ORIGINAL ARTICLE

Th17/Treg imbalance in patients with primary hyperaldosteronism and resistant hypertension

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KEY WORDS

primary hyperaldosteronism, resistant hypertension, target organ damage, Th17, Treg

ABSTRACT

INTRODUCTION Inflammation plays a pivotal role in blood pressure regulation. Data on experimental models of hypertension and hypertensive patients reflect the imbalance between T regulatory (Treg) and Th17 effector cells (Th17).

OBJECTIVES The aim of this study was to quantify peripheral blood Treg lymphocytes and Th17 subsets in individuals with primary hyperaldosteronism (PHA) and resistant hypertension (RHT) presenting with elevated blood pressure levels and augmented cardiovascular risk when compared with normotensive controls (CTRL).

PATIENTS AND METHODS Twenty CTRL participants, 21 patients with PHA, and 20 patients with RHT were enrolled. Plasma renin and angiotensin II, serum aldosterone concentration, ambulatory blood pressure monitoring (ABPM), echocardiography, clinical data, and phenotype of peripheral blood cells were assessed.

RESULTS There were no statistically significant differences in terms of age and sex between the groups. Similar systolic blood pressure (SBP) and diastolic blood pressure (DBP) levels in ABPM were observed in individuals with PHA and RHT. PHA patients had lower angiotensin II and 4-fold higher aldosterone concentrations than CTRL patients. Both, PHA and RHT were associated with cardiac hypertrophy and coronary artery disease. RHT patients presented a significantly higher $CD4^+IL\text{-}17A^+$ T cell number when compared with PHA and CTRL ones. The number of $CD4^+CD25^+FOXP3^+$ T cells did not differ between patients with secondary hypertension and normotensive controls. Finally, positive correlations between the data on 24 h SBP and the content of $CD4^+IL\text{-}17A^+$ and $CD4^+CD25^+FOXP3^+$ in the PHA were found.

CONCLUSIONS Elevated 24 h SBP in PHA was associated with the increased numbers of $CD4^+IL\text{-}17$ and $CD4^+CD25^+FOXP3^+$ T cells.

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Received: July 15, 2021.
Revision accepted: December 6, 2021.
Published online: December 10, 2021.
Pol Arch Intern Med. 2022; 132 (3): 16171
doi:10.20452/pamw.16171

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INTRODUCTION Over recent years, data obtained from experimental models of hypertension have shown the influence of immune components, and especially immune cells on the regulation of blood pressure and cardiovascular risk linked to hypertension.[1,2] Moreover, experimental studies revealed the exact function of different subsets of T cells in the primary hypertensive response.[2] The imbalance of T regulatory (Treg) and Th17 effector cells, such as $CD4^+IL\text{-}17A^+$, may lead to low-grade inflammation and progression of target organ

WHAT'S NEW?

Hypertension is considered a relevant risk factor for cardiovascular mortality and morbidity. Over the years it has been demonstrated that adaptive and innate immune responses in experimental models are modulated by aldosterone. This study aimed at demonstrating that secondary hypertension is characterized by the Th17/Treg imbalance. Moreover, it was designed to establish a positive correlation between elevated systolic blood pressure and increased numbers of $CD4^+IL\text{-}17^+$ and $CD4^+CD25^+FOXP3^+$ T cells in primary hyperaldosteronism.

damage.[3] Much less is known about the imbalance of T cells and their involvement in secondary hypertension in humans with primary hyperaldosteronism (PHA) and resistant hypertension (RHT). This is supposed to be a key issue in further research on the subject. A considerable number of studies showed that elevated renin and aldosterone levels are predictors of adverse outcome in many diseases, for example, myocardial infarction, renal insufficiency, heart failure, and insulin resistance.[4-6] Furthermore, it was demonstrated that aldosterone modulates the innate and adaptive immune responses, which promote the production of reactive oxygen species and inflammatory cytokines leading to vascular disorders.[4] It is also of vital importance that PHA serves as an excellent model for studies investigating the divergent effects of high aldosterone levels in vivo. Thus, it can be postulated that there is a close relationship between adaptive immunity and renin-angiotensin-aldosterone system (RAAS) in experimental models of hypertension.[7,8] Mineralocorticoid receptor (MR) is expressed by immune cells and may modify immune activation and functions.[9] It should be emphasized, though, that most of the reliable data are derived from animal models. Therefore, PHA and RHT effects on the imbalance of T cells in humans have not yet been estimated.

The primary aim of this study was to analyze the interplay between Th17 and Treg cells in PHA, RHT, and control (CTRL) patients. Our further research concerned the relation between the imbalance of T cells and factors such as secondary hypertension, blood pressure, and target organ damage.

PATIENTS AND METHODS

Study groups

Twenty CTRL subjects, 21 PHA patients, and 20 RHT patients were enrolled in this study. Following the Endocrine Society Guidelines we confirmed PHA on the basis of a positive saline infusion test results.[10] There were no statistically significant differences in terms of age and sex between the groups. The blood pressure values (measured by ambulatory blood pressure monitoring, [ABPM]) were similar in the PHA and RHT groups.

