T-Cell Activation and Malnutrition Adversely Impact on Endothelial Progenitor Cell Mobilization in Patients on Extracorporeal Maintenance Dialysis Therapy

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Key Words
End-stage renal disease · Endothelial progenitor cells · Haemodialysis · Haemodiafiltration · Malnutrition · T-cell activation

Abstract
Background: The number of circulating endothelial progenitor cells (EPCs) decreases on account of chronic kidney disease (CKD). ***Methods:*** Twenty patients were enrolled in this prospective and randomised study in two parallel arms: conventional haemodialysis versus online haemodiafiltration. EPCs number and T-cell activation were analysed at baseline and monthly during a 4-month period of follow-up. ***Results:*** $CD38^{bright}$ and HLA-DR+ expression among CD8 memory T cells were negatively associated with both CD34+ ($r = -0.70$, $p = 0.0006$) and CD34+ CD133+ ($r = -0.62$, $p = 0.004$) cell numbers. Conversely, a positive correlation was observed between CD34+ and CD34+ CD133+ cells with transferrin ($r = 0.75$, $p = 0.0001$ and $r = 0.47$, $p = 0.04$, respectively), and CD34+ CD133+ cells with transthyretin ($r = 0.51$, $p = 0.02$). No significant association was observed between dialysis modality and the evolution of the EPC number. ***Conclusions:*** Chronic T-cell activation may be a component of the malnutrition inflammation complex syndrome that adversely influences EPC mobilization in CKD patients.

Background

Chronic kidney disease (CKD) patients requiring dialysis are known to have a dramatic increase of cardiovascular disease burden, which represents the principal cause of death in this population [1–3]. Endothelium damage is thought to be a key mechanism in the development of coronary and peripheral arterial diseases [4]. Impairment of circulating endothelial progenitor cells (EPCs) is probably a central factor associated with the endothelial dysfunction in these patients. Most of the studies have shown a decrease in the number of circulating EPCs in CKD or an imbalance between circulating endothelial cells and the mobilization of EPCs [5–8]. In addition, alterations of EPC functions have been observed in CKD patients [9–

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11]. Such impairments may be an independent risk factor for cardiovascular events in dialysis patients [12].

Circulating EPCs are bone marrow-derived cells that represent a very small fraction of peripheral blood mononuclear cells (PBMC), estimated between 0.0001 and 0.01% as enumerated by flow cytometry [13]. The most appropriate identification of EPCs is still a subject of debate. The combined expression of CD34, CD133 and KDR (vascular endothelial growth factor receptor 2 (VEGFR2)) on CD45–/dim or CD3–, CD19– cells is currently considered the hallmark of circulating EPCs [14]. Among EPCs, CD34+, CD133+, KDR+ cells may be considered more immature EPCs than CD34+, CD133–, KDR+ cells [14–17]. CD34+, CD133+, KDR– circulating stem cells is another EPC subset that is sometimes called 'myeloid EPCs' or may be considered immature progenitors [18]. A capacity to secrete angiogenic factors to stimulate the resident endothelium and to enhance endothelial repair has been described for this last subset of EPCs [19–21].

Biological factors influencing the level of circulating EPCs in dialysis patients remain largely unidentified. Uremic toxins, oxidative stress and inflammation which contribute to uremia-induced arteriolopathy and are closely linked to the malnutrition inflammation complex syndrome [22, 23], might act, at least partially, by causing accelerated senescence and apoptosis of mature endothelial cells and depletion of EPCs. Hence, the impact of non-traditional risk factors such as inflammation, malnutrition and PBMC activation on circulating EPC levels are likely to be important. Erythropoietin supplementation [24, 25] and kidney transplantation [26, 27] may improve EPC mobilization. The dialysis modality may also notably impact on EPC functions and number either adversely via activation of PBMC due to possible bioincompatibility of dialysis procedure (membrane and dialysate purity interaction) or positively via effectiveness of the dialysis method. Hence, some authors have suggested that more frequent haemodialysis (HD) sequences, increased dialysis efficiency and a broader spectrum of uremic toxins removal may restore the function and mobilization of EPCs [5, 28]. Since the levels of uraemic toxins, particularly beta2-microglobulin (β2-M), have been reported as inversely correlated with EPC number [7, 18], it is tempting to hypothesize that change in the dialysis method may ameliorate EPC impairments and therefore improve cardiovascular outcome. Indeed, evidence for a better removal of middle molecular weight uremic toxins and reduced inflammatory profile has been reported with online haemodiafiltration (HDF) by comparison with conventional HD [29].

