Predictors of low circulating endothelial progenitor cell numbers in haemodialysis patients

Georg Schlieper¹*, Mihail Hristov²*, Vincent Brandenburg¹, Thilo Krüger¹, Ralf Westenfeld¹, Andreas H. Mahnken³, Eray Yagmur⁴, Georg Boecker⁵, Nicole Heussen⁶, Ulrich Gladziwa⁵, Markus Ketteler¹, Christian Weber² and Jürgen Floege¹

¹Department of Nephrology and Clinical Immunology, ²Institute for Molecular Cardiovascular Research (IMCAR), ³Department of Diagnostic Radiology, ⁴Central Laboratory, RWTH University Hospital Aachen, ⁵KfH Dialysis Center, Würselen, ⁶Institute of Medical Statistics, RWTH Aachen, ⁷Department of Nephrology, University Witten/Herdecke and ⁸Nephrologische Klinik, Klinikum Coburg, Coburg, Germany

Abstract

Background. End-stage renal disease (ESRD) patients exhibit increased cardiovascular mortality associated with cardiovascular calcifications and endothelial dysfunction. As circulating endothelial progenitor cells (EPCs) harbour vascular regenerative potential and are altered in uraemia, we examined clinical and biochemical factors influencing EPC levels as well as the relation between EPC numbers and function and uraemic cardiovascular calcifications.

Methods. Sixty-five haemodialysis patients were investigated. Cardiovascular calcifications were assessed by multi-slice spiral CT (MSCT, n = 44) with the calculation of coronary Agatston scores and indirectly by carotid-femoral pulse wave velocity (PWV, n = 61). EPCs were quantified in peripheral blood (CD34+/KDR+) and at day 7 after ex vivo cultivation (ac-LDL+/lectin+) by flow cytometry. In addition, colony-forming units (CFUs), migratory activity, adhesion and viability of isolated EPCs were analysed.

Results. EPC numbers were reduced (P < 0.001) compared to 27 healthy controls (−64%) or 81 patients with documented coronary artery disease and normal renal function (−58%). Coronary calcifications did not exhibit a significant association with the numbers of circulating CD34+/KDR+ or isolated ac-LDL+/lectin+ EPCs. No difference in EPC functions was observed between the 10 patients with the lowest Agatston scores (range 0–41) versus those with the highest scores (range 1181–3736). Multivariate analysis revealed low fetuin-A serum levels to be a positive predictor, while haematocrit and reticulocytes were negative predictors of reduced ac-LDL+/lectin+ EPC numbers.

Conclusions. EPC numbers and function did not correlate with the degree of coronary calcifications in haemodialysis patients. Rather they appear to be related to serum fetuin-A levels, haematocrit and reticulocytes.

Keywords: calcification; cardiovascular disease; chronic haemodialysis; endothelial progenitor cells; fetuin-A

Introduction

Dialysis patients exhibit a dramatically increased cardiovascular mortality when compared to the normal population [1]. Both vascular and valvular calcifications are important predictors of mortality in end-stage renal disease (ESRD) patients on haemodialysis [2,3]. Uraemia-associated risk factors like chronic inflammation, hyperphosphataemia and an increased calcium phosphate product contribute to progressive vascular calcifications in patients on renal replacement therapy [4–7]. However, these factors, as well as the traditional Framingham risk factors, can only partly explain the increased vascular disease burden of patients with chronic kidney disease (CKD).

Profound endothelial dysfunction is another prominent pathologic feature of uraemia [8,9]. Since the discovery of circulating endothelial progenitor cells (EPCs) in 1997 [10], these cells have been shown to effectively home to sites of endothelial damage or dysfunction and to areas of new blood vessel formation [11–15]. In general, EPCs have been proposed to display vasculoprotective properties, while their depletion may predict cardiovascular risk and events in patients with normal renal function [16–18]. Nonetheless, the exact role and regulation of EPCs in patients with coronary artery disease (CAD) remain controversial [19,20]. Whereas most but not all studies showed reduced EPC numbers in patients with CKD, the EPC function was consistently impaired in uraemia [21,22],...
and reduced levels of EPCs in uraemia were associated with a history of cardiovascular disease [23].

