Original Research

Effect of moderate-to-severe chronic kidney disease on flow-mediated dilation and progenitor cells

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Abstract

A reduction in progenitor cell populations that help preserve vascular continuity and induce vascularization may accentuate endothelial cell apoptosis and dysfunction, ultimately contributing to organ failure and increased cardiovascular disease in chronic kidney disease (CKD). We hypothesized that CD45+ myeloid and CD34+ hematopoietic circulating progenitor cell (CPC) subpopulations would be reduced, peripheral blood mononuclear cell (PBMNC) colony-forming units (CFU) would be impaired, and flow-mediated dilation (FMD) would be impaired in patients with moderate-to-severe CKD as compared with healthy controls. Eleven moderate-to-severe CKD patients (mean estimated glomerular filtration rate [eGFR]: 36 ± 5) and 14 healthy controls were studied; blood was drawn and FMD was assessed by brachial artery FMD. CPCs were quantified via flow cytometry, and isolated PBMNCs were cultured for the colony-forming assay. CKD patients had significantly impaired FMD; lower CD34+, CD34+/KDR+, CD34+/CD45− and CD34+/KDR+/CD45− hematopoietic CPCs; lower CD45+, CD45+/KDR+, CD34+/CD45+ and CD34+/KDR+/CD45+ myeloid CPCs; and impaired CFUs as compared with healthy controls. Regression analysis revealed that CD34+, CD34+/KDR+ and CD34+/CD45− hematopoietic CPCs were associated positively with eGFR and negatively with blood urea nitrogen and serum creatinine. The CD45+/KDR+ myeloid CPCs also were associated positively with eGFR and negatively with serum creatinine. CD34+ hematopoietic CPCs and CD45+/KDR+ as well as CD34+/CD45+ myeloid CPCs were associated positively with FMD. In conclusion, myeloid and hematopoietic CPCs are reduced and associated with renal function as well as FMD in CKD. Therefore, reductions in CPCs may be a potential mechanism by which vascular integrity is compromised, increasing cardiovascular disease risk and contributing to renal disease progression in CKD.

Keywords: progenitor cells, chronic kidney disease, flow-mediated dilation

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Introduction

Patients diagnosed with chronic kidney disease (CKD) are more likely to die than progress to end-stage renal disease (ESRD; stage 5; glomerular filtration rate [GFR] < 15 mL/min/1.73 m^2).[1] Evidence suggests that poor patient survival can be attributed to traditional and non-traditional cardiovascular risk factors that parallel renal disease progression resulting in an accelerated development of cardiovascular disease (CVD).[2] With CVD progression, end-organ damage ensues, manifesting in such forms as myocardial infarction, cerebrovascular insults and various ischemic diseases, which can now be considered the most significant causes of mortality and morbidity in CKD.[3] Thus, understanding the underlying mechanisms of increased CVD risk in CKD is important in order to improve patient outcomes.

Endothelial dysfunction plays a critical role in not only the development of CVD[4] but also the progression of renal insufficiency in CKD.[4,5] The endothelial monolayer lining the blood vessels helps control vascular tone and tissue perfusion through the release and regulation of various vasoactive substances.[6] Continual and/or repeated exposure to cardiovascular risk factors can compromise endothelial integrity, resulting in endothelial dysfunction, and impaired oxygen and nutrient delivery to cardiovascular and renal tissues.[3,7] In this regard, progenitor cells that help preserve vascular continuity and induce vascularization

become important in preventing endothelial dysfunction and ultimately organ failure and cardiovascular events in CKD.[3,8]

Progenitor cells have been shown to be reduced in number as well as functionally impaired in various CVDs,[9–11] ESRD[12] and progression of CKD.[8] However, subpopulations of hematopoietic and myeloid circulating progenitor cells (CPCs) in CKD have not been investigated.

Enumeration of hematopoietic and myeloid CPC subpopulations using additional cell surface markers indicative of progenitor cells including those of endothelial and myeloid lineage such as KDR (vascular endothelial growth factor-receptor II) and CD45, respectively, could be useful in accurately identifying deficiencies in cell populations that may contribute to maintaining vascular integrity in CKD.

