Altered circulating Tregs and their functional cytokines in hypertensive patients with or without left ventricular hypertrophy

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Original investigation

Keywords: hypertension, left ventricular hypertrophy, Tregs, IL-10, IL-6, TGF-β1

Posted Date: August 30th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-847306/v1

Abstract

Background:

Left ventricular hypertrophy (LVH) is the most common target organ damage in hypertension. In addition to the increased left ventricular afterload, immune injury participates in LVH pathogenesis. $CD4^+CD25^+Foxp3^+$ regulatory T lymphocytes (Tregs) are a T cell subset with immunomodulatory effects; abnormal Treg numbers or functions can cause immune disorders. This study aimed to explore the role of Tregs in LVH by investigating circulating Tregs and associated cytokine levels in hypertensive patients with LVH.

Methods:

Blood samples were collected from 69 healthy individuals (control group, CG), 91 hypertensive patients with LVH (LVH group) and 83 hypertensive patients without LVH (essential hypertension, EH group). Circulating Tregs and associated cytokines were measured by flow cytometry and enzyme-linked immunosorbent assays, respectively.

Results:

Circulating Tregs were significantly lower in EH and LVH patients than in CG subjects. The circulating Treg level was lower in LVH patients than in EH patients. No correlation between blood pressure regulation and Treg levels was found in EH or LVH patients. Furthermore, circulating Tregs in postmenopausal females were lower than those in males among LVH patients. Additionally, serum IL-10 and transforming growth factor (TGF)-β1 were decreased in EH and LVH patients, and the serum IL-6 level was significantly increased in LVH patients. Circulating Tregs were negatively correlated with CK, LDL, apoB, high-sensitivity C-reactive protein and left ventricular mass index values.

Conclusion:

Our study is the first to demonstrate the significantly decreased circulating Treg proportion in LVH patients. The protective effect of Tregs in LVH is independent of blood pressure regulation. The functional Treg cytokines IL-6, IL-10 and TGF-β1 participate in this immunomodulatory process.

Trial registration:

The study was approved by the Institutional Medical Ethics Committee of the Second Xiangya Hospital of Central South University (2018/No.046).

1. Background

Left ventricular hypertrophy (LVH) is the compensatory response of the left ventricle to the increased cardiac afterload in hypertension. LVH is an independent risk factor associated with adverse outcomes,

including myocardial infarction, heart failure and sudden death [1, 2]. It is worth noting that LVH persists in hypertensive patients even after the application of antihypertensive drugs to control blood pressure, suggesting that LVH is not just a mechanical adaptation caused by the increase in the afterload. Several studies have demonstrated that inflammation and immunoregulation are essential in pressure overload-induced LVH that occurs in response to hypertension [3, 4]. Mice that lack lymphocyte responses have a reduced hypertensive response to chronic Ang II infusion and accordingly develop less LVH [5]. This indicates that T cells participate in the onset of Ang II-induced hypertension and LVH.

$CD4^+CD25^+Foxp3^+$ regulatory T lymphocytes (Tregs), potent suppressors of effector T cells, can negatively regulate immune responses, thereby maintaining immune homeostasis and immunologic self-tolerance [6]. Decreased proportions of Tregs were observed in the renal cortex of AngII-infused mice [7] and in the peripheral blood and spleen of stroke-prone spontaneously hypertensive rats (SHRs) [8] even before the onset of hypertension. Furthermore, approaches that increase circulating Treg levels in vivo, such as adoptive transfer of Tregs and administration of an IL-2/anti-IL-2 monoclonal antibody complex, were shown to effectively reduce blood pressure in animals with hypertension [7–10]. In addition to Tregs regulating the onset and progression of hypertension, Treg depletion can promote LVH in SHRs [8]. Administration of the IL-2/anti-IL-2 monoclonal antibody complex before transverse aortic constriction was found to attenuate the development of LVH in mice [11]. In addition, adoptive transfer of Tregs reduced LVH without affecting blood pressure in AngII-infused mice [12]. Thus, attenuation of LVH by Treg transfer may be attributed to not only a decrease in blood pressure but also immunosuppressive effects independent of blood pressure regulation.

Tregs exert immunosuppressive effects mainly through the secretion of several anti-inflammatory cytokines, including transforming growth factor (TGF)-β1 and IL-10 [6]. Recent studies have confirmed that IL-10 secreted by Tregs plays vital roles in preventing hypertension [13]. Another cytokine, IL-6, can regulate immune homeostasis between T helper 17 (Th17) cells and Tregs. In hypertensive patients, increased circulating IL-6 can block the TGF-β1-mediated generation of Tregs and enhance Th17 responses [14–17], indicating that IL-6 can negatively regulate the effects of Tregs.