We raised the hypothesis that an intact endothelium or endothelial repair mechanisms mediated by EPCs are required to protect from progressive vascular calcifications in ESRD patients. We therefore specifically explored whether EPC number and functions correlate with the degree of vascular calcification in patients with uraemia and on chronic haemodialysis (CKD stage 5D) and could serve as surrogate markers predicting vascular dysfunction. The two main objectives of this study were to test (1) whether circulating and/or isolated EPCs were reduced or compromised in haemodialysis patients with arterial stiffening or with excessive coronary calcifications and (2) which clinical and biochemical parameters were related to EPC numbers in haemodialysis patients.

Subjects and methods

Patients

We studied 65 haemodialysis patients from four different dialysis centres. Patients were enrolled between October 2005 and August 2006. Characteristics of patients and control groups are depicted in Table 1. Patients with CAD and normal renal function had an angiographically documented 1-, 2- or 3-vessel disease. A healthy control group was defined as patients with the same age but normal renal function and where CAD had been excluded by angiography. Normal renal function was defined as having a normal serum creatinine and an estimated glomerular filtration rate (MDRD) >90 mL/min. The study protocol was approved by the Ethics Committee of the RWTH University Hospital Aachen and the German Federal Office for Radiation Protection, and each patient gave a written informed consent.

Isolation, culture and quantification of EPCs from peripheral blood

A volume of 30 mL peripheral blood was collected in vials anti-coagulated with 3 mL sodium citrate 3.13% after a long dialysis interval just before haemodialysis commenced. Direct measurement of CD34+/KDR+ peripheral blood cells by flow cytometry (FACS Calibur, Becton Dickinson, Franklin Lakes, USA) was performed as described [24] using direct-conjugated anti-CD34-FITC (BD Biosciences, Franklin Lakes, USA) and anti-KDR-PE (R&D Systems, Minneapolis, USA) antibodies and their respective isotype controls. In addition, remaining mononuclear cells were separated by Biocoll (Biochrom, Berlin, Germany) density gradient centrifugation and cultured on fibronectin-coated dishes in endothelial cell growth medium MCV 2 (PromoCell, Heidelberg, Germany) and treated with 0.25% trypsin for detachment. Calcein-AM (Molecular Probes, Eugene, USA) was performed. The percentage of ac-LDL+/lectin+ EPCs among all adherent cells was determined after detachment and presented as absolute EPC number per square centimetre.

Functional characterization of isolated EPCs: migration, adhesion and viability

In two subgroups of patients, namely the 10 patients with the lowest Agatston scores (range 0–41) versus 11 with very high scores (range 1181–3736), we performed a functional analysis of isolated EPCs. Transmigration was analysed using Falcon HTS FluoroBlokTM inserts (Becton Dickinson) with 8.0 µm pore size. EPCs were stained with calcein-AM (Molecular Probes-Invitrogen, Eugene, USA) and 5.0 × 10⁴ cells were plated in fibronectin-coated filter inserts in medium 199 (Gibco-Invitrogen, Karlsruhe, Germany) supplemented with 0.5% bovine serum albumin (BSA). The inserts were placed into wells of a 24-well plate, containing 100 ng/mL human recombinant SDF-1α (R&D Systems, Minneapolis, USA) in medium 199 plus 0.5% BSA. After 3 h, the fluorescence of cells migrated to the bottom surface of the filter membrane was measured using an ELISA reader.

For assessing the static adhesion, calcein-labelled EPCs (10⁴ cells/well) were plated on fibronectin-coated 96-well plates. After 1 h of incubation, cells were washed twice with PBS and the fluorescence intensity of adherent cells was measured.