This study was designed to explore the relationship between associations of EPC number with T cells activation alongside markers of inflammation and malnutrition in CKD stage 5D patients. The potential impact of dialysis modality (conventional high-flux HD versus online HDF) on the evolution of these parameters was also studied prospectively over a four-month period.

Patients and Methods

Study Design

This is a prospective, randomised and comparative study in two parallel arms, conventional HD versus online HDF. Design of the study is described in figure 1. Briefly, after a one-month washout period with HD mode, patients were randomly assigned to conventional HD (n = 10) or online HDF (n = 10) for 4 months. All the patients were dialysed with the same dialyzer, Elisio™ 210H (containing the Polynephron™ membrane based on polyethersulfone polymer, effective membrane surface area: 2.1 m^2, Nipro Europe N.V., Zaventem, Belgium) and benefited from ultrapure dialysis fluid for the entire period of the study.

Dialysis conditions remained unchanged for each patient: 3 sessions/week, 3 to 4 hours/session, with a blood flow (QB) of 350–400 ml/min, ultrapure bicarbonate buffered dialysate, dialysate flow (QD) of 500 ml/min. During the entire study, all patients were receiving erythropoietin therapy, which was adapted according to AFFSAPS recommendations.

Ethical Approval

The study was approved by our institution ethical committee and registered (AFSAPPS 2008-A00852–53, and NCT01653808). Written informed consent was obtained from all patients participating in the study.

Study Population

Twenty-stage, 5D kidney disease prevalent stable patients undergoing maintenance HD in one Montpellier dialysis facility were enrolled in this study, age (median): 66.8 (26.9–85.65); BMI: 24.97 kg/m^2 (16.89–37.57). Causes of CKD were glomerulonephritis (n = 1), cystic renal disease (n = 2), diabetic nephropathy (n = 2), diabetic and hypertensive nephropathy (n = 1), angiosclerosis and hypertensive nephropathy (n = 3), infectious/obstructive interstitial nephropathy (n = 1), genetic/congenital cause (n = 3), unknown cause (n = 2), other causes (n = 5). Vitamin D supplement was administered to 17 (85.0%) patients and 7 (35%) patients received calcium supplements. Erythropoiesis-stimulating agents were administered to all patients with a median dose of 57.1 (14.9–438.0) IU/kg/week. All subjects had a haemoglobin level >10.5 g/dl and vascular access allowing a stable blood flow of 300 ml/min during treatment and did not suffer from malignancy, chronic inflammatory or infectious diseases.

Laboratory Parameters

Serum creatinine and urea were measured using the enzymatic method (Olympus apparatus, Rungis, France). β2-M was determined by immunoturbidimetry method (Olympus apparatus, Rungis, France), and its removal rate (RR) (as a dialysis efficacy parameter) was calculated. Inflammation was estimated by high

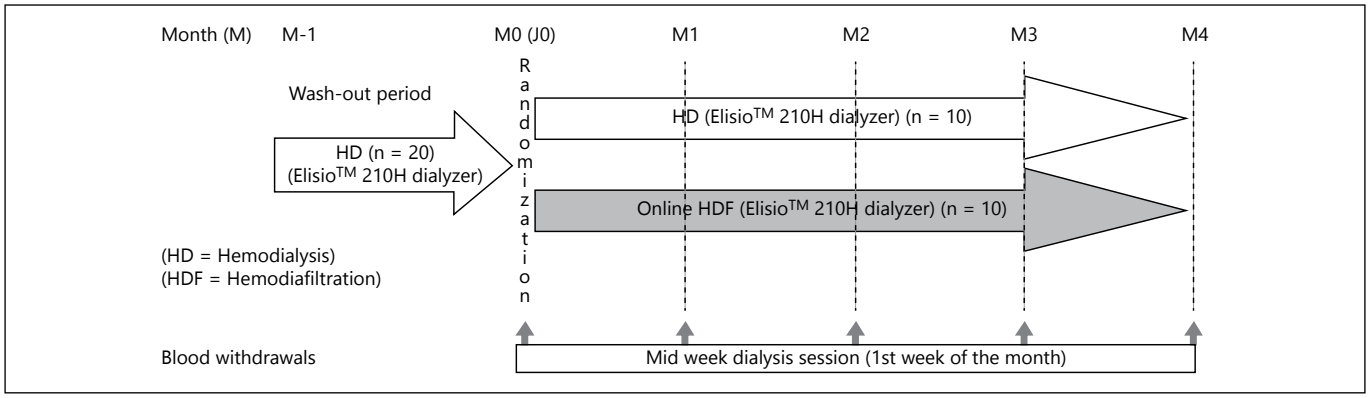

Fig. 1. Design of the study. After a one-month washout period with HD mode, patients were randomly assigned to two treatment groups, conventional HD (n = 10) and online HDF (n = 10), for 4 months. All the patients were dialysed with the same dialyzer, Elisio™ 210H (containing the Polynephron™ membrane based on polyethersulfone polymer, effective membrane surface area: 2.1 m^2) for the entire study.

