Kidney International, Vol. 67 (2005), pp. 1078–1083

Circulating endothelial cells are associated with future vascular events in hemodialysis patients

MEHMET KOC, HANNO B. RICHARDS, AZRA BIHORAC, EDWARD A. ROSS, JESSE D. SCHOLD, and MARK S. SEGAL

Division of Nephrology, Hypertension and Transplantation, Division of Rheumatology, Department of Medicine, University of Florida, Gainesville, Florida; and Department of Medicine, Marmara University, Istanbul, Turkey

Circulating endothelial cells are associated with future vascular events in hemodialysis patients.

Background. Endothelial dysfunction and injury are thought to have a key role in the pathogenesis of cardiovascular disease. We hypothesized that the presence of circulating endothelial cells, as a reflection of ongoing endothelial injury, might provide a novel means for predicting cardiovascular events in hemodialysis subjects who are known to be at marked increased risk for cardiovascular disease.

Methods. Circulating endothelial cell number was determined in 29 hemodialysis patients who were then followed for vascular events for 470 ± 172 days. In a second cohort of 44 hemodialysis patients, circulating endothelial cell number was correlated with markers of inflammation, namely high sensitivity C-reactive protein (hs-CRP), interleukin (IL)-6, IL-10, and monocyte chemoattractant protein-1 (MCP-1), and endothelial dysfunction, soluble vascular cellular adhesion molecule-1 (VCAM-1).

Results. Seven of the 19 subjects with elevated circulating endothelial cells (defined as >19 cells per mL) had cardiovascular ($N = 5$) or vascular ($N = 5$) events during follow-up, whereas no events occurred in subjects with a low number of circulating endothelial cells (≤19 CECs per mL) ($P = 0.04$ by Fisher Exact Test). In the second cohort, the number of circulating endothelial cells was independent of all markers of inflammation and endothelial dysfunction.

Conclusion. In this hemodialysis population, an increase in circulating endothelial cells was found to predict the development of cardiovascular and vascular events, and to be independent of other known markers of inflammation or endothelial dysfunction. These studies suggest that circulating endothelial cells may be a novel way to assess endothelial health and cardiovascular risk. Further studies to investigate the utility of circulating endothelial cells in predicting cardiovascular risk are needed.

Key words: circulating endothelial cells, hemodialysis, dysfunctional endothelium, inflammation.

Received for publication May 12, 2004
and in revised form August 9, 2004
Accepted for publication September 28, 2004

Patients with end-stage renal disease (ESRD) have a dramatically increased risk of cardiovascular (CV) disease. Both traditional risk factors (i.e., hypertension, hyperlipidemia, obesity) as well as factors related to uremia (i.e., anemia, calcium loading, and chronic inflammation) have been identified as key mediators [1, 2].

The "response to injury" hypothesis proposed by Ross suggests that the initial event of atherosclerotic disease is endothelial injury, leading to a local inflammatory response with macrophage infiltration, smooth muscle cell proliferation, and a fibrous cap [3]. The central role of endothelial injury in the development of atherosclerosis and vascular disease [4] has prompted the development of methods to measure endothelial injury and dysfunction. The ability of the endothelium to release nitric oxide (reflected by measurement of acetylcholine-dependent vasodilatation by brachial artery reactivity) [5] or measuring the release of endothelial cell antigens into the blood (vascular cellular adhesion molecule, etc.) represent some of the major methods currently used. However, these measures may be compromised by dietary intake or the use of medications (that affect NO production) [6], and in addition, the release of antigens may not necessarily reflect endothelial injury.

We reasoned that, if the end result of a variety of insults, hypertension, hyperlipidemia, oxidative stress, was endothelial damage, then the number of detached endothelial cells circulating in blood may be a sensitive and specific measure of endothelial injury. The number of these detached endothelial cells may not only be a direct measure of the severity of the insult, but also a direct measure of the ability of an individual's endothelium to resist that insult. We have previously reported that circulating endothelial cells (CECs) are indeed elevated in subjects with hypertension, diabetes, and in hemodialysis patients [7]. Interestingly, not all subjects in these studies had elevated CECs. We thus tested the hypothesis that those subjects who had an elevated CEC number would be at the greatest risk for CV events. We also determined how CECs compared as a risk factor with other established

risk factors. We now report in this preliminary study that in hemodialysis patients elevated CECs are strong predictors for the development of vascular events, and that this measurement appears independent of classic CV risk factors.