LVH is quite common in patients with hypertension. Previous studies have found that Tregs are involved in the progression of hypertension and LVH in different animal models and that the levels of circulating cytokines are altered in hypertension. However, the levels of circulating Tregs and related cytokines in human LVH remain unknown. Our study aimed to investigate Tregs and the associated functional cytokines in hypertensive patients with or without LVH and may offer new insight into immunotherapy for LVH.

2. Materials And Methods

2.1. Ethics statement

This study protocol was approved by the Institutional Medical Ethics Committee of the Second Xiangya Hospital of Central South University. The patients themselves or their families provided informed consent.

2.2. Subjects

Study subjects were randomly selected from hospitalized patients in the cardiology department and healthy people at the physical examination center of the Second Xiangya Hospital of Central South University from October 2018 to December 2019. In total, 243 patients were included in this study. They were divided into a control group (CG, n = 69), essential hypertension (EH) group (n = 83), and LVH group (n = 91).

2.3. Reagents and materials

2.3.1 Flow cytometry

Isolated cell suspensions were stained for fluorescence-activated cell sorting (FACS) analysis using the following antibodies: fluorescein isothiocyanate (FITC)-conjugated anti-human CD4 (clone SK3), phycoerythrin (PE)-conjugated anti-human Foxp3 (clone 206D), and allophycocyanin (APC)-conjugated anti-human CD25 (clone BC96) (all from BioLegend). samples were analyzed on a CytoFLEX (Beckman Coulter). Data were analyzed with FlowJo software.

2.3.2 Cytokine assays

Peripheral blood samples were collected using a coagulant. The serum concentrations of IL-10, IL-6 and TGF-β1 were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions.

2.4. Standards for the study subjects

2.4.1. Diagnostic criteria for the study subjects

All included subjects had satisfactory echocardiogram results (Mindray DC-8). The left ventricular end-diastolic diameter (LVEDd), interventricular septal diameter (IVSd), and posterior wall thickness (PWT) were measured. Left ventricular (LV) mass in grams was calculated according to the following formula [18]: LV mass (g) = $0.8\times1.04\times[(LVEDd + IVSd + PWT)^3 - LVEDd^3] + 0.6$. LV mass was indexed to body surface area and is reported in g/m^2: LV mass index (LVMI) = LV mass / body surface area.

The diagnostic criteria for hypertension were as follows: 1) systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg; and/or 2) a history of hypertension with current use of antihypertensive drugs to reduce the blood pressure below 140/90 mmHg, referring to the Guidelines for Prevention and Treatment of Hypertension in China (2018 edition) [19].

The diagnostic criteria for LVH were an LVMI ≥ 115 g/m^2 for males and ≥ 95 g/m^2 for females [20].

2.4.2. Inclusion and exclusion criteria for the research subjects

The inclusion criteria for the study subjects were as follows:

1. Between the ages of 25–80 years old (CG, EH, and LVH groups);
2. In line with the diagnostic criteria for hypertension (EH and LVH groups); and
3. In line with the diagnostic criteria for LVH (LVH group).

The exclusion criteria for the study subjects were as follows:

1. Secondary hypertension;
2. Valvular heart disease, myocarditis, congenital heart disease, severe arrhythmia, or congestive heart failure;
3. Acute and chronic inflammation of the heart or other organs;
4. Abnormal liver and/or kidney function;
5. Malignant tumors or other immune diseases, such as rheumatism;
6. Lung diseases that seriously affect respiratory function;
7. High fever or use of immunomodulatory drugs; and
8. Ongoing pregnancy or breastfeeding.

2.5. Statistical analysis

IBM SPSS 25.0 software (SPSS Inc., Chicago, USA) was used to analyze all the data. Enumeration data were analyzed using the X^2 test, and the results are presented as the constituent ratio. Measurement data are presented as the mean ± standard deviation (M ± SD). Kolmogorov-Smirnov normality tests were conducted for all measurement data. Comparisons among groups with normally distributed data were performed using ANOVA with the least significant difference (LSD) test as the post hoc test. Comparisons between groups with nonnormally distributed data were performed using the Kruskal-Wallis rank-sum test with the Mann-Whitney U test. Pearson's and Spearman's correlation analyses were used to calculate correlations between two variables. A two-tailed value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Basic clinical characteristics of patients

Among the patients who provided blood samples for analysis, body weight, body mass index (BMI), incidence rate of smoking, and blood pressure (including SBP and DBP) were significantly increased in the EH and LVH groups compared with the CG group (all $P < 0.05$), indicating possible roles of these comorbidities in the pathogenesis of EH and LVH. SBP was higher in the LVH group than in the EH group ($P < 0.05$). No significant differences in other characteristics, including age, body weight, BMI, incidence

rate of smoking, and DBP, were found between the EH and LVH groups (all $P > 0.05$). The clinical data of all patients are listed in Table 1.