The purpose of this investigation was to enumerate CPC subpopulations in moderate-to-severe CKD patients and apparently healthy controls using the cell surface markers CD34, KDR and CD45. Additionally, a colony-forming unit (CFU) assay was performed and flow-mediated dilation (FMD) was assessed using brachial artery FMD. We hypothesized that CPCs would be reduced, CFU ability would be impaired and FMD would be impaired in CKD patients as compared with healthy controls. We also explored the relations among CPCs, CFUs, subject characteristics and FMD measurements.

Materials and methods

Study population

Eleven moderate-to-severe CKD patients (8 men, 3 women) and 14 apparently healthy controls (10 men, 4 women) were recruited for this study. All subjects completed a medical history questionnaire and underwent resting blood pressure, heart rate, height and weight measurements. Venous blood and urine samples were taken for assessment of liver enzymes, a lipid profile, renal function tests, hemoglobin, hematocrit and blood glucose levels. Exclusion criteria for CKD patients included history of coronary artery disease, myocardial infarction and/or heart failure; untreated hypertension; current drug therapy for pulmonary, autoimmune and/or HIV diseases; presence of hepatic disease; cancer; immunosuppressant therapy; antiretroviral therapy; and current tobacco use. Patients continued all medications during the study. Healthy controls were free from disease and were not taking any medications. Estimated glomerular filtration rate (eGFR) was determined in all subjects using the modification of diet in renal disease (MDRD) equation based on serum creatinine, age, gender and race as recommended by the National Kidney Foundation.[13] CKD patients had an eGFR less than 60 mL/min/1.73 m^2, were not on dialysis, or awaiting transplantation, and healthy controls an eGFR$>$60 mL/min/1.73 m^2. This study and consent forms were approved by the University of Delaware Human Subjects Institutional Review Board. Written and verbal consent were obtained from all subjects.

Subjects reported to the University of Delaware Vascular Physiology Laboratory for the experimental visit to undergo assessment of FMD and a blood draw for CPC measurement and peripheral blood mononuclear cell (PBMNC) isolation. FMD and the blood draw were conducted after the subjects' had abstained from food for at least 4 h, alcohol and caffeine for 12 h, and strenuous physical activity for 24 h. All premenopausal women underwent FMD measurements and the blood draw during the early follicular phase of menstruation (premenopausal women: control $n = 1$; CKD $n = 1$).

CPC flow cytometry

CPCs were quantified in venous blood samples consistent with published practices and recommendations.[14,15] Control and antibody suspensions were prepared in 150 μL whole-blood aliquots using a fluorescein isothiocyanate (FITC)-conjugated-CD34 antibody (BD Biosciences, San Diego, CA, USA), phycoerythrin (PE)-conjugated-KDR antibody (R & D Systems, Minneapolis, MN, USA) and allophycocyanin (APC)-conjugated-CD45 antibody (BD Biosciences, San Diego, CA, USA) at the manufacturers' recommended concentrations. After a 15–20 min incubation period, FACS™ Lysis Solution (BD Biosciences, San Jose, CA, USA) was added and incubation continued for an additional 30–45 min, at which time the solutions were washed. The lymphocyte population – where CPCs are thought to reside – was isolated from the whole blood forward and side scatter profile[11,15] using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA) and analyzed with CellQuest™ software (BD Biosciences, San Jose, CA, USA). The number of myeloid CPCs defined as CD45+, CD45+/KDR+, CD34+/CD45+ and CD34+/KDR+/CD45+ events and hematopoietic CPCs defined as CD34+, CD34+/KDR+, CD34+/CD45−, and CD34+/KDR+/CD45− events were reported per 500,000 total events acquired.

PBMNC isolation and colony-forming assay

PBMNC isolation and the colony-forming assay were performed as described previously.[16] In summary, 15 mL of each subject's blood obtained intravenously and the equivalent of phosphate-buffered saline were added to a sterile 50 mL Accuspin™System-Histopaque® tube (Sigma-Aldrich, St Louis, MO, USA) for density-dependent centrifugation. The PBMNC layer was then removed, washed twice and re-suspended in 1–3 mL of growth media containing Medium-199, 20% fetal bovine serum and 1% penicillin/streptomycin. The maximum amount of wells of a six-well fibronectin-coated plate (BD Biosciences, Bedford, MA, USA) containing 2 mL of growth media were then seeded with 5×10^6 cells and incubated at 37°C in a 5%-CO_2 environment.

