capillary density differed significantly between patients in the high and low phosphorus groups. Patients with low phosphorus and low bicarbonate

Table 4. Associations between recruitment, demographic variables and laboratory investigations in CKD patients[a,b,c]

	Univariate model				Multivariate model			
Determinant	*B*	95% CI	P	*r*	*B*	β	95% CI	P
Age (years)	0.28	−0.24 to 0.81	0.28	0.15				
Gender (female)	0.02	0.00–0.04	0.05	0.27	3.14	0.23	−0.05 to 6.32	0.05
BMI (kg/m^2)	0.07	−0.09 to 0.23	0.38	0.12				
SBP (mmHg)	−0.08	−1.09 to 0.94	0.88	−0.02				
DBP (mmHg)	−0.19	−0.74 to 0.36	0.49	−0.10				
eGFR (mL/min)	0.18	−0.05 to 0.42	0.12	0.21				
Calcium (mmol/L)	0.01	−0.001 to 0.01	0.14	0.21				
Phosphorus (mmol/L)	−0.03	−0.04 to −0.01	0.002	−0.42	−5.20	−0.31	−9.68 to −0.72	0.02
Log_{10} 25vitD3 (nmol/L)	0.01	0.001–0.02	0.04	0.29	3.34	0.14	−3.04 to 9.71	0.30
Log_{10} 1,25vitD3 (pmol/L)	0.02	0.01–0.03	0.003	0.40	3.15	0.14	−3.56 to 9.86	0.35
Log_{10} PTH (pmol/L)	0.01	−0.01 to 0.02	0.36	0.13				
Log_{10} FGF23 (RefU/mL)	−0.02	−0.04 to 0.01	0.20	−0.18				
Bicarbonate (mmol/L)	−0.15	−0.28 to 0.02	0.022	−0.31	−0.58	−0.28	−1.07 to −0.08	0.02

[a]CI, confidence interval.
[b]Univariate and multivariate linear regression analysis.
[c]R^2 of the multivariate model = 0.37. The *B* reflects the change of recruitment (n/mm^2) related with 1 unit increment of the determinant.

Table 5. Associations between capillary density during venous occlusion, demographic variables and laboratory investigations in CKD patients[a,b,c]

	Univariate model				Multivariate model			
Determinant	*B*	95% CI	P	*r*	*B*	β	95% CI	P
Age (years)	0.06	−0.11 to 0.23	0.47	0.10				
Gender (female)	0.004	−0.003 to 0.01	0.24	0.16				
BMI (kg/m^2)	0.01	−0.04 to 0.06	0.70	0.05				
SBP (mmHg)	0.15	−0.18 to 0.47	0.37	0.13				
DBP (mmHg)	0.05	−0.13 to 0.23	0.57	0.08				
eGFR (mL/min)	0.01	−0.07 to 0.09	0.82	0.03				
Calcium (mmol/L)	0.00	−0.002 to 0.002	0.66	0.06				
Phosphorus (mmol/L)	−0.01	−0.01 to −0.003	0.003	−0.40	−21.51	−0.40	−34.29 to −8.72	0.001
Log_{10} 25vitD3 (nmol/L)	0.003	−0.001 to 0.01	0.14	0.21				
Log_{10} 1,25vitD3 (pmol/L)	0.002	−0.002 to 0.01	0.35	0.13				
Log_{10} PTH (pmol/L)	0.001	−0.004 to 0.01	0.62	0.07				
Log_{10} FGF23 (RefU/mL)	−0.01	−0.01 to 0.002	0.15	−0.20				
Bicarbonate (mmol/L)	−0.05	−0.09 to −0.01	0.01	−0.35	−2.28	−0.35	−3.84 to −0.73	0.005

[a]CI, confidence interval.
[b]Univariate and multivariate linear regression analysis.
[c]R^2 of the multivariate model = 0.28. The *B* reflects the change of venous occlusion (n/mm^2) related with 1 unit increment of the determinant.

values showed the highest baseline capillary density. Whether the association between phosphorus and microvascular function was influenced by bicarbonate levels was analysed with the interaction term 'phosphorus × bicarbonate'. In none of the multivariate models, this interaction term was statistically significant. Similar results were obtained with respect to capillary recruitment and capillary density during venous occlusion.

Discussion

This study has two main findings. First, patients with advanced CKD are characterized by a decreased functional and structural capillary density in the skin. Second, these alterations are independently associated with increased serum phosphorus and bicarbonate levels.

A novel aspect of this study is the decreased capillary density during venous occlusion, which is supposed to reflect structural aspects [17]. The discovery of a functional decrease in capillary density, as indicated by measurements at baseline and during post-occlusive reactive hyperaemia is in line with previous data on patients with CKD Stages 4 and 5 [21]. Interestingly, in non-renal patients, a progressive decrease in the functional and structural number of cutaneous capillaries was associated with an increasing risk of coronary heart disease [19, 22]. Assuming that microvascular dysfunction is a generalized feature not confined to a single organ, coronary flow reserve, reflecting the functional capacity of the microcirculation of the heart, is also decreased in patients with CKD5 [23]. Indeed, autopsy material showed not only diffuse media calcifications and thickening of the arteries but also profound rarefaction of intra-myocardial capillaries in patients with CKD [24].

