Circulating Endothelial Progenitor Cells and Endothelial Microparticles in HD Patients with Peripheral Artery Disease

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Introduction

Patients with end-stage renal disease are at high risk of developing cardiovascular and the endothelium is a key regulator of vascular homeostasis.
Chronic exposure to vascular risk factors alters the regulatory properties of the endothelium, which progresses toward a pro-inflammatory pattern, senescence, and apoptosis. This activation of the endothelium leads to the shedding of endothelial microparticles (EMP) and to the detachment of circulating endothelial cells (CEC). Microparticles bear phospholipids (e.g. the negatively charged phosphatidylserine which binds to annexin V) and membrane proteins of their mother cell.
Recent evidence suggests that MPs are abundantly released in patients with cardiovascular diseases.

Aim

- We tested the hypothesis that HD patients in particular those with peripheral vascular disease (PAD) might have increased circulating CD31+/Annexin V+ apoptotic microparticles levels, impaired repair capacity and enhanced inflammatory status.

Patients and Methods

A total of 40 HD patients with and without PAD as well as 20 age- and sex-matched controls were enrolled. Flow cytometry with quantification of EPC markers (defined as CD34+, CD34+KDR+, and CD34+KDR+CD133+)in peripheral blood samples was used to assess circulating EPC numbers.

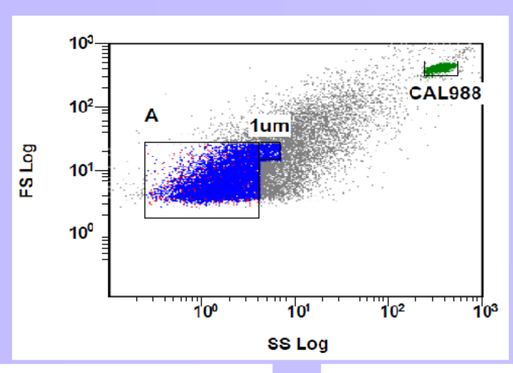
Assay of circulating EPCs

Analysis was based on the expression of surface markers CD34 and VEGFR2 on cells in the mononuclear gate where EPCs are commonly found. CD34+ and VEGFR2+ are commonly used as markers for EPCs. The percentage of double-positive mononuclear cells (CD34+/VEGFR2+) was converted to cells per ml of peripheral blood using fluorescent microbeads (Flow-Count, Beckman Coulter).

Endothelial Microparticles Analysis

Blood samples for flow cytometry were collected in Vacutainers containing citrate (Becton-Dickinson, Franklin Lakes, New Jersey) from a peripheral vein using a 21-gauge needle to minimize platelet activation and were processed for assay within 4 hours. Platelet-poor plasma (50µl) was incubated with 4µl of monoclonal antibody against CD31 (Beckman Coulter, Miami, FL, USA) followed by incubation with fluorescein isothiocyanate-conjugated Annexin V kits according to the manufacturer's instructions (Beckman Coulter, Miami, FL, USA). Fluorescence-activated cell sorter analysis was performed in a FACSCalibur cytometer. The negative control (zero value) was the value obtained using the isotype antibodies. Each result (one single value) was the average of three independent determinations of the same sample.

Representative traces of flow cytometry analysis for EMPs



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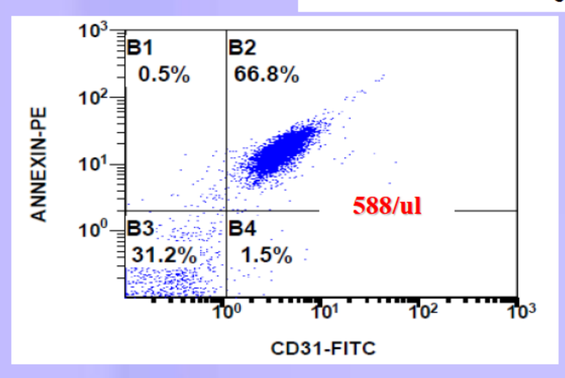

