Reduced concentration and proliferation of circulating endothelial progenitor cells in refractory hypertensives are independent of other cardiovascular risk factors

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Background

- Endothelial progenitor cells (EPC) derived from bone marrow support an important role for the integrity and functional activity of the vascular endothelium, being important to prevent atherogenesis.

- Furthermore, reduced levels and function of circulating EPCs independently predict the occurrence of cardiovascular events.

- Reduced levels of EPCs and of their functional capacity have been shown in relation to some traditional CV risk factors such as age, smoking, diabetes or hypercholesterolemia, but their independent association with hypertension is unclear.

Aim

To determine if patients with refractory hypertension (RHT) have reduced number and function of circulating EPCs independently of other CV risk factors and of some known determinants of EPCs.

Patients and Methods

Patients:

- **37 RHT** patients aged >18yr (with refractory hypertension confirmed with 24hr-ABPM in subjects treated with at least 3 antihypertensive drugs being one of them a diuretic).
- **30** normotensive control subjects (**NT**).

Analyzed parameters:

- Anthropometric and demographic features / known cardiovascular risk factors / laboratory parameters / statins treatment.
- **EPCs concentration:** EPCs (CD34+/CD133+/CD45+) were isolated from circulating polymorphonuclear cells (PMN) by flow cytometry.
- **EPCs proliferation:** these EPCs were studied *in vitro* after 7 days in culture. Double-stained cells for both acLDL-Dil and lectin were counted as EPCs.

Statistical analyses:

- **univariate analyses:** χ^2 test, exact Fisher's test, unpaired Student's *"t"*-test or Mann-Whitney "U" test were applied when appropriate.
- **multivariable analyses:** variables that achieved significance in the univariate analyses were included in a multiple linear regression model.
- A ***p*** value **≤ 0.05** was considered statistically significant

Conclusions

1) We conclude that circulating EPCs number and EPCs proliferation are significantly and independently reduced in patients with refractory hypertension.

2) If they are biomarkers or predictors of the poor cardiovascular outcomes observed in this population remains to be determined.

Results

Univariate analyses:

	HIPERTENSIVES (n=37)	NORMOTENSIVES (n=30)	p
Age (SD)	60,7 (11,5)	37.0 (9.2)	**<0.001**
Male	19 (51,4%)	11 (36.7%)	0.229
Body weight (DE)	81,5 (13.7)	66.3 (15.0)	**<0.001**
BMI (DE)	30,7 (4.1)	23.5 (3.3)	**<0.001**
Dyslipemia	24 (64,9%)	10 (33.3%)	**0.01**
Smokers	6 (16.2%)	4 (13.3%)	1
Diabetes	8 (21.6%)	0	**0.007**
Total cholesterol	205.7 ± 35.9	195.6 ± 32.4	0,236
HDL-c	53.3 ± 16.8	61.2 ± 19.6	0,082
LDL-c	132.1 ± 28.8	119.9 ± 30.0	0,098
TG *	114.0 [79.5; 137.5]	79.0 [59.5; 105.8]	**0,005**
HbA1c *	4.9 [4.5; 5.3]	4.3 [4.2; 4.4]	**<0,001**
SCr *	1.09 [0.94; 1.30]	1.08 [0.93; 1.16]	0,216
eGFR – MDRD	62.0 ± 15.9	72.3 ± 9.5	**0,003**
eGFR – CG	76.7 ± 27.6	78.6 ± 20.1	0,751
$Alb_{ur}/creat_{ur}$ *	15.6 [5.5; 44.6]	2.5 [1.9; 4.0]	**<0,001**
Hematocrit	42.5 ± 3.6	41.3 ± 3.0	0,146
Hcy	10.2 ± 3.4	9.5 ± 2.9	0,472
PCR *	0.40 [0.25; 0.80]	0.20 [0.20; 0.23]	**<0,001**
Fibrinogen	334.7 ± 71.7	276.8 ± 65.7	**0,001**
Ferritin *	60 [37; 102]	33 [16; 105]	**0,044**
EPO levels *	21.5 [15.1; 25.7]	17.0 [13.4; 22.2]	0,194

(*) Median [quartile 1;3]. Remaining parameters: mean±SD

	HIPERTENSIVES (N = 37)	NORMOTENSIVES (N = 30)	P
EPC/ 10^5 PMN median [95% CI]	**25.5** [16.5 – 39.4]	**53.2** [34.4 – 82.3]	**0.046**
EPC / field median [95% CI]	**95.8** [59.1 – 155.2]	**597.3** [322.2 – 1107.3]	**<0.001**

Age-adjusted model

Multivariate analysis:

EPCs levels:			
HYPERTENSIVES	- 25.3 EPC / 10^5 PMN →	**↓47.9%**	P=0,0236
EPCs proliferation:			
TG	(-)		P=0,0522
Body weight	(+)		P=0,0129
HYPERTENSIVES	- 427.8 EPC / field →	**↓71,6%**	P<0,0001

After adjusting for those other variables - tryglicerides and body weight - being hypertensive was the only independent predictor of reduced proliferation of EPCs in culture.