Resistant hypertension was defined as an inability to lower the office systolic blood pressure (SBP) and diastolic blood pressure (DBP) values below 140 mm Hg and/or 90 mm Hg or as inadequate control of BP confirmed in home blood pressure monitoring (HBPM) and in ABPM despite the recommended treatment and therapy adherence. The recommended treatment was described as best tolerated and optimal doses of at least 3 drugs, such as angiotensin receptor blocker / angiotensin-converting enzyme inhibitor, calcium channel blocker, and a diuretic.[11] Aldosterone to renin ratio was calculated in the RHT group in order to exclude PHA as a potential cause of resistance. The exclusion criteria were as follows: worsened renal function expressed as reduced glomerular filtration rate lower than 45 ml/min/1.73 m^2 (calculated using the Modification of Diet in Renal Disease equation), pregnancy or the first phase of the menstrual cycle in women of childbearing age, irregular menstrual bleeding, cancer (unless a disease-free period lasting more than 5 years was documented), basal cell carcinoma, cortisol cosecretion (the low dose Dexamethasone Suppression test was used in all participants), autoimmune-related diseases (systemic lupus erythematosus, type 1 diabetes, rheumatoid arthritis, multiple sclerosis, scleroderma, Sjögren's syndrome), HIV infection and/or active tuberculosis infection, type B hepatitis or type C hepatitis, oral antibiotic therapy or intravenous antibiotic therapy undergone within, respectively, 2 weeks or a month prior to the day of enrolment, tissue or organ transplant, and immunosuppressive therapy.

The following parameters were assessed in all participants: clinical evaluation, review of hypotensive treatment, biochemical evaluation, office blood pressure measurements, ABPM, echocardiography, and T-cell immunophenotype characteristics. Serum or plasma concentrations of renin-angiotensin II-aldosterone were measured in patients with PHA and RHT. Ever-smoking status was defined as smoking in the past with smoking cessation, lasting at least 1 year, whereas current smoking status was defined as active smoking. This study was approved by the Local Bioethics Committee of the Institute of Cardiology in Warsaw (Approval 1470). All procedures in this study were in accordance with the 1964 Declaration of Helsinki. Written informed consent was obtained from all patients.

Office blood pressure measurements and control of hypertension

Blood pressure was measured by a qualified nurse using an automated device (Omron 705IT, Omron Corporation, Kyoto, Japan). The measurement was taken following a 5 minute rest and each patient remained seated. Once an appropriately sized cuff had been placed on the patient's arm with the lower edge of the cuff 2 cm above the antecubital fossa, 3 readings were performed. If the difference between the readings was higher than 10 mm Hg, more measurements were carried out to obtain 3 consecutive, consistent readings. Then, the average value of such 3 readings was recorded. Hypertension was defined as office blood pressure equal or above 140 and/or equal or above 90 mm Hg on 2 separate occasions or antihypertensive treatment.[11]

Ambulatory blood pressure monitoring The ABMP measurements were recorded with the use of the SpaceLabs 90 217 device (SpaceLabs Medical Inc., Redmond, Washington, United States). Average 24 h SBP, DBP, and heart rate were measured. The blood pressure reduction during the night was defined as the relative decrease in nocturnal blood pressure for SBP and DBP. The participants were classified as dippers if the proportional decrease from awake to asleep BP was equal or above 10%. Hypertension was defined as daytime BP equal or above 135/85 mm Hg.[12]

Evaluation for plasma aldosterone (PA) and PA subtyping All eligible patients were asked to remain seated and underwent the saline infusion test (intravenous infusion of 2 l of 0.9% saline over 4 h). Postinfusion PA levels higher than 10 ng/dl confirmed a diagnosis of PHA.[13] Medication treatment was arranged according to current guidelines. As a result, in some patients diuretics, spironolactone, and other antihypertensive drugs had been withdrawn before evaluation. Next, adrenal vein sampling (AVS) was used in order to determine the subtype of PHA. A continuous intravenous infusion of cosyntropin, a synthetic adrenocorticotropic stimulating hormone (50 μg/h), was given for at least 60 minutes prior to the procedure. AVS was employed sequentially and was deemed successful if the selectivity index (SI) was higher than 5:1. A cutoff for the cortisol-corrected aldosterone ratio from high side to low side of 4:1 was used to indicate unilateral aldosterone excess. AVS was performed in order to detect unilateral aldosterone hypersecretion (aldosterone producing adenoma or bilateral adrenal hyperplasia), using the above-mentioned cutoffs.[13] Patients with severe, uncontrolled hypertension, renal insufficiency, cardiac arrhythmia, or severe hypokalemia did not undergo the saline infusion test.

Echocardiography Standard transthoracic Doppler echocardiography was performed using the GE Vivid 7 transducer (General Electric, Boston, Massachusetts, United States) (frequency 2.5 to 3.5 MHz) on the day ABPM was measured or on the following day.[14,15] The patients enrolled in the study were examined lying in the left lateral decubitus position. Left ventricular mass (LVM) was calculated applying the modified American Society of Echocardiography cube formula proposed by Devereux et al.[14] LVM was indexed to body surface area to compute the left ventricular mass index (LVMI). Left ventricular hypertrophy was defined as LVMI equal or above 95 g/m^2 for women and LVMI equal or above 115 g/m^2 for men.[14] Left ventricular systolic function was evaluated by left ventricular ejection fraction (using Simpson's method), whereas left ventricular diastolic function was assessed by mitral inflow velocity.

Renin-angiotensin-aldosterone system activity measurements RAAS activity was assessed using radioimmunoassays according to the manufacturer's instruction. Aldosterone (Active Aldosterone RIA DSL-8600, Beckman Coulter, Brea, California, United States) was measured in human serum, whereas Renin (RENIN III GENERATION, Cisbio Bioassays, Codolet, France) and Angiotensin II (BÜHLMANN, Amherst, New Hampshire, United States) were measured in human plasma.

Peripheral blood mononuclear cell isolation Blood samples were collected in ethylenediaminetetraacetic acid tubes. Whole blood was centrifuged to separate plasma, then peripheral blood mononuclear cells (PBMCs) were isolated using LSM 1077 Lymphocyte Separation Medium (PAA Laboratories GmbH, Pasching, Austria) by standard gradient centrifugation. The isolated cells were washed twice in phosphate buffered saline (PBS) solution with 1% heat-inactivated fetal bovine serum (FBS) (Gibco, Life Technologies, Carlsbad, California, United States).

