Altered monocytic phenotypes are linked to a hypertension form: A clinical observational study

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INTRODUCTION

The past decade witnessed an explosion of research interest in the role of immunity in hypertension. Accumulating evidence suggests that immune cells infiltrate the key organs contributing to target damage [1]. Although it has become evident that T cells play an important role in experimental and human hypertension [1–3], the role of monocytes subsets remains to be elucidated. In humans, elevated blood pressure (BP) is related to the increased levels of inflammatory cytokines and activated monocytes [4]. Our study aimed to examine the exact phenotype of monocyte subsets in relation to various clinical pictures — established hypertension (HTN) and secondary form — primary hyperaldosteronism (PHA), compared to newly diagnosed hypertensives (NDH).

METHODS

Sixteen NDH, 14 HTN, and 15 PHA consecutive patients were recruited for the study. PHA was confirmed by a positive saline infusion test according to the Endocrine Society Guidelines [5]. HTN was defined as established hypertension treated with β-blocker, diuretic, angiotensin-II convertase enzyme inhibitor/angiotensin-II receptor blocker (ACEI/ARB), and calcium channel antagonist (CCA). In all HTN subjects, secondary causes of hypertension were excluded. NDH was defined as newly diagnosed, untreated hypertension. All patients were matched for sex, age, body mass index (BMI), and blood pressure levels on 24-hour ambulatory blood pressure monitoring (ABPM).

In all patients, clinical and biochemical evaluation, serum/plasma level of renin-angiotensin II (Ang II) — aldosterone, albuminuria in the 24-hour sample, 24-hour ABPM, echocardiography, duplex Doppler ultrasonography of carotid arteries, and monocytes characteristics were performed (Supplementary material, *Figure S1*).

This study was approved by the Local Bioethics Committee in the Institute of Cardiology in Warsaw (approval no. 1470). All procedures in the study were in accordance with the 1964 Declaration of Helsinki. Written informed consent was obtained from all patients.

Statistical analysis

The normality of variables distribution was checked with the Shapiro-Wilk test. Continuous variables with normal distribution were compared, among the 3 groups studied, using the ANOVA test. Continuous variables with non-normal distribution, including all 44 cell characteristics, were compared, among the 3 groups studied, using the Kruskal-Wallis test. Categorical variables were compared using the χ^2 test.

Linear regression analysis adjusted for age, sex, body mass index (BMI), and current smoking status was performed to test the effect of the PHA or HTN groups on selected monocyte subpopulations in relation to the