Cell viability was determined using a specific CellTiter-Blue™ viability assay kit (Promega, Mannheim, Germany) after incubating the EPCs (10⁴ cells/well in a 96-well plate) with the CellTiter-Blue reagent for 3 h and subsequent measurement of fluorescence intensity.

Assessment of clinical parameters and measures of calcification

To determine functional parameters, we measured the systolic and diastolic blood pressure, and pulse pressure (mean value). The Compior SP system (Artech Medical, Pantin, France) was used to assess the pulse wave velocity (PWV). The PWV was measured in 61 of 65 patients. In four patients the measurement was not possible due to irregular heart rhythm (atrial fibrillation). PWV measurement was performed utilizing two electrodes (one carotid, one femoral) simultaneously to determine the velocity of the pulse in relation to the distance between femoral artery and suprasternal notch. One single observer performed two double measurements within 2 weeks. The intra-observer variability determined by six repeated measurements on 10 patients was 6%.

In order to determine coronary calcification, retrospectively, ECG-gated multislice-spiral CT (MSCT; SOMATOM Sensation 16; Siemens, Forchheim, Germany) was performed in 44 patients (61.8 ± 14.7 years, 22 males, 23 females). Twenty-one patients did not give their consent to CT scanning. A standardized scan protocol with 16 × 1.5 mm collimation, a tube voltage of 120 kV and an effective tube current time product of 150 mAseff was applied. The coronary Agatston score was calculated from images.
with a slice thickness of 3 mm and a reconstruction increment of 2 mm reconstructed at 60% of the RR interval (CalciumScore CT, version VA 60, Siemens).

Blood biochemistry

Blood was drawn from the arterial site after a long dialysis interval just before dialysis commenced. Biochemical analysis of serum risk factors (protein, albumin, HbA1c, calcium, phosphate, iPTH, triglycerides, cholesterol, LDL, HDL, erythrocytes, haematocrit, leukocytes, platelets, haemoglobin, mean corpuscular haemoglobin, reticulocytes, iron, ferritin, transferrin, erythropoietin levels) was performed by standard laboratory procedures using an automatic analyser. Serum analysis for hsCRP was performed by particle-enhanced immunonephelometry using a standard ‘CardioPhase hsCRP’ for ‘BNII’ (Dade Behring Holding GmbH, Liederbach, Germany). The nephelometric method for fetuin-A serum was adopted from a serum lectin method for human serum [26].

Statistics

Continuous variables were summarised by means and corresponding standard deviations (mean ± SD), categorical variables by relative frequencies. To compare EPC numbers between patients on haemodialysis and controls or patients with angiographically documented CAD, multivariate analysis of variance (ANOVA) was used with age, BMI, cholesterol, diabetes, gender, hypertension, smoking and dosage of statin as potentially confounding factors. All continuous variables were categorised. To describe the correlation between CD34+/KDR+ or ac-LDL+/lectin+EPCs and Agatston scores, log values of Agatston scores [=log(Agatston score + 1)] and PWV, respectively, Pearson’s correlation coefficient (r) was calculated. In addition to correlations, patients were grouped according to their degree of Agatston score (0, 1–100, 101–400, >401) for the final multivariate model. Univariate and multivariate analyses are based on 40 patients with measurements of both PWV and Agatston score. The global significance level for all statistical tests carried out was chosen to be 5%. As all statistical tests were conducted solely in an exploratory manner, no α-adjustment for multiple testing was carried out. Thus, P-values of ≤0.05 could be interpreted as statistically significant test results. All statistical analyses were performed using SAS Release 9.1.3 (SAS Institute Inc., Cary, NC, USA).

Results

The clinical characteristics of the haemodialysis patients are described in Tables 1 and 2. The mean dialysis vintage was 6.4 ± 6.0 years (median 4.5 years). Circulating CD34+/KDR+ EPCs in haemodialysis patients (n = 65) represented a small fraction of 0.13 ± 0.02% within the lymphocyte gate as revealed by the direct analysis of freshly collected peripheral blood using flow cytometry (Figure 1A). Culturing of isolated mononuclear cells under endothelial-specific conditions for 7 days identified 52.0 ± 2.8% of these cells as being ac-LDL+/lectin+EPCs by flow cytometry, defined as ‘early outgrowth’ EPCs (Figure 1B).