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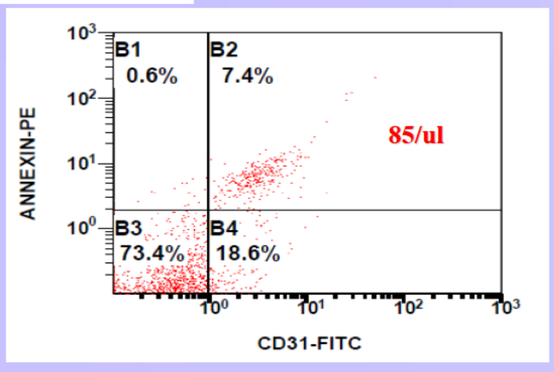
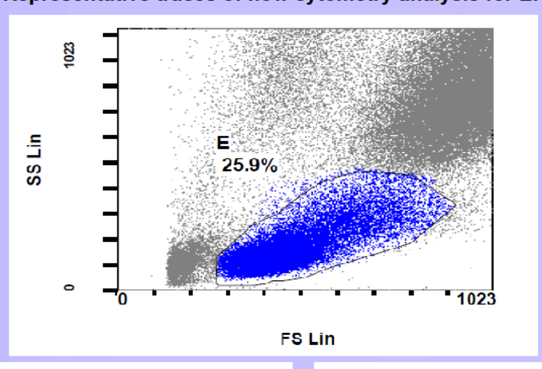


Representative traces of flow cytometry analysis for EPCs



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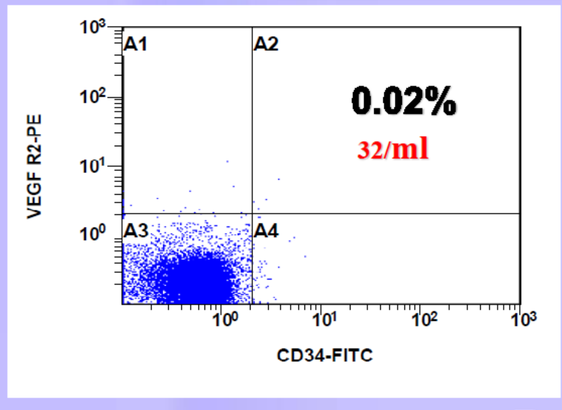

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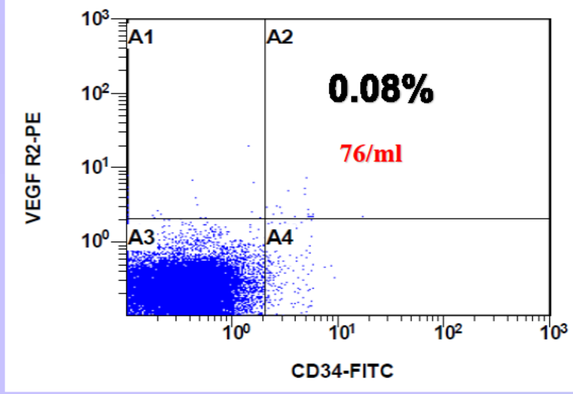


Results

HD patients had significantly increased circulating EMP levels (all p <0.05), endothelial dysfunction, and enhanced systemic inflammation compared to controls.
In addition, we found that patients with PAD had significantly elevated levels of endothelial microparticles that those without PAD.
There were no differences in all clinical parameters between HD patients with and without PAD except for HDL-Chol.
Levels of endothelial apoptotic microparticles demonstrated a significant negative correlation with circulating endothelial progenitor cells levels (*r = -0.732; p <0.001) and serum HDL-Chol levels (- 0.360 ; p =0.023)*
Furthermore, percentage of proinflammatory monocytes and neopterin (lo-transformed) positvely correlate with circulating CD31+/Annexin+ microparticles
(r =0.714, *p <0.001; r = 0.338, p= 0.035 respectively)*

Table 1. Baseline demograhic and characteristics of HD patients and healthy subjects at inclusion

	HD with PAD	HD without PAD	Healthy Subjects
n	19	21	20
Age (y)	64.1±10.5	61.1± 7.4	60.0± 5.5
Gender (M/F)	11/8	12/8	10/10
Clinical features			
BMI	23.9±4.1	23.8 ±3.5	ND
Vintage (m)	124.5± 82.1	107.1±76.6	ND
Diabetes (%)	10/19 (52.6)	6/20 (35%)	0.05
Hypertension (%)	6/19 (31.6%)	10/20 (50%)	0
Background Therapy			
erythropoietin (%)	13/19 (68.4)	18/20(90)	0
RAS blockers (%)	1/19 (5)	2/20 (10)	0
Statin (%)	2/19 (11)	2/20 (10)	0