After 48-h in culture, the non-adherent cells were removed, and 7–10 million were used for immunophenotyping, while the remainder was added to a 24-well fibronectin-coated plate (BD Biosciences, Bedford, MA, USA) at a concentration of 1×10^6 cells/well in 1 mL of growth media. Cell culture continued for a period of seven days with media being changed on days 6 and 9 until fixed and stained on day 10. CFUs were counted by

two independent investigators in three wells selected randomly. Endothelial and myeloid cell phenotypes were confirmed in a subset of subjects by incubating CFUs with fluorescent dye-labeled acetylated-LDL (DiL AcLDL; 10 μg/mL; Invitrogen, Eugene, OR, USA) at 37°C for one hour and fluorscein-isothiocyanate-conjugated Ulexeurpaeus agglutinin (UEA-1; 10 μg/mL; Sigma-Aldrich) for one hour.

Non-adherent cell immunophenotyping

Fluorescently-conjugated antibodies of cell surface markers used in this experiment are associated with cells of hematopoietic, myeloid and endothelial lineage. These included CD31-FITC (PECAM-1; BD Biosciences, San Diego, CA, USA), CD34-FITC, CD45-FITC (BD Biosciences, San Diego, CA, USA) and CD144-FITC (VE-cadherin; AbD Serotec, Raleigh, NC, USA) as well as KDR-PE. The population of cells representing the majority of cultured cells was isolated using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA) and analyzed with CellQuest™ software (BD Biosciences, San Jose, CA, USA). The percentage of positive events for each antibody was reported per 200,000 gated events acquired as compared with unlabeled controls.

Flow-mediated dilation

Brachial artery FMD was used to assess FMD using high resolution ultrasound (Sonosite Titan, Bothell, WA, USA) in accordance with published guidelines.[17] A pressure cuff was placed just distal to the olecranon process of the non-dominant arm and inflated at 200 mmHg for five minutes until released to induce reactive hyperemia. Vasodilatory responses of the brachial artery using a 10 MHz linear phased array ultrasound transducer were recorded at baseline and then continuously from 15 s pre-cuff until two minutes post-cuff release to determine the peak diameter change. Blood velocity also was assessed simultaneously with a 4MHz continuous wave Doppler probe (Multigon 500P, Yonkers, NY, USA) placed just distal to the ultrasound transducer in order to calculate shear rate. FMD is expressed as the percent increase in diameter from baseline (i.e. change in diameter divided by baseline diameter) and the magnitude of the shear stimulus as the area under the curve (AUC) of the shear rate (s^{-1} $V_{mean/vessel\ diameter}$) profile from cuff deflation to peak diameter. Analysis of brachial artery images and Doppler data collected were recorded and analyzed on custom-made LabVIEW 8.0 software programs (National Instruments, Austin, TX, USA).

Statistical analysis

Univariate analysis of variance was used to compare CPC numbers, CFUs, and FMD between groups with one-tailed probability values reported. Pearson correlations and multivariate regression analyses were conducted to examine the relationships among CPC subpopulations, CFUs, subject characteristics and FMD measurements. Statistical tests were repeated while controlling for age and sex due to their known effects on progenitor cells.[18–21] All statistical tests were performed using SPSS® statistical software version 17.0 (IBM, Armonk, NY, USA). Alpha was set at 0.05 and data are presented as mean values ± SEM.

Results

Subject characteristics

Table 1 summarizes subject demographic, hemodynamic, renal function and blood measurements as compared between groups. CKD patients had various classic cardiovascular risk factors including hypertension ($n = 11$), hypercholesteremia ($n = 6$) and diabetes ($n = 3$) that were being treated with medication. No significant mean differences were found in any of the evaluated variables between subjects with and without diabetes or hypercholesteremia (all $P > 0.05$). By design, renal function was impaired in CKD subjects as indicated by eGFR < 60 (mean: 36 ± 5; range: 12–58) as well as increased blood urea nitrogen (BUN) and serum creatinine levels.