Patients on haemodialysis displayed a significant reduction in absolute EPC numbers (P < 0.0001) when compared to control subjects (n = 27) without CAD or to patients (n = 81) with angiographically documented CAD but normal renal function, independent of other cardiovascular risk factors as analysed by multivariate analysis (Table 1, Figure 2).

Table 1. Baseline characteristics of the study and control population (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CAD</th>
<th>ESRD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>27</td>
<td>81</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>66 ± 11</td>
<td>65 ± 10</td>
<td>66 ± 15</td>
<td>0.23</td>
</tr>
<tr>
<td>Male gender</td>
<td>18 (67%)</td>
<td>55 (68%)</td>
<td>32 (49%)</td>
<td>0.65</td>
</tr>
<tr>
<td>BMI &gt; 25 kg/m²</td>
<td>18 (67%)</td>
<td>59 (73%)</td>
<td>30 (46%)</td>
<td>0.78</td>
</tr>
<tr>
<td>History of smoking</td>
<td>9 (33%)</td>
<td>28 (35%)</td>
<td>10 (15%)</td>
<td>0.59</td>
</tr>
<tr>
<td>Hypertension</td>
<td>12 (44%)</td>
<td>60 (74%)</td>
<td>60 (92%)</td>
<td>0.58</td>
</tr>
<tr>
<td>Diabetes</td>
<td>4 (15%)</td>
<td>35 (43%)</td>
<td>21 (33%)</td>
<td>0.37</td>
</tr>
<tr>
<td>Cholesterol &gt; 200 mg/dL</td>
<td>13 (48%)</td>
<td>34 (42%)</td>
<td>14 (22%)</td>
<td>0.82</td>
</tr>
<tr>
<td>Statin therapy</td>
<td>11 (41%)</td>
<td>67 (83%)</td>
<td>22 (34%)</td>
<td>0.90</td>
</tr>
<tr>
<td>EPCs/cm²</td>
<td>28 115 ± 15 475</td>
<td>23 942 ± 13 315</td>
<td>10 857 ± 7922</td>
<td>&lt;0.0001***</td>
</tr>
</tbody>
</table>

Control = healthy control with normal renal function; CAD = patients with coronary artery disease and normal renal function; ESRD = patients with end-stage renal disease on haemodialysis. ***P < 0.0001.
Fig. 1. Analysis by flow cytometry of circulating CD34⁺/KDR⁺ (A) and cultured Dil-ac-LDL⁺/lectin-FITC⁺ (B) human EPCs. Left panels: representative FSC/SSC dot plots of lysed whole blood and cultured EPCs at day 7 after the isolation. Middle panels: respective isotype control antibodies and unstained cells. Right panels: specific fluorescence staining.

Fig. 2. Absolute number of isolated ac-LDL⁺/lectin⁺ EPCs at Day 7 in healthy control subjects (n = 27), patients with coronary artery disease (CAD, angiographically documented 1-, 2- or 3- vessel CAD, n = 27 per each group) but normal renal function, and haemodialysis patients with end-stage renal disease, ESRD (n = 65). ***P < 0.0001 (ESRD versus control and CAD, respectively).