Cell culture conditions A total of 1×10^6 PBMCs were suspended in RPMI 1640 medium (Gibco, Life Technologies) with 10% FBS, 200 mM L-glutamine, and 5 mg/ml gentamicin (Sigma Aldrich, Saint Louis, Missouri, United States), and were cultured using the Leukocyte Activation Cocktail (LAC), with BD GolgiPlug from BD Biosciences (San Jose, California, United States) for 4 hours at 37 °C in a humidified atmosphere containing 5% CO_2.

Flow cytometry measurements Freshly isolated or LAC-stimulated PBMCs were washed with PBS + 1% FBS and stained with the following monoclonal antibodies provided by BD Biosciences: anti-CD3-PerCP (clone SK7), anti-CD4-APC (clone RPA-T4), anti-CD8-APC-H7 (clone SK1), and anti-CD25-PE (clone M-A251). Once the cells had been stained for 20 minutes at 4 °C in the dark, they were washed with PBS + 1% FBS and suspended in Perm/Wash Buffer (BD Bioscience). Following the process of permeabilization, the cells were washed with Perm/Wash Buffer and stained with anti-FOXP3-FITC (eBioscience, clone 236A/E7) or anti-IL-17A-PE (clone N49-653) for 20 minutes at 4° C in the dark. Subsequently, the cells were washed with Perm/Wash Buffer, suspended in PBS + 1% FBS and collected using BD FACSVerse Flow Cytometer (BD Biosciences). The results were analyzed using FlowJo v10 (Ashland, OR, United States).

First, the lymphocytes were gated on the basis of forward-scatter and side-scatter signals, and a population of CD3$^+$ T cells was selected. Then, CD4$^+$ T cells were separated within this group. Treg cells were gated based on the expression of CD25 and FOXP3 from freshly isolated PBMC, whereas Th17 cells were gated by the expression of interleukin 17A (IL-17A)

within $CD4^+$ T cell subset from LAC-stimulated PBMCs. Finally, Fluorescence Minus One controls were used to determine positivity of the evaluated antigens.

Statistical analysis Non-normal distribution of the continuous variables was defined when significant deviation from normal distribution ($P < 0.05$ using Shapiro-Wilk test) was observed in any of the investigated groups. Continuous variables with normal distribution were compared among the 3 groups using analysis of variance, whereas continuous variables with non-normal distribution were compared between the 3 groups using Kruskal-Wallis test. Then, categorical variables were compared with χ-squared test. The continuous variables are presented as mean (SD) or as median (interquartile range), whereas the categorical variables are presented as numbers and percentages. Linear regression analysis adjusted for age, sex, body mass idex (BMI), and smoking status (ex- and current smokers) was performed in order to test the effect of PHA and RHT on the selected T lymphocyte subpopulations. Correlation analysis was carried out using Spearman rank correlation test. A P value below 0.05 was considered statistically significant. All remaining analyses were performed using IBM SPSS Statistics (New York, United States) package (version 26.0) and graphs were drawn using GraphPad Prism v9.1.0.

RESULTS

Clinical characteristics of study groups We assessed 20 normotensive CTRLs, 21 patients with PHA, and 20 patients with RHT (TABLE 1). The study concluded that PHA and RHT patients presented crucial differences in ABPM values and had lower total and low-density lipoprotein (LDL) cholesterol levels than CTRL participants (TABLE 1). Moreover, PHA and RHT patients were more often diagnosed with type 2 diabetes mellitus (T2DM) and coronary artery disease (TABLE 1). It is worth mentioning that only PHA patients were characterized by significantly higher PA levels but lower plasma levels of angiotensin II (Ang II) and renin as compared with CTRL participants (TABLE 1).

Resistant hypertension is associated with high number of $CD4^+$ T cells It is interesting to note that total white blood cell count was highest in the RHT group (TABLE 1). Furthermore, an additional analysis revealed that the number of T cells was higher in RHT than in CTRL individuals (Supplementary material, *Figure S1A*). This increase was particularly marked for $CD4^+$ T cells, and not so for $CD8^+$ T cells (Supplementary material, *Figure S1B* and *S1C*). RHT patients had higher number of $CD4^+$ T cells than PHA patients (Supplementary material, *Figure S1B*).

T-cell imbalance in primary hyperaldosteronism and resistant hypertension First, we focused on analyzing the data on $CD4^+$ $CD17^+$ lymphocytes in RHT and PHA patients and compared them with the CTRL data (FIGURE 1A). As $IL\text{-}17A^+$ cells were implicated in the pathogenesis of hypertension and autoimmunity, we decided to perform the intracellular staining and proved that $CD4^+$ T cells isolated from RHT patients produced greater amounts of IL-17A in comparison with PHA patients and CTRL participants (Supplementary material, *Figure S2*). The multiple linear regression model adjusted for age, sex, BMI, and current smoking status showed that RHT was associated with higher $CD4^+IL\text{-}17A^+$ T cell number per microliter (FIGURE 1A).

No significant difference in the total number of $CD4^+CD25^+FOXP3^+$ Treg was observed among the investigated groups (FIGURE 1B, Supplementary material, *Figure S2B*).

Hyperaldosteronism promotes T-cell imbalance In the beginning, we focused on the interplay between Th17 and Treg lymphocytes in RHT and PHA patients as compared with CTRL participants (Supplementary material, *Figure S3*). The correlation between Th17 to Treg ratio and aldosterone to renin ratio (ARR) was established in order to investigate the effect of RAA system on T-cell imbalance. Finally, a strong positive correlation between Th17 to Treg ratio and ARR was found in the PHA group (FIGURE 2).