METHODS

Patients and subjects

The study protocol was approved by the Institutional Review Board at the University of Florida, and written informed consent was obtained from each patient. Patients were asked to participate without regard to their cardiovascular disease status. Exclusion criteria for the hemodialysis patients were (*1*) signs or symptoms of any clinical infection during the month previous and month after the blood draw; (*2*) patients on glucocorticoids or anti-inflammatory medications other than aspirin; (*3*) central line insertion or any invasive procedure during the month previous to the blood draw; (*4*) HIV infection; (*5*) hepatitis B or C infection; and (*6*) active or past history of neoplastic or rheumatologic disease. Sixteen of the 60 patients who agreed to participate in the study met exclusion criteria. The Cohort 2 study population consisted of 44 individuals receiving conventional hemodialysis for four hours three times weekly. Hemodialysis procedures were performed using F80 polysulfone dialyzers (Fresenius Medical Care, Lexington, MA, USA), bicarbonate dialysate, and heparin sodium as standard anticoagulant. Seventeen of these patients were among the 29 patients who previously had undergone CEC enumeration as described in our previous study [7]. Twenty-one patients in the first cohort and 18 patients in the second cohort were further classified using a modified Index of Co-Existing Disease (ICED) as previously described [7]. The score in each category of vascular disease [coronary artery disease (CAD), cerebral vascular events (CVE), and peripheral vascular disease (PVD)] was used to classify patients into three groups: No-ACVD: ICED score 0 in each of the three categories and a negative cardiac catheterization within the past year. Patients in this category have no history of any atherosclerotic cardiovascular disease, and have had a negative cardiac catheterization within the past year. Stable-ACVD: ICED score of 1 in at least one category and no ICED score of 2 in any category. Patients in this category had the diagnosis of atherosclerotic cardiovascular disease, but have been asymptomatic during the three months prior to CEC enumeration. Active-ACVD: ICED score of 2 in at least one category. Patients in this category had the diagnosis of atherosclerotic cardiovascular disease, and were symptomatic during the three months prior to CEC enumeration.

Eight hemodialysis patients in the first cohort and 25 patients in the second cohort were not included in this subgroup analysis even though they had no history of vascular disease because they did not have a recent (within past year) cardiac catheterization.

Collection of blood specimens

Blood from hemodialysis patients was withdrawn during a midweek hemodialysis session into ethylene diamine tetra-acetic acid (EDTA)-containing tubes from the arterial line of hemodialysis sets before the return of any blood to the systemic circulation. An additional 4 mL of blood was also withdrawn for separation and storage of serum and plasma.

Enumeration of circulating endothelial cells

Enumeration was performed as previously described [7]. Briefly, after lysing red blood cells from 0.5 mL of whole blood, 1×10^6 immunomagnetic beads (Dynabeads M-500, Dynal Biotech, Inc., Oslo, Norway) conjugated with P1H12 (Chemicon, Temecula, CA, USA), a murine, monoclonal antibody specific for human endothelial cells [8], were used to isolate circulating endothelial cells. The rosetted cells were cytospun onto poly-L-lysine coated slides. After drying overnight, the slides were fixed with 1% paraformaldehyde, stained with 1 μg/mL propidium iodine in phosphate-buffered saline (PBS), prior to mounting the slides in Vectashield with DAPI (Vector Laboratories, Inc., Burlingame, CA, USA). Quantitation of CEC was performed by identification of the cells using a Zeiss Axiophot microscope (Carl Zeiss, Inc., Thornwood, NY, USA).