sensitive CRP (Immunoturbidimetry, Olympus apparatus, Rungis, France), fibrinogen (Von Clauss method, STA Fibrinogen, Diagnostica Stago, Asnières, France) and serum interleukin 6 (IL-6) (Chemiluminescent immunoassay on an Access® 2 immunoassay system, Beckman Coulter, Brea, Calif., USA). Nutrition was assessed by transthyretin (Nephelometry technique, Immage Beckman Coulter, Villepinte, France), albumin, transferrin measurements, and orosomucoid (Immunoturbidimetry method, Olympus apparatus, Rungis, France). The nutritional prognostic inflammatory and nutritional index (PINI) [30] was calculated according to the following formula: orosomucoid (g/l) × CRP (mg/l)/albumin (g/l) × transthyretin (g/l). According to previous studies [31], PINI values <1 are considered to reflect a normal status, while PINI ≥1 to indicate a pathological status of malnutrition/inflammation.

Flow cytometry analysis of EPC number and T lymphocytes activation was performed on EDTA-treated fresh whole blood within 6 h of collection using a FC 500 apparatus and the CXP software (Beckmann Coulter, Miami, Fla., USA). Red blood cell lysis and cell fixation were achieved by means of an automated Multi-Q-Prep System using the ImmunoPrep Reagent System (Beckman Coulter, Miami, Fla., USA).

EPC analysis was performed on 300 µl of whole blood with the use of fluoresceine isothiocyanate (FITC)-conjugated anti-CD34 monoclonal antibody (mAb), phycoerythrine cyanine 5 (PC5)-conjugated anti-CD45 mAb (Beckman Coulter, Miami, Fla., USA), phycoerythrin-conjugated monoclonal anti-CD133 mAb (Miltenyi BiotecGmbH, Paris, France) and allophycocyanin (activated protein C)-conjugated anti-KDR mAb (R&D Systems, Minneapolis, Minn., USA). Cells with low cytoplasmic granularity, a low CD45 expression and positive for CD34 and CD133 or CD34, CD133, and KDR were considered EPCs.

Lymphocytes activation was analysed on CD4 and CD8 T cells subsets using 100 µl of whole blood. Cells were incubated with a combination of anti-CD45RO, anti-CD4, anti-CD8, anti-HLA-DR, and anti-CD38 mAbs (Beckmann Coulter, Miami, Fla., USA), conjugated, respectively with FITC, PC5, PC7, phycoerythrine (PE) and energy coupled dye (ECD). The $CD38^{bright}$ threshold corresponded to expression in greater than 8,500 CD38 binding sites per cells [32].

Statistical Analyses

Data were expressed as mean ± standard deviation (SD) for normally distributed variables and as median (min.–max.) for non-normally distributed variables.

Continuous variables were compared between genders using the Student t-test. They were normally distributed or had a log-normal distribution using Shapiro-Wilk test. Pearson's correlation coefficient was applied to measure associations between two continuous variables. Continuous variables with a log-normal distribution were log-transformed before testing, and back-transformed into natural values for presentation.

We also used a random-effect model to analyze the association between dialysis modality and 4-month change on EPCs (CD34+ CD133+) taken as continuous variables. The model included time, dialysis modality, and time/dialysis modality interaction, age and gender – two adjusted variables.

The term 'dialysis modality' represents the cross-sectional association between dialysis modality and EPCs at baseline. The term 'time' indicates the linear evolution of EPCs over time. The term 'interaction between time and dialysis modality' represents the additional monthly modification on the EPC slope.

Values were considered statistically significant at $p < 0.05$. All analyses were carried out with SAS software version 9.2 (SAS Institute, Cary, N.C., USA).

Results

Clinical and Biological Characteristics of the Patients at Baseline

The main baseline biological characteristics of the patients included in this prospective study were documented in table 1.