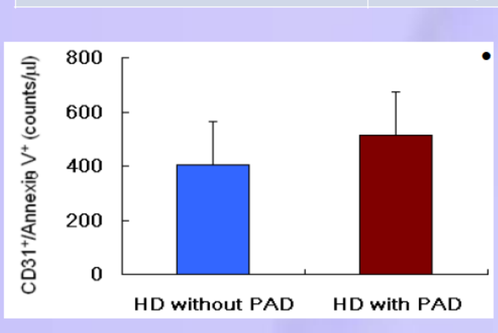
Table 2. Studied Parameters of HD patients and healthy subjects

	HD patients	Healthy Subjects
n	40	20
CD16+ Monocytes (%)	17.3±4.3	7.6±2.2 *
EMP, events/µl	456.3±168.1	21.7±5.2*
EPC, events/µl	38.3±20.8	125.3±27.1 *
EMP/EPC ratio	15.7 ±9.8	0.19± 0.08 *
Il-6, pg/mL	5.3 ±4.8	4.0±3.4

Values mean± SD; * p < 0.001

Table 3. Biochemical characteristics of HD patients with and without PAD

	HD with PAD	HD without PAD	p-value
n	19	21	
Cholesterol (mg/dL)	175.7± 39.3	174.3± 28.9	0.901
LDL-C (mg/dL)	97.0± 24.4	100.8 ±24.2	0.663
HDL-C (mg/dL)	45.6±14.4	55.1±17.4	0.069
Triglyceride (mg/dL)	144.8± 92.5 122(31-365)	107.6 ±87.4 76(37-381)	0.199
hsCRP(mg/L)	6.16 ±5.96 1.3 (0.1-20.1)	3.55±4.79 4.9 (0.1-25.4)	0.058
CD16+ Monocytes (%)	16.3 ±4.2	18.1±4.3	0.341
EMP, events/µl	514.9 ±160.4	403.3± 160.4	0.033
EPC, events/µl	38.3 ±17.6	38.4±23.7	0.987
Il-6, pg/mL	6.6 ±5.8 4.59 (0.01-20.86)	4.0 ±3.1 3.02(0.37-12.71)	0.093
Neopterin, nmol/L	165.6 ±122.1 138.2 (25.5-438.7)	78.1± 44.0 76.0 (18.0-170.0)	0.005



- Circulating endothelial MP levels in hemodialysis (HD) patients. Circulating levels of Annexin V binding MP (ANN V + MP) was significantly increased in HD patients with PAD compared with control subjects. Results for circulating CD31+/Annexin V+ apoptotic microparticles are given as means ±SD (normal distribution). **P < 0.05.*

Discussion

- Recent data support the hypothesis that MPs contribute to vascular homoeostasis and the pathogenesis of cardiovascular diseases, including inflammation and vascular dysfunction, in addition to their well-known action on the coagulation process.
- Increasing evidence suggests that the balance of EC apoptosis and EC regeneration may determine the degree and progression of atherosclerosis. Besides increased endothelial injury, patients with CKD also experience impaired repair capacity, reflected by a decrease in EPC number and functions.
- In this study, we found that HD patients in particular those with PAD have increased endothelial apoptotic microparticles and decreased circulating EPC levels, which may contribute to atherosclerotic disease progression and enhanced cardiovascular risk in these patients.
- Evidence suggest that HDL-Cholesterol is able to inhibit endothelial cell apoptosis.
- Based on our observations, EMP may enhance inflammation and increase the proportions of proinflammatory monocytes in HD patients by binding to and activating monocytes. This involves the interaction between intercellular adhesion molecule-1 (CD54) on EMPs and b2 integrins on monocytic cells . This process may enhance tissue factor activity and increased expression of monocyte CD11b, which in turn may lead to increased binding to activated endothelium.
- Neopterin levels were found to be higher in HD patients than in the healthy controls. A significant difference in neopterin levels was also found between HD patients with and without PAD. Besides, neopterin levels were found to be associated with circulating endothelial EMP levels.
- Measurement of circulating EMP may serve as a useful tool, allowing us to mirror the actual detrimental effects of cardiovascular risk factors with one integrative marker.

Conclusions:

- **HD patients in particular those with PAD have increased endothelial apoptotic microparticles and decreased circulating EPC levels, which may contribute to atherosclerotic disease progression and enhanced cardiovascular risk in these patients. Besides, circulating EMP may be associated with enhanced inflammation as well as increased proportions of proinflammatory monocytes in HD patients.**

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