T cells and blood pressure elevation In the patients with high serum aldosterone level (the PHA group), a positive correlation was found between 24 h SBP and the number of $CD4^+IL\text{-}17A^+$ lymphocytes (FIGURE 3A). This observation might point out to $IL\text{-}17A^+$ as an essential factor in developing and maintaining high blood pressure. Moreover, a positive correlation was shown between $CD4^+CD25^+FOXP3^+$ T cells and 24 h SBP in ABPM (FIGURE 3B). No significant correlation was observed in the remaining 2 groups of RHT and control patients.

T cells and target organ damage Higher LVMI, interventricular septum diameter, and posterior wall thickness were measured in the PHA group and compared with the data on the CTRL group (TABLE 2).

A significant difference in LVMI was observed for 3 investigated groups (TABLE 2). However, no significant correlation was found between Th17 and Treg cells and LVMI (Supplementary material, *Figure S4*) or aldosterone concentration (data not shown).

DISCUSSION

This study revealed that RHT patients had increased $CD4^+IL\text{-}17A^+$ T cell number as compared with CTRL and PHA participants. A positive relationship with $CD4^+IL\text{-}17A^+$ T cells and regulatory $CD4^+CD25^+FOXP3^+$ T cells was found in terms of 24 h SBP in PHA. However, no significant correlation was established between T cells and LVMI in any of the studied groups.

TABLE 1 Clinical characteristics of patients with primary hyperaldosteronism, resistant hypertension, and normotensive controls

Clinical features	CTRL (n = 20)	PHA (n = 21)	RHT (n = 20)	*P* value
Men	9 (45)	12 (57.1)	12 (60)	0.6
Age, y	56 (42.25; 58.5)	58 (50; 62.5)	58.5 (47.25; 63.5)	0.18
BMI, kg/m^2	23.7 (2.5)	29.2 (5.6)	32.2 (6.1)	<0.001
Characteristics of blood pressure				
24 h ABPM, systolic mm Hg	114 (7.0)	140.1 (17.1)	144.2 (8.0)	<0.001
24 h ABPM, diastolic mm Hg	73.05 (4.7)	83.2 (8.6)	85.3 (12.4)	<0.001
Daytime ABPM, systolic mm Hg	117.5 (7.6)	141.8 (17.5)	146.4 (21.5)	<0.001
Daytime ABPM, diastolic mm Hg	76.5 (5.0)	85.0 (9.7)	88.8 (12.8)	0.001
Nocturnal ABPM, systolic mm Hg	100.7 (7.7)	133.0 (22.2)	136.6 (17.4)	<0.001
Nocturnal ABPM, diastolic mmHg	62.1 (4.9)	77.1 (9.6)	79.1 (11.7)	<0.001
24 h heart rate, n/min	73 (68.25; 78.5)	65 (61;75)	69.5 (63; 83)	0.10
Aldosterone, pg/ml	113 (90; 158.5)	300 (212.5; 479)	119.5 (100.65; 190.75)	<0.001[a]
Angiotensin II, pg/ml	6.44 (4.23; 11.3)	2.5 (1.29; 5.13)	3.77 (2.52; 12.02)	<0.02[a]
Renin, pg/ml	8.9 (5.1; 12.55)	3.4 (1.95; 4.9)	11.5 (6.38; 23.3)	<0.001[a]
Characteristics of renal function				
GFR, ml/min per 1.73 m^2	75.05 (63.67; 91.3)	84.5 (71.15; 95.8)	92.5 (67.98; 102.93)	0.25
Uric acid, µmol/l	297.2 (99.8)	341.0 (81.2)	351.6 (107.3)	0.28
Characteristics of cardiovascular risk				
Current smokers	2 (10)	3 (14.3)	3 (15)	0.88
Past smokers	3 (15)	6 (29)	7 (35)	0.34
Total cholesterol, mmol/l	5.5 (0.9)	4.6 (1.1)	4.5 (1.3)	0.01
LDL cholesterol, mmol/l	3.4 (0.7)	2.7 (0.8)	2.6 (1.1)	0.007
Type 2 diabetes	0	6 (28.6)	9 (45)	0.004
Inflammatory disease				
hs-CRP, mg/dl	0.19 (0.08; 0.79)	0.17 (0.08; 0.3)	0.14 (0.1; 0.25)	0.54
WBC count, cell/µl	6.1 (1.7)	6.7 (1.6)	7.9 (2.3)	0.01
Medical history				
Prior myocardial infarction	0	0	3 (15)	0.04
Coronary artery disease	0	2 (9.5)	7 (35)	0.005
Stroke	0	2 (9.5)	0	0.14
Heart failure	0	1 (4.8)	1 (5)	0.6
Medications				
ACE inhibitors	0	6 (28.6)	10 (50)	0.001
Angiotensin II receptor blockers	0	8 (38.1)	7 (35)	0.008
β-Blockers	0	14 (66.7)	19 (95)	<0.001[a]
Calcium channel blockers	0	14 (66.7)	17 (85)	<0.001
α-Blockers	0	9 (42.9)	8 (40)	0.003
Thiazide diuretics	0	7 (33.3)	14 (70)	<0.001[a]
Loop diuretics	0	3 (14.3)	6 (30)	0.028
Statins	0	9 (42.9)	14 (70)	<0.001

a Data reflecting PHA vs RHT comparison

Continuous variables are shown as mean (SD) or as median (Q1;Q3), whereas categorical variables are shown as number (percentage)

Abbreviations: ABPM, ambulatory blood pressure monitoring; ACE, angiotensin-converting enzyme; BMI, body mass index; CAD, coronary artery disease; CTRL, normotensive controls; GFR, glomerular filtration rate; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; PHA, primary hyperaldosteronism; RHT, resistant hypertension; WBC, white blood cell