Access and cardiovascular events

Twenty-nine patients enrolled in our previous study [7] were followed for an average of 470 ± 172 days (range 40 to 588), and the incidence of access related events, cardiovascular events, or death was recorded. The determination of cardiovascular and access events were made blinded to CEC number. Cardiovascular events were defined as myocardial infarction (1 patient); cardiac arrest (2 patients); ischemic colitis (1 patient); transient ischemic attack or cerebrovascular accident (1 patient); the need for coronary angioplasty or coronary bypass surgery (0 patients); or the need for peripheral artery angioplasty, bypass surgery, or amputation due to peripheral arterial disease (0 patients). We did not include death due to sepsis (1 patient, high CEC group), chronic claudication (present before CEC enumeration) secondary to an old clot in a femoral access (1 patient, low CEC group), or CHF exacerbation due to increased fluid intake (1 patient, high CEC group). Nor did we include a reversible ischemic defect found on an adenosine thallium done on an asymptomatic patient during transplant screening (1 patient, high CEC group). Access events included need for AV fistula angioplasty (4 events in

3 patients) and access revision (1 patient). We did not include any angioplasty that was in a fistula that had poor flows prior to CEC enumeration (1 patient, high CEC group), or thrombosis of a graft placed after CEC enumeration (1 patient, low CEC group).

Laboratory tests

Levels of plasma IL-6, IL-10, MCP-1 (BD Biosciences Pharmingen, San Diego, CA, USA), and serum soluble VCAM-1 (BioSource International, Inc., Camarillo, CA, USA) were determined by enzyme-linked immunosorbent assay (ELISA). The assays were performed as per manufacturer instructions, and developed with tetramethylbenzidine dihydrochloride substrate (Pierce, Rockford, IL, USA), and read on a PowerWave 200 Scanning Spectrophotometer (Bio-Tek Inst., Winooski, VT, USA). hs-CRP levels were determined on a BN ProSpec® System Nephelometer (Dade Behring, Deerfield, IL, USA).

Statistical analysis

Statistical analysis was performed with Prism Statistical software (version 3.02; Graphpad, San Diego, CA, USA). Continuous variables were reported as mean ± standard deviation unless otherwise specified. Comparisons of continuous variables between the groups were performed by Mann-Whitney U test or unpaired t test where appropriate. Since MCP-1, hs-CRP, IL-6, and IL-10 values and IL-6 to IL-10 ratios are non-normally distributed, they were log transformed, and log transformed values were used for statistical analysis. The Fisher exact test was used to compare the categorical variables. Correlation analyses were performed by Spearman's rank correlation analysis. Multivariate regression analyses were employed when evaluating the effect(s) of continuous variables on the number of CECs used as the dependent variable. Survival curves were calculated by the Kaplan-Meier method, and compared by log rank test using SAS (version 8.02, Cary, NC, USA). Results were regarded as significant at $P < 0.05$.

RESULTS

Cohort 1: Elevated CECs predict vascular events in hemodialysis subjects

We previously described CEC enumeration in 29 hemodialysis patients [7]. These patients had a mean CEC number of 31 ± 19 per mL compared to the mean of healthy control patients of 19 ± 7 per mL [7]. Given our normal mean of 19 cells per mL in healthy subjects, we used the CEC cutoff of 19 cells per mL to categorize patients into high ($N = 19$) and low ($N = 10$) CEC number groups. Table 1 shows the baseline characteristics in these two subgroups in terms of dialysis adequacy, presence of established CV risk factors, and use of angiotensin-converting enzyme inhibitor (ACEI)/angiotensin II receptor blocker (ARB) and HMG CoA reductase therapy. There were 7 patients with hypertension and 4 patients with glomerulonephritis as the etiology of their ESRD. The mean number of CECs among patients with hypertension (30 ± 8.9) was similar ($P = 0.20$) to the mean number of CECs among patients with glomerulonephritis (33 ± 34.0) as the etiology of their ESRD. There was also no statistical difference in the number of patients with hypertension or glomerulonephritis in the high and low CEC groups (Table 1). In the first cohort we found a higher calcium phosphorous product in the higher CEC group (Table 1). However, while evaluating all hemodialysis patients in cohort 1 and 2 as a whole, we did not find a correlation between CEC number and calcium phosphorous product (data not shown).