Haemodialysis patients showed a wide range of coronary Agatston scores between 0 and 4920 (mean value 926 ± 1155, median 461). Values obtained for PWV ranged from 5.8 to 15.8 m/s (mean 9.4 ± 2.5 m/s). We could not detect a significant correlation between circulating CD34⁺/KDR⁺ EPCs and coronary Agatston scores (Figure 3, top) or log-values of Agatston scores (r = −0.05; P = 0.75), respectively. The number of isolated ac-LDL⁺/lectin⁺ EPCs also did not show a significant correlation with coronary calcification scores (Figure 3, bottom) or log-values of Agatston scores (r = 0.05; P = 0.74), respectively. EPC levels (CD34⁺/KDR⁺ and ac-LDL⁺/lectin⁺) did not differ significantly when patients were grouped according

Table 2. Serum and blood parameters of the 65 ESRD patients (mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
</tr>
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<tbody>
<tr>
<td>Serum protein (g/L)</td>
<td>67.7 ± 4.8</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>39.8 ± 2.8</td>
</tr>
<tr>
<td>Highly sensitive C-reactive protein (mg/L)</td>
<td>11.5 ± 22.8 (median 5.1)</td>
</tr>
<tr>
<td>Serum fetuin-A (g/L)</td>
<td>0.59 ± 0.15</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>111 ± 11</td>
</tr>
<tr>
<td>Reticulocytes (g/L)</td>
<td>58.7 ± 21.3</td>
</tr>
<tr>
<td>Iron (µmol/L)</td>
<td>11.1 ± 4.4</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>465 ± 271</td>
</tr>
<tr>
<td>Transferrin (g/L)</td>
<td>1.82 ± 0.39</td>
</tr>
<tr>
<td>Erythropoietin (U/L)</td>
<td>17.2 ± 23.7 (median 10.0)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.4 ± 0.8</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.32 ± 0.17</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.77 ± 0.56</td>
</tr>
<tr>
<td>Ca × PO₄ product (mmol²/L²)</td>
<td>4.1 ± 1.29</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>247 ± 280 (median 143)</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>174 ± 107</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>171 ± 59</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>47 ± 15</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>110 ± 49</td>
</tr>
</tbody>
</table>

Table: |
Predictors of low circulating EPC numbers

Fig. 3. Percentage of circulating CD34+/KDR+ EPCs and absolute numbers of cultured ac-LDL+/lectin+ EPCs at Day 7 in relation to the coronary Agatston score, respectively (n = 44).

Fig. 4. Percentage of circulating CD34+/KDR+ EPCs and absolute numbers of cultured ac-LDL+/lectin+ EPCs at Day 7 in relation to pulse wave velocity (PWV), respectively (n = 61).

to their level of coronary calcification (Agatston scores: 0, 1–100, 101–400, >400; P = 0.40 and P = 0.55 for CD34+/KDR+ and ac-LDL+/lectin+, respectively). When correlating CD34+/KDR+ and ac-LDL+/lectin+ EPCs to PWV, we could not detect a significant correlation between these two parameters (Figure 4). Patients with high Agatston scores and low PWV that might represent a subgroup of patients with intima rather than media calcification did not show altered levels of CD34+/KDR+ and ac-LDL+/lectin+ EPC numbers when compared to the other patients of our study (data not shown).

In order to analyse the clonogenic potential of EPCs, we further investigated whether cultured EPCs of haemodialysis patients exhibited the capacity to develop CFUs. Notably, only 18 of 65 haemodialysis patients (28%) demonstrated the ability to form EPC-CFUs. However, when comparing patients with and without the potential to develop EPC-CFUs, no differences in PWV values (9.50 ± 2.28 m/s versus 9.16 ± 3.10 m/s; P = 0.12) or Agatston score (908 ± 1163 and 982 ± 1186; P = 0.86) could be detected.

Next, we compared functional characteristics of cultured EPCs between subgroups, namely the 10 patients with the lowest Agatston scores (0–41, mean age 53 ± 20 years, four males) versus 11 patients with the highest Agatston scores (1181–3736, 64 ± 13 years, nine males). EPC functions, as assessed by migratory capacity towards an SDF-1α gradient (164 ± 196 versus 102 ± 64; P = 0.33), adhesion on a fibronectin matrix (17 ± 24 versus 31 ± 44; P = 0.40) and cell viability (24 ± 9 versus 32 ± 27; P = 0.35, all arbitrary fluorescence units) did not differ significantly between these two groups.