To our knowledge, this is the first study showing the association of peripheral T-cell phenotypes with cardiac hypertrophy in humans with PHA and RHT. It is true that a substantial number of experimental studies have already documented the pathogenic role of T cells in the development of hypertension.[2,16,17] We should bear in mind, though, that the data derived from currently available clinical studies seem to be rather limited.[1,18,19]

T lymphocyte subsets play a pivotal role in eradication of invading bacteria, viruses, and

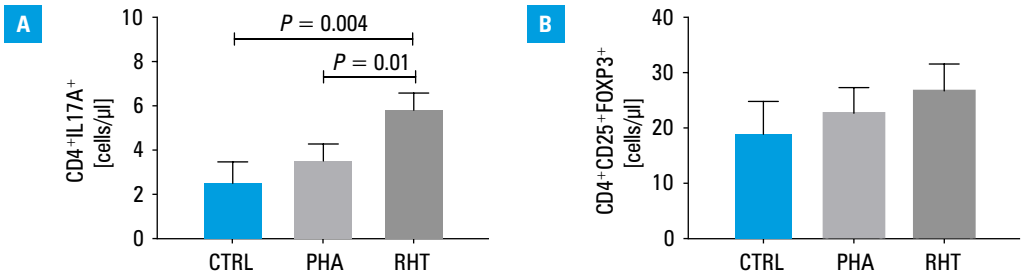

FIGURE 1 T helper 17 and T regulatory subsets in patients with primary hyperaldosteronism (PHA), resistant hypertension (RHT), and normotensive controls (CTRL). **A** – number of $CD4^+IL17A^+$ (Th17) cells per µl, n = 20 for CTRL, n = 21 for PHA, n = 20 for RHT; **B** – number of $CD4^+CD25^+FOXP3^+$ (Treg) cells per µl, n = 18 for CTRL, n = 19 for PHA, n = 18 for RHT. Values indicate adjusted mean (standard error of the mean) estimated for the mean values of all covariates.

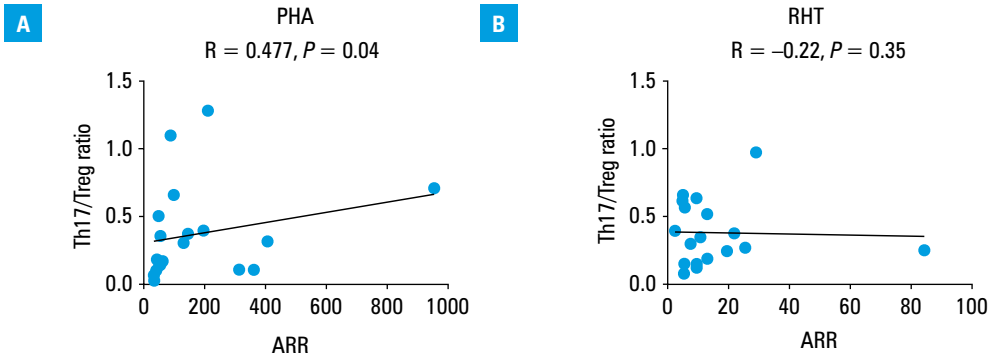

FIGURE 2 Correlations between the Th17/Treg ratio and aldosterone to renin ratio (ARR) in patients with primary hyperaldosteronism (PHA) and resistant hypertension (RHT). **A** – Spearman rank correlation between the Th17/Treg ratio and ARR in patients with PHA (n = 19); **B** – Spearman rank correlation between the Th17/Treg ratio and ARR in patients with RHT (n = 18)