Mean comparisons

Brachial artery FMD was significantly lower in the CKD patients (controls: $n = 14$; CKD: $n = 11$; see Figure 1). Shear rate AUC, an estimate for the stimulus for dilation,

Table 1 Subject characteristics

N	Control 14	CKD 11
Demographic information		
Age (year)	50 ± 3	55 ± 6
Height (cm)	173 ± 2	172 ± 3
Weight (kg)	78 ± 4	87 ± 5
BMI (kg/m^2)	26 ± 0.88	30 ± 1.8*
Hemodynamic measurements		
Resting HR (BPM)	63 ± 4	64 ± 2
Systolic blood pressure (mmHg)	116 ± 3	140 ± 4***
Diastolic blood pressure (mmHg)	68 ± 2	78 ± 3**
Mean arterial pressure (mmHg)	84 ± 2	99 ± 3***
Renal function		
Blood urea nitrogen (mg/dL)	16.5 ± 0.95	38.8 ± 6.3**
Serum creatinine (mg/dL)	0.97 ± 0.04	2.4 ± 0.37**
eGFR (mL/min/1.73 m^2)	>60	36 ± 5
Blood lipids, cells and glucose		
Total cholesterol (mg/dL)	204 ± 11	206 ± 15
High-density lipoprotein (mg/dL)	55 ± 3	57 ± 8
Low-density lipoprotein (mg/dL)	131 ± 9	121 ± 12
Triglycerides (mg/dL)	86 ± 8	141 ± 25*
Hemoglobin (g/dL)	14.3 ± 0.21	12.8 ± 0.52*
Hematocrit (%)	42.1 ± 0.57	38.9 ± 1.4*
Glucose (mg/dL)	83 ± 1	100 ± 10*
Medications (number of patients)		
Antihypertensives		
ACE inhibitors	0	6
ANG II-receptor antagonists	0	2
Beta-blockers	0	5
Diuretics	0	6
Calcium antagonists	0	7
Statins	0	6
Insulin	0	3
Erythropoiesis-stimulating agents	0	1
Allopurinol	0	2

Values are means ± SEM or number. *n*, number of subjects; CKD, chronic kidney disease; BMI, body mass index; ACE, angiotensin-converting enzyme; ANG, angiotensin; HR, heart rate; eGFR, estimated glomerular filtration rate

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

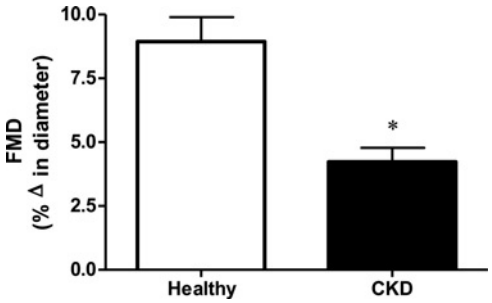

Figure 1 Flow-mediated dilation (FMD) responses in healthy controls and CKD subjects. Values are mean ± SEM. CKD, chronic kidney disease
*$P < 0.001$

was not different between groups (AUC: 32297 ± 7347 versus 27749 ± 8305, $P = 0.345$).

Myeloid (Figures 2a and c) and hematopoietic CPCs (Figures 2b and c) were lower in the CKD group including the CD34+/CD45+ myeloid CPCs (218 ± 40 versus 137 ± 23) and CD34+/CD45− hematopoietic CPCs (22 ± 1 versus 9 ± 1; all $P < 0.05$). CFUs were impaired in CKD patients as compared with apparently healthy controls (Figure 2d; Controls: n = 13, CKD: n = 10), and the non-adherent PBMNCs that formed CFUs expressed a lower percentage of endothelial cell markers, CD31 and CD144, and the hematopoietic marker, CD34 (see Table 2). No significant differences were observed in the amount of cells expressing the myeloid cell marker, CD45, or the endothelial marker, KDR ($P = 0.08$), between groups.