Concentrations of β2-M, CRP, IL-6 and percentage of $CD38^{bright}$ CD8 memory T cells are frequently high in CKD subjects, above the normal ranges for 100, 35, 45

Table 1. Biological characteristics of the population at baseline (pre-dialysis levels) (n = 20)

Variable	Normal range of concentration	Mean ± SD median (min.–max.)	% of patients below the normal range of concentration	% of patients above the normal range of concentration
Beta2-microglobulin, mg/l	0.8–2.4	26.59±5.93 27.16 (16.31–39.57)	0.0	100.0
C reactive protein, mg/l	0.3–5.0	5.40±4.04 3.85 (0.80–17.00)	0.0	35.0
Interleukin 6, pg/ml	for patients <50 years old (1.9–4.0)	6.27±4.53 5.74 (0.87–18.39)	25.0	50.0
	for patients ≥50 years old (4.1–6.5)		31.0	44.0
Fibrinogen, g/l	1.9–4.0	3.94±1.09 3.65 (2.30–6.20)	0.0	35.0
Transferrin, g/l	2.3–6.0	1.57±0.18 1.58 (1.27–1.87)	100.0	0.0
Lymphocytes count, /mm^3	1,200–4,000	1,246.88±319.30 1,287.00 (731.00–1,932.00)	47.0	0.0
Transthyretin, g/l	0.19–0.35	0.32±0.06 0.33 (0.21–0.43)	0.0	25.0
Erythropoietin resistance index, IU/kg/g Hb		2.99±0.99 2.68 (1.81–5.88)		
CD8+ CD45RO+ CD38^{bright}, %	<13	25.30±17.96 20.60 (2.40–59.50)	–	60.0
CD34+ CD133+, /ml		323.97±168.68 322.60 (60.30–691.50)		

and 60%, of patients, respectively. By contrast, low lymphocyte count and low transferrin concentration that are associated with poor nutritional status are observed below the normal range in 47 and 100% of subjects, respectively.

The median BMI of the patients was 25.0 (16.9–37.6) kg/m^2 with 2 patients between 16.0 and 18.5 kg/m^2 (underweight), 8 patients between 18.5 and 25.0 kg/m^2 (healthy weight), 4 patients between 25.0 and 30.0 kg/m^2 (overweight) and 6 patients between 30.0 and 35.0 kg/m^2 (obese class I). The median PINI index was 0.33 (0.06–1.93) with 16 (80%) patients presenting a PINI value <1 (normal nutritional status).

EPC Number, PBMC and T Lymphocytes Activation

The mean (SD) numbers of CD34+ cells, CD34+ CD133+ cells and CD34+ CD133+ KDR+ cells were 1,227.3 (680.05)/ml, 324.0 (168.7)/ml and 61.9 (46.28)/ml, respectively. The numbers of CD34+ cells and CD34+ CD133+ cells exhibit a good linear relationship (r = 0.72, p = 0.0003), while a non-significant trend was observed between CD34+ CD133+ double stained cells and CD34+ CD133+ and KDR triple stained cells (r = 0.39, p = 0.09), (data not shown).

Endothelial Progenitor Cells Are Negatively Associated with Cell Activation Markers

As shown in table 2 parameters reflecting cell activation were found to be negatively associated with EPC numbers at baseline. Hence, CD38^{bright} and HLA-DR+ expression among CD8 memory T cells were negatively associated with both CD34+ and CD34+ CD133+ cell numbers. A trend was observed between HLA-DR+ CD4 memory T cells and CD34+ cells. No significant association was found between EPCs and markers of inflammation in serum, but a trend was observed between IL-6 and CD34+ CD133+ cells (r = –40, p = 0.08).

Table 2. Associations between endothelial progenitor cells and other biological variables at baseline

	CD34+ (/ml)		CD34+ CD133+ (/ml)		CD34+ CD133+ KDR+ (/ml)	
	correlation coefficient or mean ± SD	p value	correlation coefficient or mean ± SD	p value	correlation coefficient or mean ± SD	p value
Gender						
Male (n = 11)	1,299.7±880.55[b]	0.60	346.1±193.7	0.53	50.13±32.88[b]	0.12
Female (n = 9)	1,144.2±466.15[b]		296.9±138.4		80.15±62.14[b]	
Age, years	–0.24	0.30	–0.38	**0.10**	–0.005	0.98
BMI, kg/m^2	0.03	0.90	–0.03	0.89	–0.08	0.74
Beta2-microglobulin, mg/l	–0.03	0.89	0.05	0.84	–0.31	0.19
C reactive protein, mg/l[a]	0.06	0.80	–0.13	0.59	0.02	0.93
Interleukin 6, pg/ml[a]	–0.27	0.25	–0.40	**0.08**	0.17	0.49
Fibrinogen, g/l	–0.05	0.83	–0.08	0.74	–0.10	0.68
Transferrin, g/l	0.75	**0.0001**	0.47	**0.04**	0.04	0.86
Lymphocytes count, /mm^3	0.30	0.25	0.33	0.20	0.15	0.58
Transthyretin, g/l	0.25	0.29	0.51	**0.02**	0.02	0.92
ERI, IU/kg/g Hb[a]	–0.18	0.45	–0.05	0.82	0.21	0.38
CD8+ CD45RO+ CD38^{bright}, %[a]	–0.70	**0.0006**	–0.62	**0.004**	–0.24	0.31
CD8+ CD45RO+ HLA-DR+, %[a]	–0.49	**0.03**	–0.55	**0.01**	–0.35	0.13
CD4+ CD45RO+ CD38^{bright}, %[a]	–0.38	0.10	–0.07	0.78	0.20	0.41
CD4+ CD45RO+ HLA-DR+, %[a]	–0.38	0.11	–0.13	0.60	0.06	0.80