Patients were followed for a mean of 470 ± 172 days (range 40 to 588 days). All five cardiovascular events (2 cardiovascular deaths, 1 myocardial infarction, 1 cerebral vascular accident, 1 ischemic colitis) and five access related events (4 angioplasties and 1 arteriovenous fistula revision) occurred in patients with an elevated CEC number ($P = 0.04$ by Fisher exact test). Using Kaplan-Meier plot, a 30% difference in morbidity and mortality between the two groups was observed at 20 months (Fig. 1, $P = 0.035$). The Kaplan-Meier plots showed a trend toward more cardiovascular events ($P = 0.085$) in patients who had greater than 19 CECs per mL (Fig. 1). The seven patients who had an event had a statistically higher CEC number than all of the rest of the patients (44.3 ± 22.6 vs. 26.5 ± 16.4; $P = 0.03$ by Student t test).

Cohort 2: Correlation of CECs with inflammation and endothelial dysfunction

In the original study, a correlation of CECs with markers of systemic inflammation and endothelial dysfunction had not been performed. We therefore studied a second cohort (44 subjects) on hemodialysis, and measured classical markers of inflammation (hs-CRP, IL-6, and MCP-1), anti-inflammation (IL-10), and endothelial dysfunction (serum VCAM-1), and evaluated their relationship with the number of CECs.

Baseline characteristics of the new and old patient cohort are shown in Table 1. Of the 44 hemodialysis subjects, 22 had an elevated CEC number (above 19 cells per mL), and 22 had a low CEC number (19 cells per mL or less) (Table 2). As shown in Table 2, there was no difference in the levels of systemic inflammatory markers, and VCAM were seen in subjects with elevated CECs compared to those with a low CEC number. A stepwise multiple regression analysis model created for the 44 hemodialysis patients also did not find that these markers were significant predictors of pre-HD CEC number

Table 1. Demographic and clinical characteristics of the first and second cohort of hemodialysis patients

	First cohort as a group ($N = 29$)	First cohort: Low CEC group ($N = 10$)	First cohort: High CEC group ($N = 19$)	Second cohort ($N = 44$)
Age *years*	52 ± 15	51 ± 20	53 ± 13	51 ± 14
Male%	52	60	47	45
Ethnicity%				
African American	66	60	68	66
Caucasian	24	30	21	30
Hispanic	10	10	11	5
End-stage renal disease etiology %				
Diabetes mellitus	34	50	26	34
Hypertension	24	0	37	23
Glomerulonephritis	17	30	5	18
Other	25	20	32	25
ACVD score[b] *N* (%) [c]				
0	10 (34)	1 (10)	9 (47)	1 (2)
1	6 (21)	5 (50)	1 (5)	16 (36)
2	5 (17)	0 (0)	5 (26)	2 (5)
# of patients classified % *of cohort*	21 (72)	6 (60)	15 (79)	19 (43)
Time on RRT *months*[a]	41 (14–98)	37 (12–106)	47 (14–97)	36 (12–78)
Arteriovenous fistula %	66	70	53	55
Pre-hemodialysis SBP *mm Hg*	150 ± 34	157 ± 41	134 ± 25[f]	148 ± 27
Pre-hemodialysis DBP *mm Hg*	79 ± 19	81 ± 18	74 ± 16	80 ± 21
Urea reduction ratio %	74	75	74	74
Hemodialysis dose *Kt/V*	1.6 ± 0.4	1.7 ± 0.2	1.5 ± 0.5	1.7 ± 0.3
Use of ACEI/ARB %	72	70	74	66
Use of statin therapy %	41	40	42	50
HgbA1c of diabetic patients %	6.1 ± 1.2	5.6 ± 0.9	6.4 ± 1.4	6.1 ± 0.6
Calcium *mg/dL*	8.6 ± 0.6	8.6 ± 0.5	8.5 ± 0.7	8.9 ± 0.9
Phosphorus *mg/dL*	4.7 ± 1.8	3.6 ± 1.4	5.3 ± 1.8[d]	5.6 ± 1.7
Calcium and phosphorus product mg^2/dL^2	40 ± 15	31 ± 12	45 ± 15[f]	50 ± 13
Intact parathyroid hormone *ng/mL*[a]	290 (132–580)	345 (205–591)	290 (42–610)	244 (132–333)
Hemoglobin *g/dL*	11.2 ± 0.8	11.3 ± 0.7	11.2 ± 0.8	11.4 ± 1.2
Total weekly erythropoietin dose *U/kg/wk* [a]	173 (51–300)	182 (43–309)	183 (62–332)	143 (55–241)
Albumin *g/dL*[a]	3.9 (3.5–3.9)	3.9 (3.8–3.9)	3.7 (3.5–3.9)	3.9 (3.6–3.9)
Total cholesterol *mg/dL*	130 ± 33	113 ± 26	139 ± 33[e]	149 ± 40
High-density lipoprotein cholesterol *mg/dL*	48 ± 19	43 ± 15	51 ± 21	51 ± 21
Low-density lipoprotein *mg/dL*	60 ± 29	47 ± 16	67 ± 31	74 ± 34.3
Triglycerides *mg/dL*	118 ± 71	141 ± 92	105 ± 56	125 ± 65

