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

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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.

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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