Table 2 Non-adherent PBMNC expression of endothelial, myeloid and hematopoietic cell surface makers

	Control (%) (n = 13)	CKD (%) (n = 10)
CD31	40 ± 4	28 ± 5*
CD144	30 ± 3	15 ± 4**
CD45	94 ± 1	92 ± 1
CD34	6 ± 1	2 ± 0.4**
KDR	23 ± 6	12 ± 5

Values are the mean ± SEM % of cells expressing cell surface markers per 200,000 flow cytometric events. PBMNC, peripheral blood mononuclear cell; CKD, chronic kidney disease; n, number of subjects
*$P < 0.05$, **$P < 0.01$

FMD remained impaired significantly ($P < 0.01$) and all CPCs reduced significantly after adjusting for age and sex (all $P < 0.05$) with the exception of the CD34+/CD45+ myeloid CPCs ($P = 0.07$) and CD34+/KDR+/CD45− hematopoietic CPCs ($P = 0.05$). CFUs were no longer significantly different between groups with age and sex adjustment ($P = 0.07$).

Univariate analysis

Univariate analysis of the relations between FMD and other evaluated variables is shown in Tables 3 and 4. FMD was associated significantly with all measures of renal function in addition to systolic blood pressure (SBP) and body mass index (BMI). Increasing hemoglobin ($r = 0.428$, $P < 0.05$)

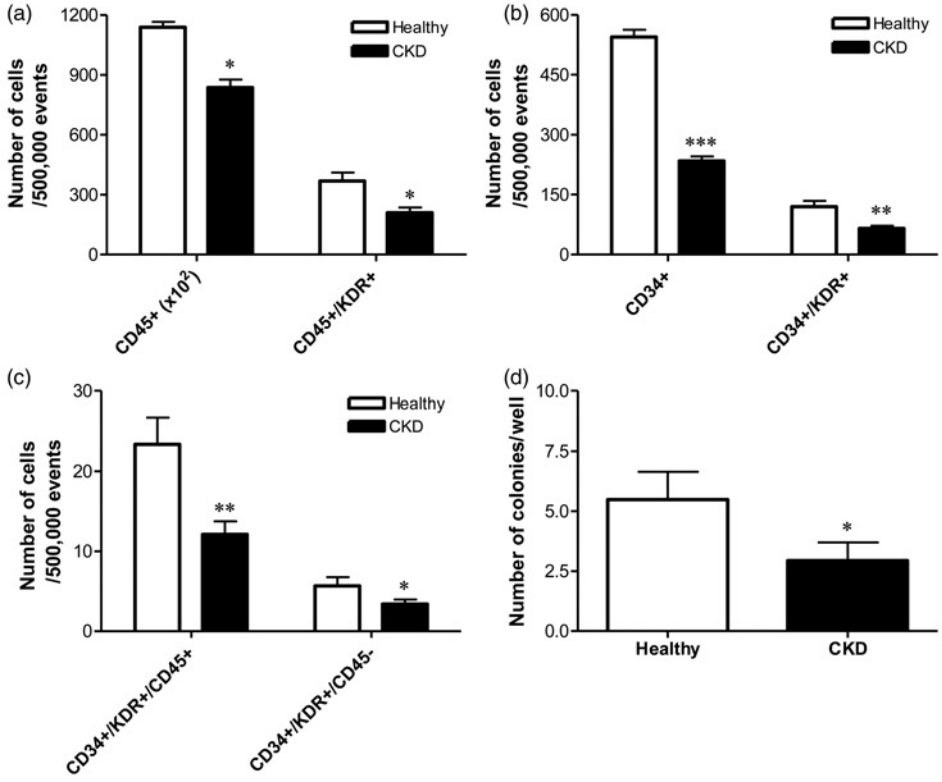

Figure 2 (a) Myeloid CD45+ and CD45+/KDR+ CPCs; (b) hematopoietic CD34+ and CD34+/KDR+ CPCs; (c) triple-antibody stained myeloid CD34+/KDR+/CD45+ and hematopoietic CD34+/KDR+/CD45− CPCs; and (d) colony-forming units in healthy controls and CKD patients. Values are mean ± SEM. CKD, chronic kidney disease; CPC, circulating progenitor cell
*$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$