Abbreviations are: RRT, renal replacement therapy; Hgb, hemoglobin. Values are expressed as mean ± SD except for [a]those that are expressed as median (lower and upper quartile). Conversion factors from conventional to SI units for serum calcium, serum phosphorus, total-cholesterol, and triglycerides (mg/dL to mmol/L) are 0.2495, 0.3229, 0.0259, and 0.0113, respectively, and the conversion factor for serum albumin and hemoglobin (g/dL to g/L) is 10.

[b]See **Methods** for definition of subgroups.

[c] Twenty-one patients were given ACVD score in the first cohort, and 19 patients were given ACVD score in the second cohort (see **Methods** section).

[d] $P < 0.05$ vs. low CEC group; [e] $P = 0.054$ vs. low CEC group; [f] $P = 0.07$ vs. low CEC group.

(data not shown). In addition, there was no difference in the presence/absence of known risk factors between the two groups (Table 3).

DISCUSSION

The number of CECs is increased in a variety of conditions, and may reflect the activation state of the endothelium [8]. This study is the first to report on the long-term follow-up of vascular events in hemodialysis patients after enumeration of CEC number. Previously, we reported that hemodialysis patients have a higher mean value of CECs per mL as compared to control patients. In this study, we used the mean CEC of healthy control patients from the previous study to divide hemodialysis patients into two groups, patients with "high" versus "low" number of CECs. In the ensuing 20 months, all 10 vascular events occurred in patients that were in the "high" CEC group. Three of the patients who had an event were in the active atherosclerotic disease group in the previous study [7] and would predictably have a higher risk of mortality. The other four patients who had a vascular event had an elevated CEC with no history of atherosclerotic disease. Interestingly, no patient previously classified as having stable ACVD, who as a group had a statistically lower CEC number, had an event. Despite the small number of patients in the present study, there is a statistically significant increased mortality and morbidity from vascular events in hemodialysis patients with a "high" number of CECs and a trend toward increased cardiovascular events. To our knowledge, this is the first study to determine the predictive value of CECs with regard to cardiovascular morbidity and mortality. However, results of previous studies were suggestive that CECs could be a