[a] log-transformation applied; [b] geometric mean ± SD. ERI = Erythropoietin resistance index.

Endothelial Progenitor Cells Are Positively Associated with Nutritional Parameters

A positive correlation was observed between both CD34+ and CD34+ CD133+ cells with transferrin (r = 0.75, p = 0.0001, r = 0.47, p = 0.04, respectively). The concentration of transthyretin was also positively associated with the number of CD34+ CD133+ EPCs (r = 0.51, p = 0.02). Finally, we observed a negative association between CD38^{bright} CD8 memory T cells with transferrin at baseline (r = –0.50, p = 0.025) (see fig. 2) and a trend with transthyretin (r = –0.33, p = 0.15).

Based on these results, a specific profile may be established to distinguish CKD patients with EPC number below or above the median CD34+ CD133+ cells count. Patients with EPC number below the median CD34+ CD133+ cell count were generally older with higher levels of inflammation/cell activation markers and a worst nutritional status with lower levels of lymphocyte; transferrin and transthyretin (see fig. 3).

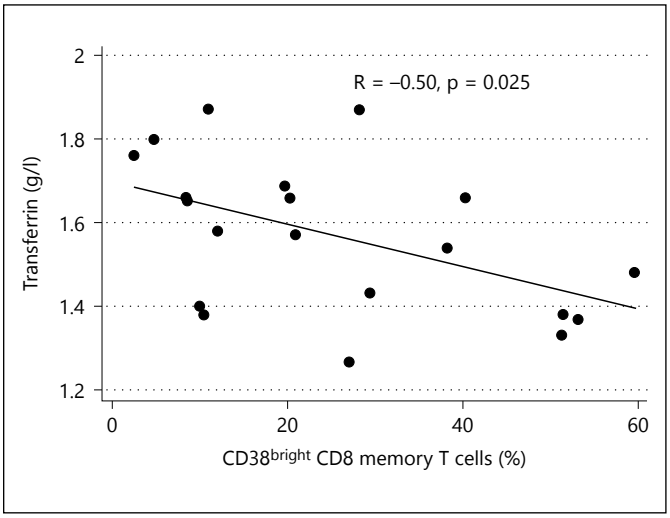

Fig. 2. Inverse correlations between CD38^{bright} CD8 memory T cells and transferrin at baseline.

Association between Dialysis Modality and CD34+ CD133+ Cells Over Time, Random-Effect Model

Three patients left the study before the end of the follow-up, two in the 'online HDF' group (one transplantation, one moving) after M1 visit, and one in the 'conventional HD' group (for personal decision), after M2 leaving 17 patients for the statistical analysis of the crossover study. Analyses of CD34+ CD133+ circulating cells were performed monthly over the follow-up period (see fig. 4). No significant association was found between dialysis