Table 3 Univariate analysis of renal function

	eGFR	BUN	Serum creatinine
FMD	0.617**	−0.445*	−0.382**
Hematopoietic progenitors			
CD34+	0.689***	−0.470*	−0.435*
CD34+/KDR+	0.562**	−0.404*	−0.455*
CD34+/CD45 −	0.574**	−0.398*	−0.344*
CD34+/KDR+/CD45 −	0.243	−0.261	−0.209
Myeloid progenitors			
CD45+	0.21	0.086	−0.123
CD45+/KDR+	0.456*	−0.251	−0.361*
CD34+/CD45+	0.247	−0.241	−0.291
CD34+/KDR+/CD45+	0.373*	−0.163	−0.211
Colony-forming units	0.198	−0.102	−0.144

Pearson's correlation coefficients (*r* value) of associations between flow-mediated dilation (FMD; $n = 24$), circulating progenitor cells (CPC) ($n = 25$), colony-forming units ($n = 23$) and various measures of renal function. *n*, number of subjects; eGFR, estimated glomerular filtration rate; BUN, blood urea nitrogen
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

and hematocrit ($r = 0.327$, $P < 0.05$) were associated positively with FMD, and the negative association between FMD and increasing blood glucose approached significance ($P = 0.06$). FMD also was associated positively with the CD34+ hematopoietic CPCs ($r = 0.353$, $P < 0.05$) as well as CD45+/KDR+ ($r = 0.474$, $P < 0.05$) and CD34+/CD45+ ($r = 0.358$, $P < 0.05$) myeloid CPCs. The association between FMD and CD34+/CD45− hematopoietic CPCs approached significance ($r = 0.318$, $P = 0.065$), but FMD was not associated significantly with any other CPC subpopulations or CFUs (all $P > 0.05$).

CD34+, CD34+/KDR+, and CD34+/CD45− hematopoietic CPCs were associated significantly with all measures of renal function (see Table 3). CD45+/KDR+ myeloid CPCs were associated significantly with increasing eGFR and decreasing serum creatinine, and CD34+/KDR+/CD45+ myeloid CPCs were also associated significantly with increasing eGFR. All other CPCs and CFUs were not associated significantly with any measures of renal function (all $P > 0.05$), with the association between CD34+/CD45+ myeloid CPCs and decreasing serum creatinine approaching significance ($P = 0.07$).

Subject characteristics that were associated with myeloid and hematopoietic CPCs included BMI and SBP (see Table 4). CD34+ and CD34+/CD45− hematopoietic CPCs were associated negatively with increasing BMI and SBP, and CD45+/KDR+ myeloid CPCs were also associated negatively with SBP. CD45+, CD34+/KDR+/CD45+ and CD34+/KDR+/CD45− CPCs, as well as CFUs, were not associated significantly with BMI or SBP (all $P > 0.05$). Blood glucose, hemoglobin and hematocrit were not associated significantly with CFUs or any of the CPCs (all $P > 0.05$), but the negative association between increasing blood glucose and CD34+ ($P = 0.06$) and CD34+/KDR+ ($P = 0.05$) hematopoietic CPCs, as well as CD34+/CD45+ myeloid CPCs ($r = -0.311$, $P = 0.07$), approached significance.

Multivariate analysis

CD45+/KDR+ myeloid and CD34+/CD45− hematopoietic CPCs were used as representatives of the two distinctive groups of CPCs for multivariate analysis due to their significant associations with different demographic factors and renal function measurements (see Tables 3 and 4). High colinearity existed between the different measurements of renal function as well as the different demographic factors. Therefore, the strongest correlates of each cell population were selected and used with eGFR for multivariate analysis in the absence and presence of age and sex listed as covariates. CD45+/KDR+ myeloid CPCs were no longer associated significantly with increasing eGFR after performing multivariate analysis without (see Table 4) and with age and sex adjustment ($r = 0.407$; $P = 0.137$). However, increasing eGFR remained associated significantly with CD34+/CD45− hematopoietic CPCs both when not adjusted (see Table 4) and adjusted for age and sex ($r = 0.513$; $P < 0.05$).