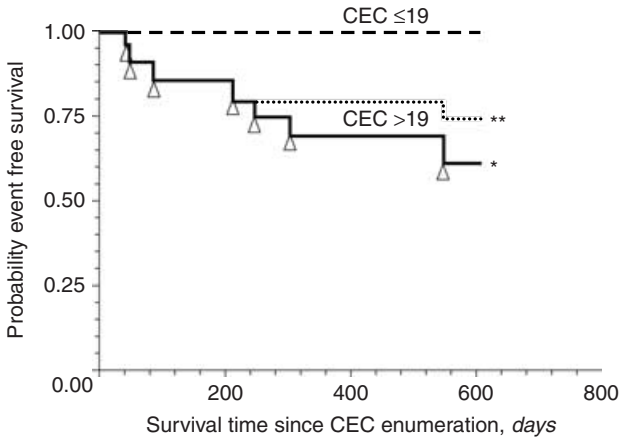

Fig. 1. Time of onset to first cardiovascular event in patients with high and low number of CECs. Kaplan-Meier curve showing the time to onset of first cardiovascular event (. . .) or time to onset of first vascular (cardiovascular or access-related event; Δ) (▬) in patients who had greater than 19 CECs per mL, and in those who had less than or equal to 19 CECs per mL (- - -). $^{*}P = 0.035$ or $^{**}P = 0.085$ by log-rank test compared to patients with less than or equal to 19 CECs per mL.

Table 2. Relationship between CEC and endothelial and inflammatory markers

	Low CEC group ($N = 22$)	High CEC group ($N = 22$)	P value
Mean CEC number	11.8 ± 4.4	29.8 ± 12.4	<0.0001
VCAM-1 *ng/mL*	1961 ± 1309	1646 ± 851	0.55
Serum MCP-1 *pg/L*[a]	1077 ± 486	1590 ± 2825	0.4
	875 (715–1439)	976 (869–1339)	
Serum hs-CRP *mg/dL*[a]	15.5 ± 19.9	9.6 ± 6.8	0.73
	8.38 (3.42–22.6)[a]	6.19 (4.24–15.1)[a]	
Plasma IL-6 *pg/mL*[a]	4.58 ± 7.6	2.05 ± 2.46	0.94
	0.55 (0.28–7.8)[a]	1.77 (0.21–2.8)[a]	
Plasma IL-10 *pg/mL*[a]	4.95 ± 3.87	0.92 ± 1.07	0.26
	1 (0.5–1)	0.5 (0.5–1)	

Statistical analyses were performed using Mann-Whitney U test.
[a]Values are also given as median (upper-lower quartile).

predictor of cardiovascular events. Patients who suffer from unstable angina pectoris have an increased number of CECs compared to patients with stable angina [9], and CECs are increased in those patient who suffered an acute myocardial infarction [9, 10]. Also, serial determination of CEC number demonstrates a decline in CEC number with improvement of disease processes, such as ANCA-associated vasculitis [11], sickle cell crisis [8], or acute myocardial infarction [9]. Our preliminary study suggests that an elevated CEC number may be a predictor of future cardiovascular morbidity and mortality in HD patients.

In cohort 2, half of the patients had a "high" CEC number and half a "low" CEC number; however, there was no statistical difference in VCAM-1 levels between the two groups. We also did not find a difference between the two groups with regard to hs-CRP, IL-6, or IL-10 levels, nor did we find a correlation between these markers and

Table 3. Cardiovascular risk factors in patients with low and high CEC numbers in the second cohort

	Low CEC	High CEC	P value
Age	53.3 ± 14.1	49.5 ± 14.6	0.38
Male N (%)	13, (59)	10, (45)	0.54
Total cholesterol	162 ± 46	139 ± 29	0.079
LDL-cholesterol	83 ± 38	65 ± 27	0.11
HDL-cholesterol	53 ± 25	50 ± 15	0.94
Diabetes mellitus N (%)	5, (23)	10, (45)	0.20
Smoker N (%)[a]	4, (18)	5, (23)	1.0
SBP	149 ± 22	148 ± 32	0.92
DBP	82 ± 19	78 ± 23	0.56

Values are expressed as mean ± SD.
[a]Any individual currently using tobacco was considered a smoker. There were no individuals with a remote tobacco history.

CEC number. One explanation for this disparity is that in the absence of an acute injury there is no increase in markers of inflammation detected in the serum. Perhaps in the chronic state, CEC number reflects the level of inflammation integrated over days-to-weeks and not one determination.

In the first cohort, we found a higher calcium phosphorous product in the higher CEC group. Although we are not aware of any published data demonstrating direct endothelial injury of an elevated calcium phosphorous product, considering its role in the pathogenesis of vascular calcifications observed in uremic patients, its association with a higher CEC number would seem reasonable. However, evaluating the two cohorts as a whole, we did not find a correlation between CEC number and calcium phosphorous product, perhaps emphasizing that CEC number likely reflects a large number of factors.