Table 1
Information of clinical characteristics in CG, EH and LVH groups

Characteristics	CG	EH	LVH
Gender (M/F)	45/24	55/28	59/32
Age (years)	58.45 ± 12.86	58.30 ± 10.88	61.34 ± 11.17
Height (cm)	162.34 ± 7.92	163.06 ± 7.19	163.86 ± 8.78
Weight (kg)	60.58 ± 11.43	67.91 ± 11.18*	69.91 ± 14.08*
BMI (kg/m^2)	22.86 ± 3.12	25.47 ± 3.30*	25.88 ± 3.90*
SBP (mm Hg)	122.41 ± 16.19	141.30 ± 17.75*	148.40 ± 23.68*#
DBP (mm Hg)	78.45 ± 9.18	85.25 ± 13.01*	87.01 ± 15.74*
Smoking (n, %)	18 (26.09%)	40 (48.2%) *	43 (47.3%) *

Compared with the CG group, the EH group did not show any significant differences in the white blood cell count (WBC), neutrophil percentage (N%), high-sensitivity C-reactive protein (hs-CRP) or N-terminal brain natriuretic peptide (NT-proBNP) (all $P > 0.05$). However, these characteristics were increased in the LVH group compared with the CG group (all $P < 0.05$), indicating upregulated inflammation in the LVH patients. The level of NT-proBNP was higher in the LVH group than in the EH group ($P < 0.05$), but no significant differences in the WBC, N% or hs-CRP level were found (all $P > 0.05$) (Table 2).

Table 2
Information of blood biochemical indexes in CG, EH and LVH groups

Indexes	CG	EH	LVH
WBC ($\times 10^9$/L)	6.10 ± 1.37	6.53 ± 1.57	6.68 ± 1.66*
N (%)	61.04 ± 9.33	63.84 ± 7.84	64.79 ± 9.23*
hs-CRP (mg/dL)	1.43 ± 2.37	3.99 ± 7.34	6.01 ± 14.30*
NT-proBNP (pg/ml)	162.16 ± 196.15	165.76 ± 231.22	624.93 ± 1114.08*#
cTnT (μg/L)	8.96 ± 16.83	35.36 ± 160.69	38.49 ± 82.31
CK (pg/ml)	79.73 ± 29.54	96.37 ± 48.34	86.52 ± 42.00
CK-MB (pg/ml)	15.51 ± 10.96	16.20 ± 8.48	15.97 ± 10.02
TG (mmol/L)	1.53 ± 1.09	2.19 ± 1.45*	2.04 ± 1.75
TC (mmol/L)	4.41 ± 0.97	4.03 ± 0.94*	3.94 ± 0.98*
HDL-C (mmol/L)	1.09 ± 0.36	0.95 ± 0.28*	0.96 ± 0.21*
LDL-C (mmol/L)	2.78 ± 0.83	2.51 ± 0.76	2.42 ± 0.85*
HDL-C/TC	0.26 ± 0.10	0.24 ± 0.07	0.26 ± 0.08
FFA (mmol/L)	0.29 ± 0.19	0.34 ± 0.17	0.37 ± 0.18*
Lp(a) (mg/L)	131.14 ± 129.80	310.39 ± 296.31*	332.30 ± 265.49*
apoA (g/L)	1.07 ± 0.25	0.98 ± 0.21	0.98 ± 0.19
apoB (g/L)	0.89 ± 0.27	0.82 ± 0.22	0.79 ± 0.25*
HbA1c (%)	5.41 ± 0.28	6.54 ± 1.29*	6.81 ± 1.13*#

In addition, no significant differences were observed among the three groups for myocardial enzymological indexes, including cardiac troponin T (cTnT), creatine kinase (CK) and an isoenzyme of creatine kinase-MB (CK-MB) (all $P > 0.05$) (Table 2).