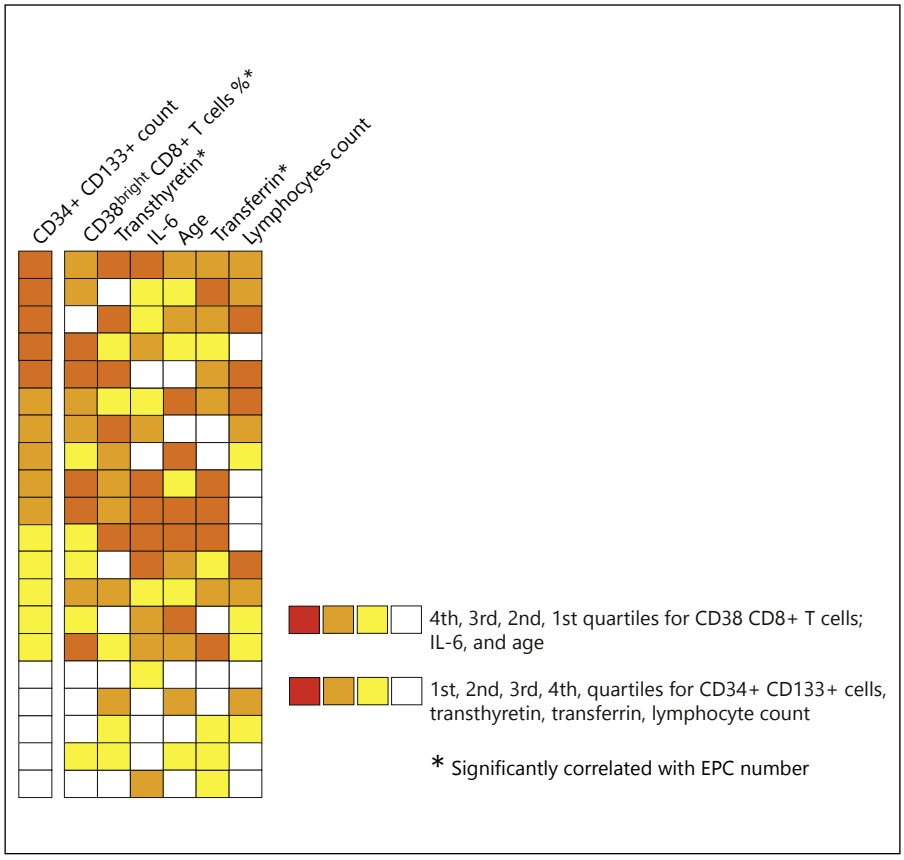

Fig. 3. Inflammation and nutrition profiles according to the EPC number. Factors potentially related to EPC mobilization are shown as a heat map. Subjects were ranked according to circulating CD34+ CD133+ cell number, from the lowest values in the first line of the map to the highest value in the last line. Quartiles were used to define a color scale for the levels of lymphocytes count (/mm^3); transferrin (g/l); transthyretin (g/l); IL-6 (pg/ml); age and CD38^{bright} CD8 memory T-cell number (%). Colors ranging from white blocks (most favorable level) to dark red blocks (less favorable level) were assigned for each marker.

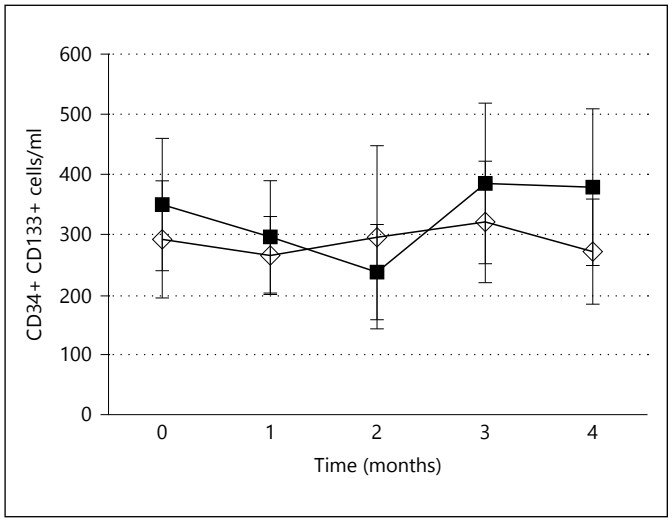

Fig. 4. Evolution of CD34+ CD133+ cells over the study period according to dialysis modality. White line: HD; dark line: HDF. Performing multivariate-adjusted random-effect model, no significant association was found between dialysis modality and the CD34+ CD133+ cells evolution (β = –5.40, SE = 19.24, p = 0.78) and no significant effect of dialysis modality at baseline or through the follow-up was observed.

modality and the CD34+ CD133+ cells evolution (β = –5.40, SE = 19.24, p = 0.78) and no significant effect of dialysis modality at baseline or through the follow-up was observed. Likewise we did not identify significant changes for transferrin, transthyretin, and percentage of activated CD8 memory T cells according to dialysis modality (data not shown). Finally, we did not observe any significant interaction overtime between transthyretin (0.14), transferrin (0.18) and EPC number.

Discussion

Impairment in kidney function is responsible for the creation of an environment favoring atherogenesis. Inflammation and malnutrition, recognised as important risk factors for cardiovascular diseases in HD patients, may be involved in endothelium dysfunctions, which are thought to play a major role in the mechanism of atherogenesis. Our data suggest that activation of the immune system as well as malnutrition state may be implicated in the cardiovascular pathogenesis of CKD patients through

reduction in EPC mobilization from bone marrow. In this prospective randomised study, we observed a significant inverse correlation between T-cell activation and the number of circulating EPCs. The strongest relationship was observed between CD34+ CD133+ EPCs and $CD38^{bright}$ expression on CD8 memory T cells, which have been analysed by using a standardised flow cytometric assay. Besides, a significant association was observed when exploring HLA-DR expression on CD8 memory T cells and CD34+ or CD34+ CD133+ cells. A trend in the correlation with inflammatory parameters (IL-6) was also observed. Positive correlations between CD34+ CD133+ ECPs and nutritional markers (transthyretin, transferrin), suggest that EPC impairment is a component of the malnutrition inflammation complex syndrome.