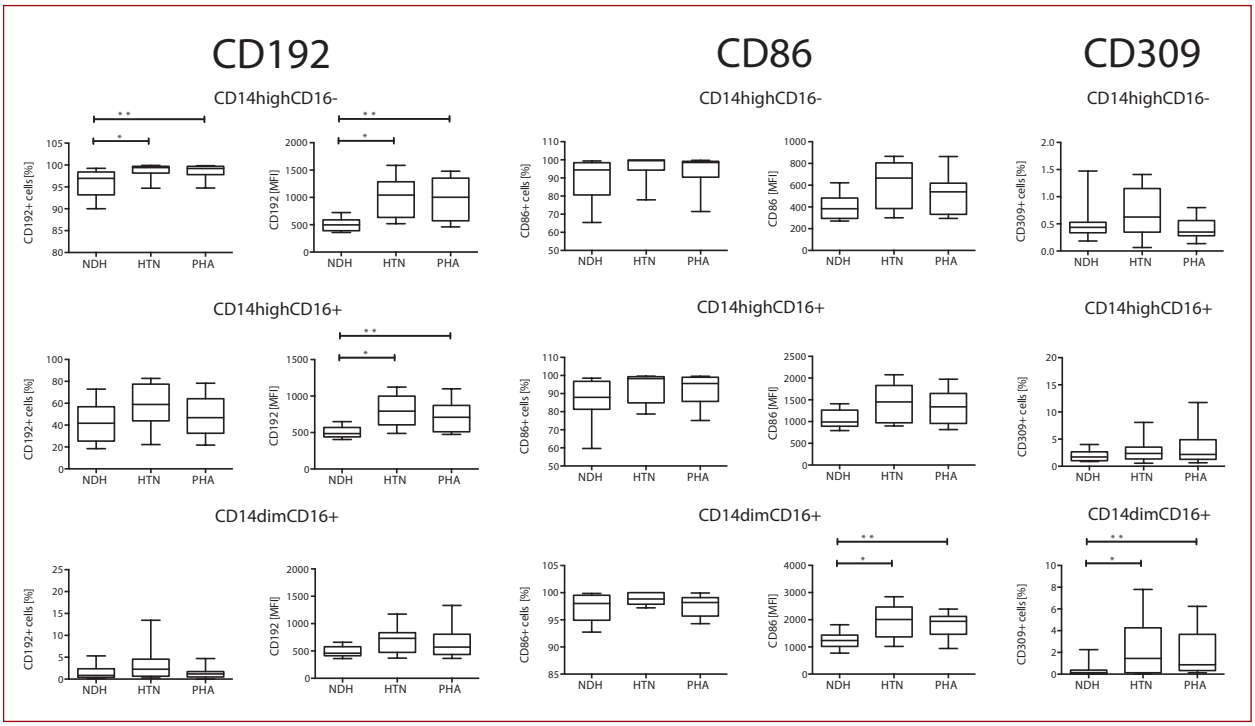

Figure 1. Expression of selected markers in monocyte subsets in patients with hypertension. The percentages of CD192, CD86, and CD309 positive cells within individual monocyte subsets including CD14highCD16-, CD14highCD16+, and CD14dimCD16+ cells are shown in patients with NDH, HTN, and PHA. MFI indicates mean fluorescence intensity. Boxes represent the 25th and 75th percentiles and horizontal lines the median

Abbreviations: HTN, hypertension; NDH, newly diagnosed hypertensives; PHA, primary hyperaldosteronism

NDH group. The false discovery rate (FDR) correction was applied while testing differences with respect to all 44 cell characteristics among all subgroups (Supplementary material, *Figure S2*).

RESULTS AND DISCUSSION

We assessed NDH, HTN, and PHA subjects matched for sex, age, BMI, and daily systolic and diastolic BP on 24-hour ABPM (Supplementary material, *Table S1*). Subjects with PHA were characterized by a significantly higher level of plasma aldosterone and a lower plasma level of Ang II as compared to NDH and HTN subjects. Diabetes mellitus (DM) 2 and ischemic heart disease (IHD) were observed more often in HTN and PHA compared to NDH. A higher left ventricular mass index (LVMI) was observed in the HTN and PHA groups as compared to NDH subjects (Supplementary material, *Tables S1, S2*).

We observed no differences in the content of total monocytes or their subsets including classical (CD14high-CD16-), intermediate (CD14highCD16+), and nonclassical (CD14dimCD16+) cells among the groups (Supplementary material, *Figure S1*). Our data are in line with results obtained by van der Heijden et al. [6] who did not observe significant differences in the peripheral monocytes subsets between PHA and NDH patients [6].

As monocyte chemoattractant protein-1 (MCP-1) is involved in monocyte infiltration into the inflamed tissue, we analyzed the role of its receptor — C-C chemokine receptor 2 (CCR2) among monocytes. We noticed that PHA and HTN subjects presented a higher percentage and mean fluorescence intensity (MFI) of CD192 among total monocytes, classical and intermediate subsets compared to NDH patients (Figure 1, Supplementary material, *Figure S2,* and *Table S3*). The multiple linear regression model showed that advanced forms of hypertension — HTN and PHA were linked to a higher percentage of CD14highCD16-CD192+ monocytes and higher surface expression of CD192 among classical and intermediate monocytes (Supplementary material, *Table S4*).