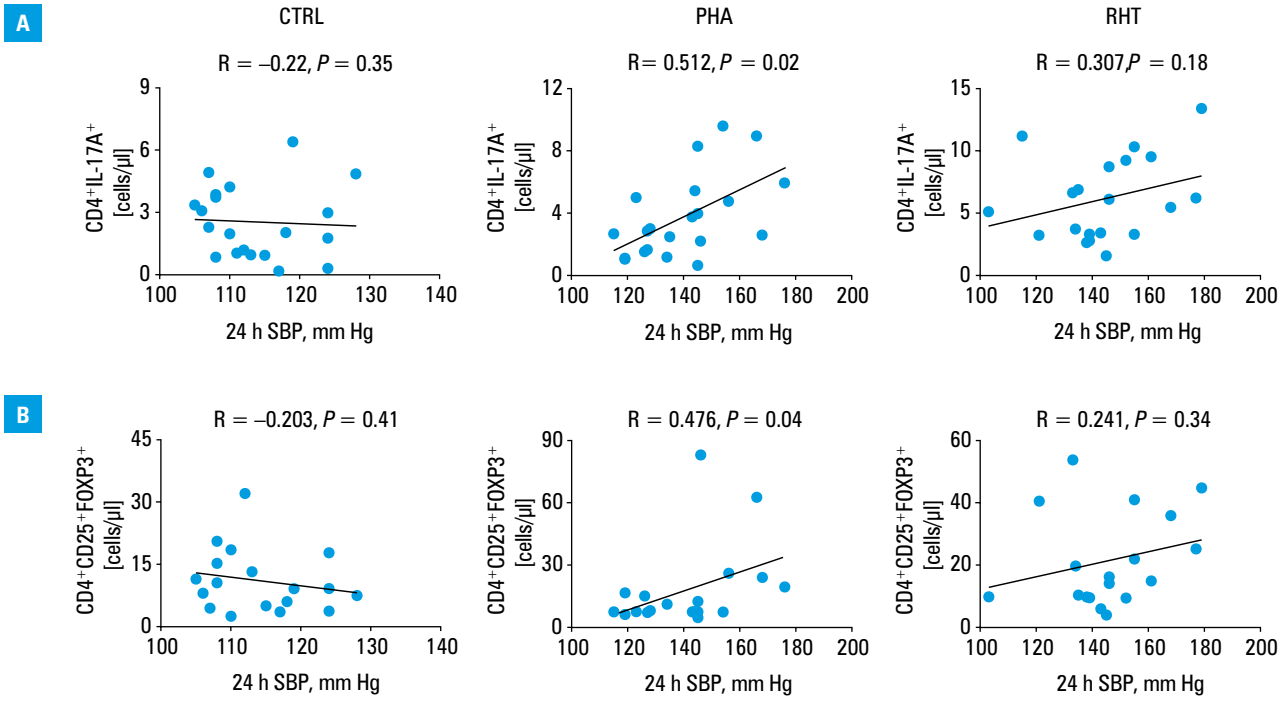

FIGURE 3 Correlations between Th17 or Treg content and 24 h systolic blood pressure in patients with primary hyperaldosteronism (PHA), resistant hypertension (RHT), and in normotensive controls (CTRL). **A** – Spearman rank correlations between $CD4^+IL\text{-}17A^+$ T-cell content and 24 h systolic blood pressure (24 h SBP), n = 20 for CTRL, n = 21 for PHA, n = 20 for RHT; **B** – Spearman rank correlations between $CD4^+CD25^+FOXP3^+$ content and 24 h systolic blood pressure (24 h SBP), n = 18 for CTRL, n = 19 for PHA, n = 18 for RHT

TABLE 2 Echocardiographic characteristics of patients with primary hyperaldosteronism or resistant hypertension in comparison with normotensive controls

Echocardiographic characteristics	CTRL (n = 20)	PHA (n = 21)	RHT (n = 20)	*P* value
LVEF, %	66.7 (6.7)	67.9 (5.1)	63.4 (7.0)	0.08
IVSD, mm	8.4 (7; 9.75)	11.7 (9.6; 13.2)	13.55 (11.2; 14.33)	<0.001
PWD, mm	8.7 (1.5)	11.5 (1.7)	12.6 (2.1)	<0.001
LVMI, g/m^2	77.0 (21.2)	122.3 (30.7)	138.0 (34.2)	<0.001
LAVI, ml/m^2	28.7 (9.8)	36.2 (9.5)	37.0 (15.7)	0.07
GLS	20.3 (2.6)	18.1 (3.3)	16.3 (3.5)	0.001

Values are shown as mean (SD) or median (Q1;Q3)

Abbreviations: GLS, global longitudinal strain, IVSD, interventricular septum diameter; LAVI, left atrial volume index; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; PWD, posterior wall diameter; others, see **TABLE 1**

other pathogens by migrating into injured tissues and producing inflammatory cytokines to combat specific pathogens.[20]

Activation of the majority of naïve T lymphocytes requires 2 signals from antigen presenting cells. Initially activated by dendritic cells, naïve CD4$^+$ T lymphocytes undergo differentiation into Th1, Th2, Treg, Th17, Th9, or T follicular helper cells. It was proved that each subset of differentiated T cells fulfils its own unique function and can secrete its own cytokine panel.[20]

Th17 lymphocytes produce a wide variety of proinflammatory cytokines, including IL-17A, interleukin 21, interleukin 22, and play a significant role not only in preventing autoimmune disorders but also in defending exogenous pathogens. Recently, IL-17 has been closely investigated in various models of hypertension. Th17 and IL-17 perform multiple functions, such as orchestrating dysfunction of endothelial cells and regulating sodium homeostasis in kidneys.[21,22]

Ang II–induced hypertension is associated with increased production of IL-17A by T cells. Madhur et al[21] discovered that, similarly to wild type (WT) mice, IL-17A$^{-/-}$ mice were capable of increasing their blood pressure quite significantly, but not to sustain elevated blood pressure response. Moreover, the study provided evidence that, in comparison with WT mice, IL-17A$^{-/-}$ mice were less prone to developing vascular inflammation and preserved vascular function in response to Ang II administration.[21]

Amador et al,[23] in a study in DOCA salt treated rats, showed a substantial increase in Th17 lymphocytes. Furthermore, it was indicated that the use of antibodies against IL-17A decreased organ fibrosis and reduced blood pressure response.[23]

Yet another study, conducted by Kamat et al,[24] revealed that, in contrast to hypertensive WT mice, IL-17A$^{-/-}$ mice treated with Ang II presented preserved diuresis and natriuresis in response to acute saline challenge. Interestingly, Nguyen et al[25] focused in their study on impaired vessel relaxation due to reduced nitric oxide production and on the development of blood pressure response, following the infusion of recombinant IL-17A in mice.

In the study undertaken by Saleh et al,[17] the usage of antibodies against IL-17A or IL-17RA, but not IL-17F, generated a decrease in blood pressure and renal fibrosis in comparison with control IgG1 antibodies.

Herrada et al[8] provided evidence that modulation of the dendritic cell function by aldosterone enhanced activation of CD8$^+$ T cells and produced CD4$^+$IL-17$^+$ polarized responses, which in turn could have contributed to the inflammatory damage, eventually leading to cardiovascular disease and hypertension. According to their study, the application of spironolactone, a MR antagonist, prevented the effects induced by aldosterone. Moreover, aldosterone limited the induction of suppressor FOXP3-positive Treg cells.[26] Scientific research revealed a crucial role of the MR expression in immune cells, brain, heart, and vasculature.[9,27,28] In this study, we also observed elevated parameters of cardiac hypertrophy, such as LVMI in patients with PHA and RHT. Our research findings concerning a significant correlation between IL-17A and LVMI in the hypertensive patients were consistent with the data collected by Gackowska et al[19] in their study in children with primary hypertension. Furthermore, similarly to Itani et al,[18] we reached the conclusion that hypertensive humans had a higher percentage of CD4$^+$IL17-A T cells than normotensive control participants.