Our observations were in line with a previous study observing an inverse correlation between β2-M and EPC numbers [18]. β2-M is not only a soluble marker of inflammation but also a component of MHC class 1 molecules which are involved in antigen presentation and T cells activation. In this study, we investigated the activation of the immune system by directly analysing HLA-DR and CD38 expression on T-cell rather than β2-M serum concentration, which is affected by dialysis procedure and especially by HDF technique [31]. Although circulating activated CD8 T cells may be directly involved in endothelial dysfunction, this cellular marker can also be viewed as a proxy of exposure to inflammation and an overall marker of PBMC activation in CKD patients. The chronic activation of PBMC in CKD patients may impair endothelial differentiation of EPCs by modifying the homeostasis of different leukocyte subsets and leukocyte progenitors involved in endothelium repair. Hence, some CD14+ progenitor cells have the capacity to enhance endothelial regeneration and to accelerate the restoration of vascular function [19]. On contrary, CD14+ CD16+ monocytes subsets that are observed in high proportion in CKD patients may be involved in vascular impairment [34]. An inverse correlation between CD14+ CD16+ level and EPCs number has been observed [35–37]. Our results are in line with these observations, suggesting that both circulating pro-inflammatory monocytes and activated T cells are associated with a poor mobilization of EPCs. CD14+ CD16+ cells have enhanced capacity to secret proinflammatory TNF-α and IL-6 cytokines [37]. Inflammatory cytokine produced by macrophages may trigger T-cell activation via a bystander mechanism without engagement of the T-cell receptor. Macrophages are the major source of proinflammatory cytokines, but activated T cells contribute to produce inflammatory cytokines such as TNF-α and IL-6. It was established that TNF-α markedly reduced the proliferation of EPCs [38–40] and increased their apoptosis [41, 42].

In vitro studies and animal models of atherosclerosis also showed that inflammatory cytokines such as TNF-α and IL-6 induced vascular smooth muscle cells differentiation. By contrast with the reduction of EPCs, the number of circulating smooth muscle progenitor cells is unaffected in patients with end-stage renal disease [43]. Vascular smooth muscle cells probably play a key role during adverse vascular remodeling and vascular calcification through phenotypic transformation into osteogenic cells. Pro-inflammatory cytokines increased the endothelial expression of the bone morphogenetic protein-2 (BMP-2), which is a key molecule driving osteogenic cell differentiation. EPCs and endothelial microparticles by themselves may also be directly involved in vascular calcification since an osteoblast-like phenotype has been recently reported in patients with atherosclerosis and end-stage renal disease [44, 45].

The second major point of this study is the positive correlation observed between EPC number and transferrin and transthyretin concentrations. Transferrin is a glycoprotein responsible for iron transport. The malnutrition-inflammation score (MIS) of the International Society of Renal Nutrition and Metabolism, which is one of the CKD-specific nutritional scoring systems includes this parameter in its biochemical panel. Serum concentration of this molecule is diminished from 30 to 50% in CKD patients by comparison with healthy controls [46]. Inflammatory process and protein-energy wasting are potential responsible factors of such impairment. As chronic inflammation impacts on protein-energy wasting in CKD [47], it may be therefore a main factor influencing transferrin concentration [26, 27]. Evidences of this impact are the negative correlations between transferrin and CRP or IL-6 concentrations in HD patients [46, 48]. The negative correlation between $CD38^{bright}$ expression on CD8 memory T lymphocytes and transferrin concentration corroborated this negative influence of chronic inflammation on nutritional status. Low transferrin concentration may also be directly involved in the endothelium impairment, since the transferrin receptor plays a key role in angiogenesis process [49, 50]. In CKD patients, this endothelial dysfunction has been recently evidenced by measuring flow-mediated vasodilatation by Doppler and soluble intercellular adhesion molecule-1 (sICAM-1) level [5]. The concept of malnutrition inflammation complex syndrome (MICS) defined by

Kalantar-Zadeh et al. [47] suggests that beyond inflammation, amplification loops involving malnutrition could drive numerous endothelial defects in CKD patients. The reduction of circulating EPC number appears as one of those. Alongside transferrin, we observed a negative correlation between CD34+ CD133+ cells and transthyretin. Transthyretin is a negative acute phase protein known as a complex transporter of thyroxine, which showed interesting correlations with nutritional indices and quickly reflected nutritional status changes [51]. Such finding reinforces this hypothesis. Finally, we observed a trend between the low number of CD34+ CD133+ cells and low lymphocyte count, which also represents an indicator of poor nutritional status and is associated with the MIS score [50]. Hence, a low number of EPCs in CKD patients seems to be associated with T cells activation, inflammation, elderly and poor nutritional status [53].