Compared with the CG group, the EH group exhibited increased triglycerides (TG), and the LVH group showed increased free fatty acids (FFA) (all $P < 0.05$). Total cholesterol (TC) was lower while lipoprotein (a) (Lp(a)) was higher in the EH and LVH groups than in the CG group, and low-density lipoprotein cholesterol (LDL-C) and apoprotein B (apoB) were lower in the LVH group than in the CG group (all $P < 0.05$). High-density lipoprotein cholesterol (HDL-C) was lower in the EH and LVH groups than in the CG group (all $P < 0.05$), but there were no differences in apoprotein A (apoA) or the HDL-C/TC ratio among the three groups (all $P > 0.05$) (Table 2).

Compared with the CG group, the EH and LVH groups showed increased levels of glycosylated hemoglobin A1 (HbA1c) (all $P < 0.05$). The level of HbA1c was much higher in LVH patients than in EH patients ($P < 0.05$) (Table 2).

3.2. Analysis of patient echocardiograms

The left atrium (LA) was increased in the EH group compared with the CG group ($P < 0.05$). Compared with the CG and EH groups, the LVH group showed higher LA and right atrium (RA) values (all $P < 0.05$).

The IVSd, PWT and LVMI were increased in the EH group compared with the CG group (all $P < 0.05$). Additionally, compared with the CG and EH groups, the LVH group showed higher LVEDd, IVSd, PWT and LVMI values (all $P < 0.05$).

In addition, the ejection fractions (EF) of the three groups were in the normal range (50–70%), but the EF in the LVH and EH groups were both decreased compared with that in the CG group, and the EF was lower in the LVH group than in the EH group (all $P < 0.05$). These data are listed in Table 3.

Table 3
Characteristics of echocardiogram in CG, EH and LVH groups

Characteristics	CG	EH	LVH
IVSd (mm)	8.95 ± 1.01	10.11 ± 1.00*	11.56 ± 1.41*#
PWT (mm)	8.74 ± 0.96	9.41 ± 0.90*	10.97 ± 1.34*#
LVEDd (mm)	45.43 ± 4.37	46.18 ± 3.09	50.54 ± 4.18*#
LVMI (g/m^2)	93.92 ± 19.13	105.11 ± 12.16*	148.70 ± 21.41*#
LA (mm)	31.38 ± 3.35	34.80 ± 3.91*	38.02 ± 3.85*#
RA (mm)	31.05 ± 3.09	30.89 ± 2.98	32.36 ± 3.20*#
RV (mm)	29.86 ± 3.60	30.18 ± 2.85	30.98 ± 3.04
EF (%)	63.93 ± 4.76	62.30 ± 3.69*	57.80 ± 7.98*#

3.3. Circulating $CD4^+$ T cells and Tregs in patients

The levels of circulating $CD4^+$ T cells and Tregs were analyzed by flow cytometry, and the results showed that circulating $CD4^+$ T cells were similar among the CG (41.66 ± 8.54%), EH (42.34 ± 8.03%) and LVH (41.14 ± 9.11%) groups ($P > 0.05$) (Fig. 1(a), 1(b)). Compared with the levels in the CG group (5.17 ± 2.16%), the Treg levels in the EH (4.29 ± 1.86%) and LVH group (3.68 ± 1.51%) were significantly decreased (Fig. 1(a) and 1(c)), and the Treg level was lower in LVH patients than in EH patients (all $P < 0.05$) (Table 4).

Table 4
Tregs and serum cytokine levels in CG, EH and LVH groups

Characteristics	CG	EH	LVH
Treg (%)	5.17 (4.43–5.91)	4.29 (3.86–4.72) *	3.68 (3.35–4.02) *** #
IL-10 (pg/ml)	9.14 (6.92–11.36)	6.12 (4.52–7.72) *	6.11 (4.67–7.56) *
TGF-β1 (ng/ml)	42.92 (39.46–46.38)	37.73 (33.61–41.84) *	37.09 (33.68–40.49) *
IL-6 (pg/ml)	6.52 (3.67–9.38)	7.45 (4.77–10.12)	11.54 (8.10-14.99) * #

3.4. Correlation between blood pressure control and circulating Tregs in hypertensive patients

Blood pressure control is closely related to the occurrence of hypertensive myocardial hypertrophy. To explore whether the decreased level of Tregs in hypertensive patients is affected by blood pressure regulation, the EH and LVH groups were further divided into well-controlled and poorly controlled groups according to each patient's blood pressure control situation (24-hour ambulatory blood pressure was measured). The well-controlled group was defined by a mean SBP < 140 mmHg and mean DBP < 90 mmHg, while the poorly controlled group was defined by a mean SBP ≥ 140 mmHg and/or mean DBP ≥ 90 mmHg. The results showed no difference in Treg levels between the well-controlled and poorly controlled subgroups in either EH patients or LVH patients (all $P > 0.05$) (Fig. 2(a)). Moreover, no correlation was found between Tregs and the duration of hypertension in LVH patients ($P > 0.05$) (Fig. 2(b)). These results reveal that there is no correlation between blood pressure control and circulating Treg levels in hypertensive patients.