The univariate analysis failed to reveal a significant association of EPC numbers with classical risk factors for calcification (including diabetes) or serum risk markers of uraemia-associated calcification (including hsCRP, data not shown). To exclude that individual predictors were masked by independent variables in a univariate model we performed a multivariate analysis. Notably, the multivariate model identified serum fetuin-A levels as an independent positive predictor (P = 0.0365) for numbers of ac-LDL+/lectin+ EPCs while haematocrit and reticulocytes were negative predictors for ac-LDL+/lectin+ EPC numbers (P = 0.0376 and P = 0.0476, respectively; Table 3). CD34+/KDR+ EPCs were only influenced by serum protein levels but not by other independent predictors as assessed by multivariate analysis (data not shown). Interestingly, EPC numbers were not affected by serum levels of erythropoietin or the weekly dosage of erythropoietin in our study.
Discussion

In this study, we have investigated numbers and functional characteristics of circulating and isolated EPCs of chronic haemodialysis patients in relation to coronary calcification (Agatston scores) and PWV values and analysed clinical and biochemical risk factors related to EPCs. We first confirmed that Agatston scores and PWV were increased in CKD patients due to mediasclerosis and accelerated calcification [27].

Since the identification of bone marrow-derived EPCs [10] many animal studies have shown that these cells can integrate into new and existing blood vessels with a positive impact on neovascularization, e.g. by intimal lesion repair [11–13]. In humans low levels of circulating EPCs were associated with Framingham risk factors [16] and with increased cardiovascular events [18]. To date, only a few studies have investigated EPCs in the context of ESRD.

We found reduced EPC numbers in haemodialysis patients as compared to healthy controls or patients with documented CAD. This finding is in line with other studies in ESRD patients, which mostly revealed reduced levels of EPCs, possibly due to a deficiency in erythropoietin [22,28,29]. These patients also displayed impaired EPC functions [21,22]. In contrast, two other studies showed slightly increased or similar levels of EPCs in uraemic patients, compared to patients with normal renal function [21,23]. Differences in EPC quantification methods and/or erythropoietin dosage may account for the inconsistent findings. In this context, it should be noted that the characterization of cultured EPCs is somewhat limited as colony-forming units may also reflect other cell populations like myeloid cells and as ac-LDL+/lectin+ cells may also bear monocytic features. A recent study in peritoneal dialysis patients demonstrated that a history of cardiovascular disease was associated with reduced EPC numbers [23]. In this concept, it appears possible that an intact endothelium provides an efficient barrier protecting from calcification processes within the arterial wall, in particular the vascular intima. However no study assessed the relation between measures of calcification and EPC numbers or functions in uraemic patients so far.

The first major finding of the present study was that EPC numbers and functions were significantly reduced in dialysis patients but not associated with parameters of vascular damage, such as increased PWV or the extent of coronary calcifications. Moreover, even patients with a severe degree of coronary calcification did not show any significant impairment in EPC function (clonogenic potential, adhesion, migration, viability) when compared to patients with a lower degree of calcification. Recent studies in non-uraemic patients also failed to detect a significant association between total EPCs and coronary atheroma category (i.e. history of CAD or calcification score ≥ 400 HU) [30] or calcification [20,24]. Another recent study in non-uraemic patients noted a significant reduction of EPCs only in the presence of carotid plaque formation but not for changes in other vascular sites (femoral artery, aorta), when adjusted for cardiovascular risk factors [31]. A previous study of EPCs in peritoneal dialysis patients showing an association between the history of vascular disease and EPC numbers failed to show a correlation of EPC with other cardiovascular risk factors or endothelial (dys-)function [23]. From these and our results it might be concluded that EPCs do not seem to play a major pathomechanistic role in the development of uraemic calcifications.