It is worth mentioning that, being a key parameter in the diagnosis of PHA, ARR is also considered a basic parameter indicating a direct relationship between high aldosterone and low renin plasma levels in PHA. In this study, we established a positive correlation between aldosterone to renin and CD4$^+$IL-17/Treg ratios. However, no correlation was found between aldosterone level and Th17 cell content in the PHA group (data not shown). The possibility that aldosterone exerts an impact on T-cell phenotype, and especially, on CD4$^+$IL-17A$^+$ differentiation, should not be excluded. We decided not to conduct any experiments on isolated T cells cultured

in the presence of high aldosterone levels. Based on the outcomes of our study, a conclusion might be drawn that further research is needed to investigate the direct effect of aldosterone level on $CD4^+IL\text{-}17A^+$ content.

Despite the increase in circulating Th17 cells in RHT, the level of high sensitivity C-reactive protein (hs-CRP) in those patients did not differ from its levels in PHA and CTRL participants. The level of hs-CRP can increase in many conditions, such as bacterial and viral infections, pregnancy, cancer, or obstructive sleep apnea. Literature data show that patients with high cardiovascular risk and especially patients with PHA or RHT are characterized by higher hs-CRP levels in comparison with healthy controls. Moreover, patients with the metabolic syndrome present higher hs-CRP levels than healthy controls. In our study, no significant difference in hs-CRP levels between the study groups and normotensive controls was noticed. Its level was the lowest in the RHT group which might be associated with the highest cardiovascular risk and result from the intensive pharmacotherapy for hypertension. Moreover, RHT patients presented the lowest LDL cholesterol level. Thus, it is possible that statins could influence not only LDL cholesterol level, but also the level of hs-CRP in this group. Interestingly, Junqueira et al[29] provided evidence that the levels of CRP differed in patients with RHT, depending on whether they were or were not treated with statins.

Data collected from experimental rat models indicated that administration of spironolactone could prevent vascular damage, regardless of hypertension.[23] This finding might lead to the conclusion that aldosterone contributes to target organ damage through nonhemodynamic effects.[9, 30] Clinical trials, such as RALES (Randomized Aldactone Evaluation Study), 4E (4E – Left Ventricular Hypertrophy Study), and EPHESUS (Eplerenone Post–Acute Myocardial Infarction Heart Failure Efficacy and Survival Study) showed that MR blockade could markedly improve cardiovascular and renal functions in patients suffering from illnesses characterized by higher RAAS activity, for example, heart failure, hypertension, or acute myocardial infarction.[31,32] Examination of diabetic humans revealed that serum levels of proinflammatory IL-17A were significantly higher in hypertension accompanied by T2DM than in DM alone.[33]

In our study, the percentage of T2DM was rather high in both study groups. The multicenter Japan Primary Aldosteronism Study (JPAS) showed that the prevalence of T2DM in the Japanese population with primary aldosteronism reached 28.7% in comparison with 13.2% in the Nagahama city population with hypertension (the Nagahama Study).[33] Similarly, in our study group, the percentage of T2DM was 28.6%. It is well known that aldosterone can contribute to a significant reduction in insulin secretion due to hypokalemia. Thus, hyperaldosteronism plays a pivotal role in developing insulin resistance. Chronically elevated aldosterone levels are associated with higher risk of the metabolic syndrome development and poor hypertension control.[17]

Interestingly, in our study, patients with RHT were characterized by even higher incidence of T2DM (45%). Overall, in the RESIST-POL study, the percentage of the metabolic syndrome among the 204 patients with RHT was about 65%. True resistant hypertension was associated with a significant overlapping of the metabolic syndrome, obstructive sleep apnea, and primary hyperaldosteronism, all frequently found in the study patients.

In our study, the patients diagnosed with RHT presented higher BMI than those with PHA.

Our analysis additionally revealed a significant correlation between $CD4^+CD25^+FOXP3^+$ T lymphocytes and 24 h SBP in PHA. Regulatory T cells are a subpopulation of T cells comprising 5%–10% of all peripheral $CD4^+$ T cells. They are crucial for the induction and maintenance of immune tolerance.[34]

Our research demonstrated that dysregulated Treg cells might play an important role in the development of infection, chronic inflammation, or autoimmune disorders. Treg cells develop mainly in the thymus, but a small percentage can also differentiate in peripheral sites (from naïve $CD4^+$ T cells). It is worth noticing that Treg cells can perform their functions in varied microenvironments.[34] Moreover, it seems evident that Treg cells may protect the body against cardiovascular diseases due to their ability to reduce inflammation.[35]

The first study reporting on the role of Treg cells in pathogenesis of experimental, genetic hypertension was conducted by Viel et al.[36] Research undertaken afterwards by Barhoumi et al[37] proved that adoptive transfer of Treg cells in C57BL/6 mice blunted Ang II–induced oxidative stress, hypertensive response, and endothelial dysfunction. Furthermore, such a transfer reduced the plasma cytokine level and macrophage infiltration in the aorta.[37] Similar conclusions were drawn based on the experimental models of aldosterone--induced hypertension.[4] Moreover, Kasal et al[4] emphasized that blood pressure elevation, oxidative stress, and vascular remodeling could be prevented by previous adoptive transfer of Treg cells. In a study conducted by Matrougui et al,[38] Treg cells were presented as the key cells mediating endothelial dysfunction in coronary arteries.