Discussion

In the present investigation, we support previous findings showing reduced FMD in moderate-to-severe CKD patients (eGFR: 36 ± 5).[22] The novel findings of the current study are: (1) myeloid and hematopoietic CPC subpopulations, identified using the cell surface markers KDR and CD45 in addition to CD34, are reduced in number in moderate-to-severe CKD; (2) cultured non-adherent PBMNC CFUs are impaired in moderate-to-severe CKD; (3) FMD and select myeloid and hematopoietic CPCs are associated with renal function as assessed by eGFR, BUN and serum creatinine; and (4) CD34+ as well as CD45+/KDR+ and CD34+/CD45+ myeloid CPCs are associated positively with FMD. Taken together, these findings suggest that production, mobilization and/or differentiation of hematopoietic and myeloid CPCs may be impaired in moderate-to-severe CKD. However, CPC function was not

Table 4 Univariate and multivariate analysis of subject characteristics

		Univariate analysis				Multivariate analysis	
	FMD	CD34+	CD34+/KDR+	CD34+/CD45 −	CD45+/KDR+	CD45+/KDR+	CD34+/CD45 −
SBP	−0.638**	−0.402*	−0.270	−0.359*	−0.398*	−0.184	
BMI	−0.449*	−0.373*	−0.231	−0.382*	−0.138		−0.139
Glucose	−0.262	−0.352	−0.347	−0.245	−0.154		
eGFR	0.617**	0.689***	0.562**	0.574**	0.456*	0.322	0.507*

Pearson's correlation coefficients (*r* value) of univariate and multivariate analysis between FMD, circulating progenitor cells and subject characteristics ($n = 25$). *n*, number of subjects; FMD, flow-mediated dilation; SBP, systolic blood pressure; BMI, body mass index; eGFR, estimated glomerular filtration rate
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

assessed in the present study. Impaired CPC function has the potential to compromise vascular integrity and may explain the association between myeloid and hematopoietic CPC deficiencies and impaired endothelial function as assessed by FMD.[23]

Endothelial dysfunction has been reported in different stages of renal insufficiency and is associated with not only renal disease progression but also increased CVD risk in CKD patients.[4,22,24–26] Here, we show impaired FMD, a measure of endothelial-dependent dilation, in moderate-to-severe CKD. We did not measure endothelial-independent dilation so we cannot definitively state that reduced FMD is due to endothelial dysfunction or impaired smooth muscle responsiveness. However, no differences in endothelial-independent dilation between control and CKD subjects have been reported previously.[22]

CD34+ hematopoietic and CD45+ myeloid CPC subpopulations were reduced in CKD patients. Furthermore, CD34+ hematopoietic CPCs, with the exception of the CD34+/KDR+/CD45− subgroup, and select CD45+ myeloid CPC subpopulations were associated with different measurements of renal function. The lack of association between CD34+/KDR+/CD45− hematopoietic CPCs and renal function could be the result of the rarity of these cells in the peripheral circulation. It is also important to note that the MDRD equation, as well as other equations to predict GFR, show a bias when estimating GFRs above 60 mL/min/1.73 m^2.[27]

The CD34+ hematopoietic and CD45+ myeloid subpopulations explored potentially represent various cell populations of hematopoietic stem cell origin that are thought to migrate to sites of hypoxia and vascular injury and construct scaffolds for new capillary formation, release proangiogenic factors and/or differentiate into mature endothelial cells either replacing damaged cells or forming new capillaries.[28–30] The CD34+/KDR+, CD34+/CD45 − , CD45+/KDR+, CD34+/CD45+ hematopoietic and myeloid CPCs, respectively, most likely represent populations of immature cells still with the ability to differentiate into a variety of cell types of hematopoietic lineage, whereas the CD34+/KDR+/CD45− and CD34+/KDR+/CD45+ cell populations are more rare CPCs that may participate in re- and neovascularization directly and/or in a paracrine fashion.[29]