It has been suggested that the enhancement of uraemic toxin removal by dialysis treatment may restore EPC functions, increase EPC number and finally, improve cardiovascular outcome [7, 26]. This hypothesis has been proposed to explain significant advantages of increased middle molecule removal with respect to survival and to a combined cardiovascular endpoint cardiovascular events in HD patients with type 2 diabetes mellitus [6]. Uremic toxins may have a deleterious role on progenitor cells since a negative correlation was observed between CD34+ CD133+ progenitor cell number and uraemic toxin levels including β2-M and indole-3 acetic acid [18]. The same authors also reported a pro-apoptotic effect of indole-3 acetic acid on CD34+ CD133+ cells in vitro. Although low-, high-flux HD and HDF have different capacities in uraemic toxins removal, here we did not observe any effect of dialysis modality on circulating EPCs over the treatment period even though a significant increase in β2-M removal was observed using HDF. Likewise, we did not observe any change in T lymphocytes activation and soluble markers of inflammation, transferrin and transthyretin concentrations over the 4-month period of the study according to the dialysis method used. Results of our exploratory study could not draw definitive conclusions on the influence of HDF versus conventional HD on these parameters. Cross-sectional longitudinal studies have explored changes under different HD techniques in CKD subjects. Using this study design each patient acted as his own control, which improves reproducibility and facilitates explorations of biological modifications associated with dialysis modalities. Previous studies have observed the reduction of the percentage of proinflammatory CD14+ CD16+ monocytes using high-flux one line hemofiltration [34, 35, 37]. This reduction of microinflammation may improve EPCs mobilization and endothelial damage/repair imbalance. Authors have recently reported a rise of CD34+/CD133+/KDR+ cells alongside with a reduction of endothelial microparticles number after the shift from high-flux HD to HDF with high convective transport post-dilution [37]. In another cross-over study, authors were unable to demonstrate a different effect of high-flux HD versus HDF on circulating EPC number [7]. Higher number and better functionality of EPCs have also been reported in CKD patients under nocturnal HD compared to conventional HD [28]. A recent study had observed that after adjusting for other potential confounders, EPC number was higher in CKD persons under continuous ambulatory peritoneal dialysis than in HD patients [54].

In line with our observation, Krieter et al. reported that protein-bound uremic toxins including indoxyl sulfate and para-cresyl sulfate were not efficiently removed on regular three times weekly maintenance HDF [33]. These protein-bound uremic toxins have been shown to have endothelial toxicity and inhibit endothelial proliferation [55]. Adsorption-based dialysis techniques and novel membranes need to be developed and evaluated for their capacity to more efficiently remove uremic toxins [56, 57].

Our study acknowledged some limitations, especially the relatively small sample size and short time of exposure of this prospective randomised study, which may have influenced the significance or nonsignificance of the relationships observed. It could also be argued that EPCs and progenitor cells were enumerated by immunophenotyping without in vitro analysis to identify EPC Colonies Forming Cell (CFU) or VEGF release. The enumeration of CFU allows the identification of EPCs with capacity of differentiation into endothelial lineage cells, but previous studies showed that the EPC numbers exhibited a strong correlation with endothelial-colony forming units in culture [52, 56].

Conclusions

Our results suggest that chronic T-cell activation and malnutrition, which are involved in amplification loops, adversely influence EPC number in CKD patients. Attempts to reduce chronic T-cell activation, inflammation and to improve nutritional status of CKD patients may improve EPC mobilization. The potential benefit of in-

creasing uremic toxins removal by convective modalities to improved EPC circulation still needs to be demonstrated in more powered cross-over studies.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

E.T., M.M., N.K. and A.R. carried out the laboratory assays. E.T., B.C. and J.-P.C. conceived and designed the experiments. E.T., M.M., N.K. and I.J. analysed the data. I.J. performed the statistical analysis. E.T., M.M., B.C., J.P.C. and I.J. wrote the manuscript. L.C., A.R., M.M., J.-P.V. and F.G. contributed to reagents, materials and helped to draft the manuscript. All authors read and approved the final manuscript.

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