Another key and novel finding of the present study is that hyperphosphataemia was associated with microvascular dysfunction in patients with advanced CKD. Previous studies have indicated that high serum phosphorus levels are strongly associated with peripheral artery disease in

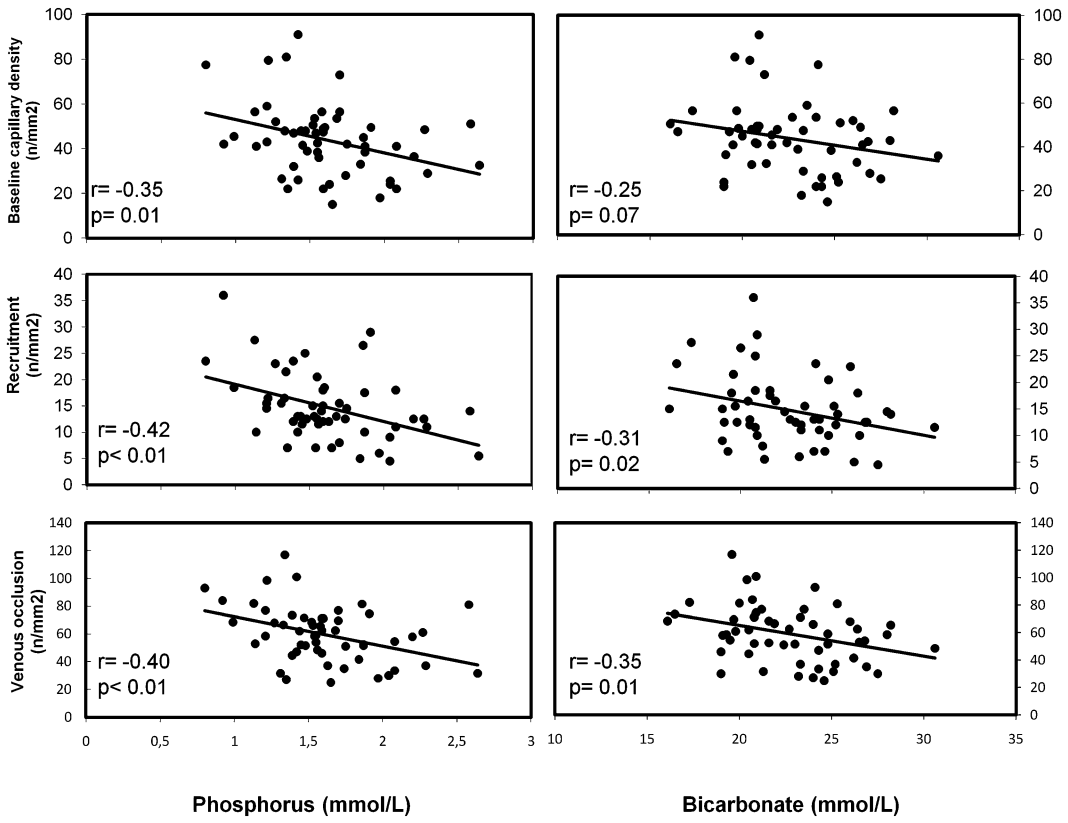

Fig. 3. Correlations between baseline capillary density, recruitment and venous occlusion on the one hand and phosphorus and bicarbonate at the other. For description see text.

humans with moderate CKD [25]. In long-term HD patients, elevated serum phosphorus levels were independently correlated with cardiovascular morbidity and mortality [26–29]. In this study, we showed that serum phosphorus is independently and inversely related to both functional and structural capillary density of the skin. At first glance, the strong and inverse correlation between baseline capillary density, capillary recruitment and capillary density during venous occlusion and serum bicarbonate seems surprising, as severe acidosis has been associated with mortality in the Dialysis Outcomes and Patterns Study (DOPPS) [30] and correction of acidosis improved the nutritional state and slowed the progression of CKD [31]. However, in the DOPPS, moderate acidosis was associated with a lower risk for both mortality and hospitalization. In uraemic rats, it was recently demonstrated that calcitriol-induced soft tissue calcification is inhibited by experimental metabolic acidosis [32]. As the expression of the sodium-dependent phosphorus cotransporter Pit-1 was down-regulated by acidosis, its protecting effect on calcification was attributed to a reduced cellular uptake of phosphorus. Alternatively, acidosis may enhance the dissolution of deposited calcium-containing salts. In humans, calcific uraemic arteriolopathy is commonly treated with sodium thiosulphate [33], which has been shown to lower both pH and bicarbonate levels [34]. The formation of soluble calcium thiosulphate complexes is pH dependent, as its formation was reduced in an alkaline milieu. Currently, it is unknown if, and to what extent, the abovementioned mechanisms affect vascular calcifications in humans with advanced CKD.