It is important to note that calcification in ESRD patients can take place both in the intima (i.e. atherosclerosis) and in the media (i.e. arteriosclerosis) of the vessel wall [2,32]. An increased carotid-femoral PWV (i.e. arterial stiffening) is associated with mediasclerosis while coronary Agatston scores reflect intimal and medial calcification and do not allow distinguishing between calcification processes of the media and the intima. Both PWV and Agatston scores were not related to EPC numbers in our study. While medial calcifications do not seem to be associated with EPCs in our study, we cannot exclude that an association between intimal calcification and EPC numbers might have been overlooked as we could not assess intimal calcification selectively. Nevertheless, patients with high Agatston scores and low PWV in our study should reflect patients with intimal rather than medial calcifications and these patients also did not exhibit altered EPC levels.

One reason for the lack of association between calcification and EPCs in our cohort could be that a subgroup of patients might have exhibited increased EPC levels in response to subacute clinical events and thereby blunted any statistical association between low EPC numbers and vascular wall changes. It is known that acute events like myocardial infarction are associated with increased EPC levels in patients with normal renal function [33,34]. Similarly some CKD patients with more severe calcifications might display sub-clinical myocardial stress. However, we found no evidence to support this hypothesis as hsCRP levels did not correlate with the extent of calcification or EPC numbers in our study. Moreover, our patients were investigated under stable conditions and no acute events occurred clinically.

Another major aim of our study was to investigate other clinical or laboratory parameters that might relate to circulating EPC numbers. In these analyses we noted that serum levels of the calcification inhibitor fetuin-A were an independent positive predictor of ac-LDL+/lectin+ EPC numbers. Two recent studies favour a protective role of fetuin-A for the endothelium in uraemic patients [35,36]. As serum fetuin-A is a negative acute-phase protein [37] a potential link between EPCs and fetuin-A might be inflammation. EPC levels can be down- or upregulated in experimental settings of inflammation and clinical sepsis, respectively [38,39]. However, we could not detect a significant
association between EPCs and CRP serum levels in our study, potentially suggesting that fetuin-A levels can be affected by non-inflammatory changes as well.

Other factors influencing EPC levels in our study were haematocrit and reticulocyte counts, both of which were negative predictors of ac-LDL"/lectin" EPC numbers. It was recently shown that erythropoietin could lead to increases in EPC numbers and function [40]. A recent study administered 5000 units of erythropoietin per week to dialysis patients for 8 weeks and found that EPC levels increased by 200% while haematocrit rose by 17% [40]. However, despite the negative association of EPC numbers and haematocrit and reticulocyte counts, no correlation between EPC counts and erythropoietin levels or dosage was detected in our study. One explanation could be that EPC and red blood cell numbers might be regulated by independent mechanisms and/or different erythropoietin blood concentrations. However, our finding is remarkable in the context of recent studies showing no survival benefit of higher target haemoglobin levels in CKD patients [41,42]. Our study thereby raises the possibility that high haemoglobin levels might exert a negative effect in CKD patients by inducing a reduction of circulating EPC numbers.

Not only erythropoietin but also ACE inhibitors, statins, PPAR-gamma agonists or exercise exert a potential benefit on EPC biology in non-uraemic patients [14,43–45]. However, in dialysis patients with advanced diabetes mellitus and thus with extremely advanced vascular disease statins did not lead to improvements in mortality in the 4D study [46]. In such cases with advanced vascular disease statins may be less beneficial with regard to EPC modulation. Interestingly, other modalities to alleviate uraemia, such as nocturnal haemodialysis or kidney transplantation, are associated with increased EPC levels and improved EPC function, respectively [29,47]. Whether modulation of EPCs leads to increased survival in uraemic patients needs to be addressed in large outcome studies.

In conclusion, we could not detect a significant association of EPC number and function with the degree of coronary calcification in our cohort of haemodialysis patients. Thus, EPC do not serve as a ‘biomarker’ for the degree of vascular calcification in patients with ESRD. Rather we identified new predictors for EPC numbers in haemodialysis patients including serum fetuin-A, haematocrit and reticulocyte counts.

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