The investigation of cardiovascular disease in hemodialysis patients has found many markers that correlate with an increased risk of atherosclerotic events. Previously, blood pressure [12], low serum albumin [13], anemia [14], hs-CRP [15], and IL-6 [16, 17], among others [2, 18, 19], have been shown to be related to mortality. CEC may reflect a unique cardiovascular risk factor. We envision the endothelium to be an integrator of a variety of different influences. Forces such as changes in shear stress [20], elevated glucose [21, 22], and oxidative stress [23] tend to "activate" the endothelium, while elevated nitric oxide and heme oxygenase-1 levels may reflect the inherent ability of an individuals endothelial cell to resist that activation. CEC number may provide insight into the product of forces that activate the endothelium and the forces that act to resist that activation.

Just as CECs are an indicator of endothelial damage, endothelial progenitor cells have increasingly been viewed as a mechanism of endothelial repair (reviewed in [24, 25]). Endothelial progenitor cells originate from the bone marrow, and can differentiate into endothelial cells. These cells are thought to be important in processes such as vasculogenesis and endothelial repair (reviewed in

[24, 25]). Recently, endothelial progenitor cells have been shown to be inversely proportional to cardiovascular risk factors [26]. Consistent with this finding, hemodialysis patients have decreased endothelial progenitor number and function [27–29]. Thus, the marked vascular disease seen in hemodialysis patients is likely due to increased endothelial damage, represented by an elevated CEC number, as well as a decreased ability to repair the endothelial damage.

CONCLUSION

This study demonstrated that a high CEC number in hemodialysis patients may be an independent predictor of cardiovascular events. Since this is a small study, the findings need to be confirmed in larger prospective study in hemodialysis patients and in patients at high risk for vascular events but with normal renal function.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grant DK02537, Gatorade Research funds, and Baxter Renal Discovery Grant to M.S.S. M.K. is the recipient of an ISN/ASN Training Grant and Turkish Society of Nephrology fellowship support. The authors would like to thank the dialysis nurses for help collecting the specimens, and Dr. R.J. Johnson for critical reading of the manuscript.

Reprint requests to Dr. Mark S. Segal, P.O. Box 100224, Gainesville, FL 32610.
E-mail: segalms@medicine.ufl.edu