In line with previous reports [35], low levels of both 25vitD3 and 1,25vitD3 were found in patients with advanced CKD. In the univariate model, both 25vitD3 and 1,25vitD3 were positively correlated with capillary recruitment. In this respect, it is interesting to note that low serum 25vitD3 levels are associated with coronary calcifications [36, 37] and mortality [38] in patients with CKD, whereas treatment with calcitriol was associated with survival both in individuals not yet on dialysis [39] and in HD patients [40].

Based on current literature and the abovementioned findings, we propose the following mechanism. With advancing CKD, generalized calcifications arise in the vascular tree, mainly due to a decline in inhibitors, such as fetuin-A, and an increase in promoters of this process, particularly phosphorus [41]. As a result, osteogenic transformations occur in vascular smooth muscle cells, not only in large and medium-sized arteries but also in small resistance vessels and arterioles. Due to a reduced mobilization of bone marrow-derived endothelial progenitor cells [42, 43], partly as a result of the uraemic milieu itself and partly due to a diminished production of vascular endothelial growth factor (VEGF), vessel regeneration and repair is disturbed. In this respect, it is interesting to note that VEGF released from osteoblastic cells only occurs in the presence of 1,25vitD3 [44, 45]. As a result of these processes, rarefaction and reduced capillary recruitment may occur in various

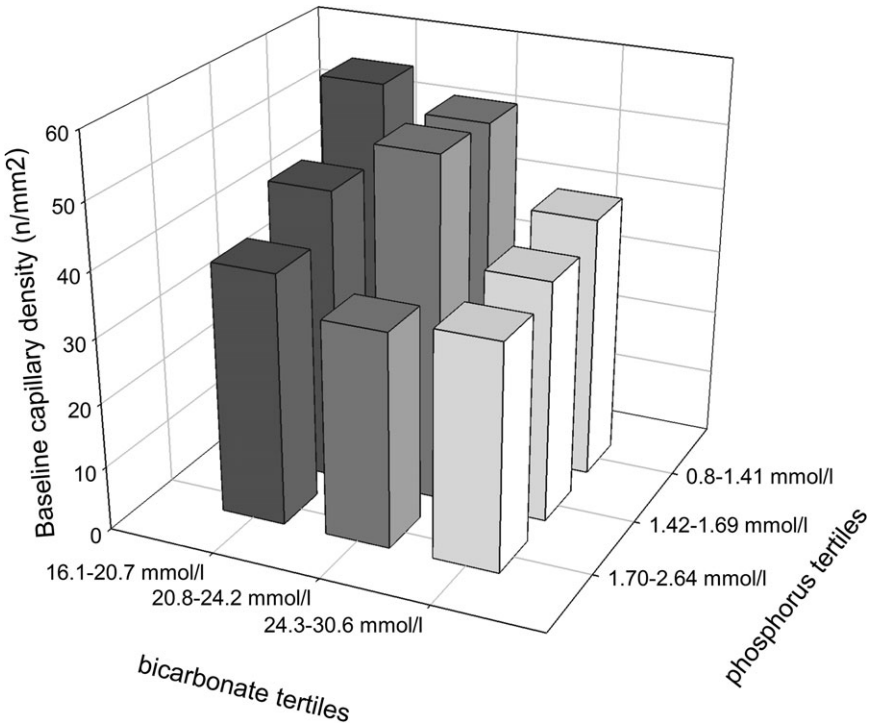

Fig. 4. Relation between baseline capillary density, bicarbonate and phosphorus in patients with advanced kidney disease. The latter two variables were divided into tertiles. The interaction term phosphorus × bicarbonate was not significant. Similar results were obtained with respect to recruitment and capillary density during venous occlusion.

organs, including the skin. In support of such a concept, we recently showed that inhibitors of VEGF/vascular endothelial growth factor receptor (VEGFR)-2 signalling reduce structural skin capillary density [46].

Our study has important limitations. Previously, it was demonstrated that long-term treatment with angiotensin-converting enzyme inhibitors (ACEI), but not β-blockers, may normalize the structure of the microcirculation in hypertensive patients and reduce vascular rarefaction [47]. As many patients with advanced CKD are treated with antihypertensive medication, it is conceivable that these drugs influenced capillary measurements. Furthermore, most patients with advanced CKD receive phosphate binders in combination with vitamin D supplementation, which may also influence vascular reactivity, especially in combination with ACEI [48]. However, as the study group was relatively small and the amount of tablets prescribed was >10/day in many patients, the analysis of the influence of different drug categories on capillary density was rather crude. Lastly, as several parameters were analysed (multiple testing), the data must be interpreted with caution.

To summarize, patients with advanced CKD are characterized by an impaired functional and structural capillary density in skin, which may compromise tissue perfusion and oxygenation. In these patients, strong inverse and independent associations were found between baseline capillary density, capillary recruitment and capillary density during venous occlusion on the one hand and serum phosphorus and bicarbonate concentrations at the other. As microvascular dysfunction of the skin is a strong and independent predictor of cardiovascular disease and death in patients with advanced CKD [12], it is tempting to speculate that besides optimal correction of serum phosphorus, persistent moderate acidosis may alleviate the high incidence of cardiovascular disease in these patients.

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Conflict of interest statement. None declared.

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