Reductions in CD45+ myeloid and CD34+ hematopoietic CPCs of our CKD cohort could be the result of multiple mechanisms affecting both the vascular milieu as well as progenitor cell populations in CKD. Nitric oxide (NO) has been shown to mobilize progenitor cells. NO is synthesized by endothelial nitric oxide synthase via the growth factor, stromal derived factor-1alpha (SDF)-1α, and its receptor, CXCR4, in a PI3K/Akt-dependent manner.[31,32] The CKD patients in our study showed impaired FMD, a response that is partially mediated by NO.[7] If FMD represents a systemic reduction in NO production and/or bioavailability, then decreased NO could decrease CPC mobilization from the bone marrow in CKD.

Other mechanisms of progenitor deficiencies in CKD include decreased erythropoietin (EPO) production and increased BUN. EPO has been shown to mobilize progenitor cells *in vitro*[33] and *in vivo*.[34] In our investigation, EPO was not directly measured in the blood, but hemoglobin and hematocrit were significantly reduced in the CKD patients. Yet, these two measures were not associated with CPC numbers. Conversely, hematopoietic CPCs were associated negatively with BUN. Recent investigation has suggested urea to be indicative of uremic-toxin burden in renal disease patients by inducing macrophage proliferation *in vitro*.[35,36] Consequentially, myeloid and hematopoietic CPCs could be reduced due to transdifferentiation of progenitor cells and differentiation of hematopoietic stem cells into proinflammatory monocytes and macrophages instead of vascular progenitors.

Consistent with a reduction in CPCs, colony-forming ability was impaired in the CKD sample. The colony-forming assay that we performed has been proposed to yield a heterogeneous population of cells consisting of monocytes, macrophages and colonies of CD14+/CD31+/CD45+/CD144+/KDR+ myeloid progenitor cells[29,37] that are inversely correlated with CVD risk.[11,16] Our results showed decreased expression of CD31, CD144 and CD34 in the non-adherent cell population that formed these colonies in the CKD patients. Yet, CFUs were not associated significantly with FMD or any of the subject characteristics, a finding that contrasts previous reports in CVD populations.[11,16]

The current experimental design has determined if FMD and CFUs are impaired and CPC subpopulations reduced in moderate-to-severe CKD patients. Our findings should be interpreted with some caution due to the small sample size. However, our results for FMD and CD34+/KDR+ CPCs are consistent with previous studies,[8,22] and *post hoc* power analysis ($\alpha = 0.05$) for both FMD and CD34+/KDR+ CPCs indicated an achieved power ($1 - \beta$ error probability) of 0.92 or higher. Patients were taking a variety of medications known to have positive effects on vascular function, but despite taking these medications, FMD and CFUs were impaired and CPC numbers reduced in CKD. The mechanisms responsible for reduced CPC populations still remain unclear and findings are limited to rare event flow cytometric analysis and *in vitro* cell culture techniques. Although flow cytometry and CFU assays are used consistently in the literature, uncertainty exists about the appropriate identification of progenitor cells and the relevance of these measurements to *in vivo* physiological functioning. In particular, we did not use CD133, another hematopoietic stem cell marker that is commonly associated with different vascular progenitor cell populations, when determining the amount of progenitor cells in the circulation.[14]

Conclusions

In conclusion, different myeloid and hematopoietic CPCs are reduced and FMD is impaired in moderate-to-severe CKD patients without known CVD as compared with apparently healthy controls. Select myeloid and hematopoietic CPCs are also correlated with renal function and positively associated with FMD. Reductions in progenitor cells are accompanied by impaired CFU ability and

decreased expression of hematopoietic and myeloid cell surface markers in non-adherent cultured PBMNCs. These observations suggest that impaired CPC subpopulations may be related to renal disease progression and increased CVD risk in CKD. Therefore, therapies that re-vitalize progenitor cells in CKD could be important in improving both renal and cardiovascular outcomes.

Author contributions: JMK, CRC, WBF and DGE designed the study; all authors participated in analysis and interpretation of the data and review of the manuscript; JMK, MDD and JJD conducted the experiments; CRC and RAS supplied critical space and supplies for cell culture; and JMK and DGE wrote the manuscript.

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