MCP-1 plays a key role in atherosclerosis and insulin resistance, and it is responsible for recruiting monocytes to the site of inflammation [7]. Infiltration of monocytes into the heart and vasculature in the course of hypertension depends on the CCL2 (C-C chemokine ligand)-CCR2 axis [1]. Pharmacological inhibition of CCR2 prevents the recruitment of monocytes into the aortic wall and heart [1]. Interestingly, Zaldivia et al. [8] have shown the reduction of classical monocyte activation in HTN patients after renal denervation procedure. Poor BP control might induce monocytes infiltration and progression of target organ damage (TOD) stated by albuminuria and higher LVMI and IMT. The chronic inflammatory state could create a vicious circle and further progression of TOD and the development of complications such as DM type 2 or IHD.

Secondly, we assessed CD309, known as vascular endothelial growth factor receptor 2 (VEGF-R2), which is expressed by endothelial cells and is a pro-angiogenic factor involved in neovascularization and atherosclerosis [9–10]. We proved that patients with HTN and PHA were characterized by a higher percentage of CD14dimCD16+ CD309+ monocytes in comparison to patients with NDH (Figure 1, Supplementary material, *Figures S1,* and *S2*). However, the result was not significant in the multiple linear regression model (Supplementary material, *Table S4*). We indicated that HTN and PHA subjects are characterized by higher intima-media thickness (IMT) which is considered a surrogate marker of atherosclerosis. *In vivo* models showed that VEGF induces the angiogenic cascade with hypoxia-inducible factor 1 (HIF-1). In hypertensive rats, VEGF expressed by macrophages and T lymphocytes stimulates endothelial cells to produce MCP-1, attracting monocytes and enhancing cell migration by increasing permeability of the endothelial layer [11]. There is evidence that VEGF induces migration and activation of monocytes through induction of MCP-1 [12]. Zhao et al. [13] have shown that VEGF acts as a mediator of Ang II-induced vascular inflammation. Stumpf et al. [12] have observed that young subjects with hypertension are characterized by higher plasma levels of VEGF and MCP-1. Elevated VEGF levels in hypertensive patients support the concept of abnormal angiogenesis in the pathophysiology of hypertension [14].

In the current study, we assessed CD86 expression as a key protein that provides the costimulatory signals necessary for T-cell activation and survival. The expression of CD86 was higher in the population of non-classical monocytes in HTN and PHA as compared to NDH subjects (Figure 1, Supplementary material, *Figures S1* and *S2*). The multiple linear regression model showed that patients with HTN and PHA were characterized by higher surface expression of CD86 among CD14dimCD16+ monocytes (Supplementary material, *Table S4*).

We observed a preactivated monocyte phenotype in hypertensives. This is consistent with other studies. Dorffel et al. have demonstrated the increase of interleukin (IL-1β) secretion from peripheral monocytes after Ang II stimulation in comparison to normotensive subjects [4].

The limitations of our study include the small sample size and the limited number of monocyte phenotypes analyzed. We also observed a high incidence of DM type 2 in PHA and HTN patients. Our observations were in line with results obtained in the JPAS and RESIST-POL registries (further discussed in Supplementary material, *Section S3*). Moreover, our study groups were treated with hypotensive drugs that could have influenced the phenotype of monocytes (Supplementary material, *Section S3*).

CONCLUSION

Monocytes isolated from patients with an advanced form of hypertension present features of increased cell activation measured as a higher percentage and surface expression of MCP-1 receptor among classical and intermediate subsets. Patients with poor hypertension control and features of TOD exhibit higher surface expression of costimulatory marker CD86 among nonclassical monocytes and a higher percentage of nonclassical cells positive for VEGF-R2.

Supplementary material

Supplementary material is available at https://journals.viamedica.pl/kardiologia_polska

Article information

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