REFERENCES

1. Cheung AK, Sarnak MJ, Yan G, *et al*: Atherosclerotic cardiovascular disease risks in chronic hemodialysis patients. *Kidney Int* 58:353–362, 2000
2. Parfrey PS: Cardiac disease in dialysis patients: Diagnosis, burden of disease, prognosis, risk factors and management. *Nephrol Dial Transplant* 15:58–68, 2000
3. Ross R: Atherosclerosis—An inflammatory disease. *N Engl J Med* 340:115–126, 1999
4. Ross R: The pathogenesis of atherosclerosis: A perspective for the 1990s. *Nature* 362:801–809, 1993
5. Panza JA, Quyyumi AA, Brush JE, Jr., *et al*: Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 323:22–27, 1990
6. Higashi Y, Sasaki S, Nakagawa K, *et al*: A comparison of angiotensin-converting enzyme inhibitors, calcium antagonists, beta-blockers and diuretic agents on reactive hyperemia in patients with essential hypertension: A multicenter study. *J Am Coll Cardiol* 35:284–291, 2000
7. Koç M, Bihorac A, Segal M: Circulating endothelial cells as potential markers of the state of the endothelium in hemodialysis patients. *Am J Kidney Dis* 42:704–712, 2003
8. Solovey A, Lin Y, Browne P, *et al*: Circulating activated endothelial cells in sickle cell anemia. *N Engl J Med* 337:1584–1590, 1997
9. Mutin M, Canavy I, Blann A, *et al*: Direct evidence of endothelial injury in acute myocardial infarction and unstable angina by demonstration of circulating endothelial cells. *Blood* 93:2951–2958, 1999
10. Makin AJ, Blann AD, Chung NAY, *et al*: Assessment of endothelial damage in atherosclerotic vascular disease by quantification of circulating endothelial cells: Relationship with von Willebrand factor and tissue factor. *Eur Heart J* 25:371–376, 2004
11. Woywodt A, Streiber F, de Groot K, *et al*: Circulating endothelial cells as markers for ANCA-associated small-vessel vasculitis. *Lancet* 361:206–210, 2003
12. Foley RN, Parfrey PS, Harnett JD, *et al*: Impact of hypertension on cardiomyopathy, morbidity and mortality in end-stage renal disease. *Kidney Int* 49:1379–1385, 1996
13. Foley RN, Parfrey PS, Harnett JD, *et al*: Hypoalbuminemia, cardiac morbidity, and mortality in end-stage renal disease. *J Am Soc Nephrol* 7:728–736, 1996
14. Foley RN, Parfrey PS, Harnett JD, *et al*: The impact of anemia on cardiomyopathy, morbidity, and mortality in end-stage renal disease. *Am J Kidney Dis* 28:53–61, 1996
15. Zimmermann J, Herrlinger S, Pruy A, *et al*: Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. *Kidney Int* 55:648–658, 1999
16. Bologa RM, Levine DM, Parker TS, *et al*: Interleukin-6 predicts hypoalbuminemia, hypocholesterolemia, and mortality in hemodialysis patients. *Am J Kidney Dis* 32:107–114, 1998
17. Pecoits-Filho R, Barany P, Lindholm B, *et al*: Interleukin-6 is an independent predictor of mortality in patients starting dialysis treatment. *Nephrol Dial Transplant* 17:1684–1688, 2002
18. Stenvinkel P, Pecoits-Filho R, Lindholm B: Coronary artery disease in end-stage renal disease: No longer a simple plumbing problem. *J Am Soc Nephrol* 14:1927–1939, 2003
19. Himmelfarb J, Stenvinkel P, Ikizler TA, *et al*: The elephant in uremia: Oxidant stress as a unifying concept of cardiovascular disease in uremia. *Kidney Int* 62:1524–1538, 2002
20. Mohan S, Mohan N, Valente AJ, *et al*: Regulation of low shear flow-induced HAEC VCAM-1 expression and monocyte adhesion. *Am J Physiol* 276:C1100–C1107, 1999
21. Kado S, Wakatsuki T, Yamamoto M, *et al*: Expression of intercellular adhesion molecule-1 induced by high glucose concentrations in human aortic endothelial cells. *Life Sci* 68:727–737, 2001
22. Yngen M, Ostenson CG, Li N, *et al*: Acute hyperglycemia increases soluble P-selectin in male patients with mild diabetes mellitus. *Blood Coagul Fibrinolysis* 12:109–116, 2001
23. Annuk M, Zilmer M, Lind L, *et al*: Oxidative stress and endothelial function in chronic renal failure. *J Am Soc Nephrol* 12:2747–2752, 2001
24. Schatteman GC, Awad O: Hemangioblasts, angioblasts, and adult endothelial cell progenitors. *Anat Rec* 276A:13–21, 2004
25. Asahara T, Kawamoto A: Endothelial progenitor cells for postnatal vasculogenesis. *Am J Physiol Cell Physiol* 287:C572–579, 2004
26. Vasa M, Fichtlscherer S, Aicher A, *et al*: Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 89:E1–7, 2001
27. Choi J-H, Kim KL, Huh W, *et al*: Decreased number and impaired angiogenic function of endothelial progenitor cells in patients with chronic renal failure. *Arterioscler Thromb Vasc Biol* 24:1246–1252, 2004
28. Eizawa T, Murakami Y, Matsui K, *et al*: Circulating endothelial progenitor cells are reduced in hemodialysis patients. *Curr Med Res Opin* 19:627–633, 2003
29. de Groot K, Hermann Bahlmann F, Sowa J, *et al*: Uremia causes endothelial progenitor cell deficiency. *Kidney Int* 66:641–646, 2004