A randomized, double-blind study conducted in hypertensive humans with atherosclerosis showed that a combination treatment with telmisartan and rosuvastatin reduced blood pressure and increased Treg cell count.[39] However, other experimental works in this field reported conflicting results. A study by Kvakan et al[40] demonstrated that adoptive transfer of Treg cells could prevent Ang II–induced cardiac fibrosis and hypertrophy. Nevertheless, it had no impact on hypertensive response development.[40] Furthermore, administration of Treg cells did not exert any effects on blood pressure in aortic coarctation-induced hypertensive murine models.[41] These findings were

in line with other comprehensive research studies which showed no significant effects of Treg cells on SBP in response to Ang II infusion in Apoe$^{-/-}$ mice.[34,42]

In general, we did not observe any major differences in Treg cell count between RHT, PHA, and normotensive controls. However, a trend towards a higher number of peripheral Treg cells in the hypertensive groups was detected. Certain discrepancies between the studies could be accounted for by diverse experimental conditions and different mechanisms underlying hypertension.

It should be strongly emphasized that data in humans remain limited and concern mainly humans with primary hypertension. Thus, it can be hypothesized that a higher Treg cell number might be a compensation reaction in the advanced form of hypertension with target organ damage, and that it is related to hypertension duration.

Data collected from experimental models of hypertension indicated that the Th17/Treg imbalance, described as an increase in Th17 level and a decrease in Treg cells level, could contribute to the development of a hypertensive response, that is, spontaneously induced hypertension, Ang II–induced hypertension, and DOCA salt–induced hypertension.

Katsuki et al[43] noticed a significant reduction in the Th17/Treg imbalance in the spleen of spontaneously hypertensive rats after renal denervation. Moreover, spironolactone and anti-17A antibody ameliorated T-cell imbalance in the kidney and heart of DOCA salt–induced hypertensive rats.[23]

The Th17/Treg imbalance was also confirmed by Du et al,[44] who reported lymphocyte infiltration in the renal and cardiac tissues resulting in decreased expression of interleukin 10 and increased expression of proinflammatory IL-17A, IL-23, and tumor necrosis factor α.

It is worth noting that the Th17/Treg imbalance in Ang II–induced hypertension was caused by serum / glucocorticoid-regulated kinase 1 (SGK-1). Administration of EMD638683 (SGK-1 inhibitor) prevented the Ang II–induced pathological changes in the heart and kidney.[44]

Based on numerous findings, it can be postulated that immune cells might perform an important role in the pathogenesis of secondary hypertension and hypertension-related cardiac hypertrophy. Further exploration of the exact role of T cells might be crucial for future immunomodulatory therapies (as shown in the CANTOS [Canakinumab Anti-Inflammatory Thrombosis Outcomes Study]).[45]

Additionally, a substantial number of studies demonstrated hypertension to be a relevant risk factor for premature death among patients diagnosed with COVID-19.[46-49] It could be assumed that activation of innate immune mechanisms and more exaggerated systemic inflammatory response were associated with worse clinical outcome in patients diagnosed with COVID-19 and hypertension.[46,47] Studies by Bartoloni et al[47] and Bienvenu et al[48] provided valuable evidence that components of the adaptive immune system, especially CD8$^+$ T cells, were dysfunctional in hypertensive patients. Moreover, boosted inflammatory responses as well as accelerated aging in patients with hypertension might contribute to the higher risk of severe COVID-19 complications.[47,49]

Our study has several limitations. Firstly, relatively few patients were enrolled. Nonetheless, it should be stated that other authors were able to reach valid conclusions in their research in groups of similar size.[18,19] Secondly, we observed a high percentage of patients suffering from T2DM in both study groups. The fact that PHA and RHT are often related to T2DM was also confirmed by Hall et al.[50] It must be emphasized that we examined a limited number of T-cell phenotypes and studied the T-cell imbalance in relation to the parameters of cardiac hypertrophy. Moreover, the role of T cells in hypertension was analyzed mainly in animal models. Just a few studies were conducted in humans with primary hypertension, and there were almost no data regarding T-cell characteristics in secondary hypertension. Since we analyzed only circulating T lymphocytes at one time point, our findings should be interpreted with caution.

Another limitation of this study is a lack of live / dead staining. However, our previous studies revealed an extremely high percentage of live cells isolated from peripheral blood.

It should be also underlined that no biopsies were performed to examine the phenotype of T cells in human organs. Hence, more studies are urgently required to define the direct role of adaptive immunity in hypertension related target organ damage.

This is the first study demonstrating that human T cells provide evidence of IL-17A production in patients with advanced hypertension. Presumably, an increase in peripheral Treg cells, seen as a defensive reaction, is the answer to a long-standing question of hypertensive stimulation. On the whole, the results of our study support previous research findings on T-cell phenotype in animal models with primary hypertension. Undoubtedly, further studies are required to confirm our conclusions and provide a sufficient explanation of as yet unresolved issues.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

ARTICLE INFORMATION

ACKNOWLEDGMENTS The study was conducted thanks to the Polish Ministry of Science and Higher Education Diamond Grant to AMI and AJ (2015–2018; DI 2013 – 014043) and the Polish National Centre for Research and Development (ERA-CVD/NEMO/7/2019 – TPM). We would like to thank Monika Paciorek for providing technical assistance.

CONTRIBUTION STATEMENT AMI and TPM designed and conducted the experiments and wrote the manuscript. AMI, AJ, TJG and AP worked on the clinical part of the study. TPM and AKR performed the flow cytometric analysis. MS, PD and MJ were responsible for methodology and interpreted the data. AMI provided the clinical samples. BG and EBK collected the samples in the outpatient clinic. PD performed echocardiography examinations. AW recorded RAAS measurements. AP, TJG and AJ supervised the project and provided comments on the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST None declared.

HOW TO CITE Imiela AM, Mikołajczyk TP, Siedliński M, et al. Th17/Treg imbalance in patients with primary hyperaldosteronism and resistant hypertension. Pol Arch Intern Med. 2022; 132: 16171. doi:10.20452/pamw.16171

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