3.5. Circulating Tregs in male and female hypertensive patients

In the EH and LVH groups, we further compared the proportion of circulating Tregs between male and female patients, but no sex difference in circulating Tregs was found ($P > 0.05$) (Fig. 3). Several studies have revealed that estrogen could protect the murine heart from pressure overload-induced LVH [21–23]. Considering the possible role of estrogen in attenuating LVH, we compared the Treg levels in hypertensive patients between males (> 49 years old) and postmenopausal females aged over 49 years old (the median age at onset of menopause in Asia) [24].

The results showed that circulating Tregs were lower in postmenopausal females than in males (> 49 years old) in both the LVH and EH groups (all $P < 0.05$) (Fig. 3), indicating that Tregs may participate in the modulatory mechanism through which estrogen protects female patients from hypertension and LVH. In addition, in postmenopausal females, circulating Treg levels were still lower in the LVH group than in the EH group ($P < 0.05$), indicating that decreased circulating Treg levels may participate in the onset of LVH in hypertensive patients even without the effect of estrogen.

3.6. Serum cytokine concentrations in patients

To investigate the expression of functional cytokines related to Tregs, serum IL-10, IL-6, and TGF-β1 levels were measured by ELISA. Decreased IL-10 and TGF-β1 concentrations were observed in the EH and LVH groups compared with the CG group (all $P < 0.05$) (Fig. 4(a-b) and Table 4); no significant differences were observed between the EH and LVH groups for these cytokines (all $P > 0.05$). The serum IL-6 level was increased in the LVH group compared with the EH and CG groups (all $P < 0.05$) (Fig. 4(c) and Table 4). No significant difference in IL-6 was found between the EH and CG groups ($P > 0.05$).

We then assessed whether the level of circulating Tregs was associated with functional cytokines in the serum in CG, EH and LVH patients, and correlation analysis showed that circulating Tregs were negatively correlated with the serum concentration of IL-10 and positively correlated with the serum concentration of TGF-β1 in LVH patients (all $P < 0.05$). No correlation was found between Tregs and serum IL-6 among the three groups (all $P > 0.05$) (Fig. 4(d-f)).

3.7. Associations between circulating Tregs and clinical characteristics

We assessed whether circulating Tregs were associated with the clinical characteristics of all 243 individuals. Correlation analysis showed that circulating Tregs were negatively correlated with LVMI, hs-CRP and CK levels (all $P < 0.05$) but not with age, smoking index, BMI, SBP, DBP, WBC, HbA1c, NT-proBNP, cTnT, CK, CK-MB, TC, TG, HDL-C, LDL-C, Lp(a), apoA, or apoB levels (all $P > 0.05$) (Table 5 and Fig. 5(a-c)). In addition, circulating Tregs were negatively correlated with CK, LDL and apoB in EH patients (all $P < 0.05$) (Table 6 and Fig. 5(d-f)). In the LVH group, circulating Tregs were negatively correlated with CK ($P < 0.05$) (Table 7 and Fig. 5(g)).

Table 5
Correlations between Tregs and clinical characteristics were assessed by Pearson and Spearman's correlation analysis in total 243 patients.

Characteristics	R	P value
Age	-0.0313	0.646
BMI	0.092	0.178
Smoking index	0.0688	0.312
LVMI	-0.1632	0.025*
SBP	0.0083	0.903
DBP	-0.0150	0.825
WBC	0.1262	0.064
hs-CRP	-0.1520	0.049*
cTnT	-0.0634	0.393
CK	-0.1909	0.011*
CK-MB	-0.1040	0.139
NT-proBNP	0.0600	0.402
Lp(a)	-0.1278	0.085
HbA1c	0.0090	0.922
TG	-0.0378	0.588
TC	0.0309	0.658
HDL-C	-0.107	0.123
LDL-C	0.0598	0.392
apoA	-0.0260	0.727
apoB	0.0716	0.334

Table 6
Correlations between Tregs and clinical characteristics were assessed by Pearson and Spearman's correlation analysis in EH patients.

Characteristics	R	P value
CK (pg/ml)	-0.2621	0.028*
LDL-C (mmol/L)	-0.2394	0.041*
ApoB (g/L)	-0.2398	0.046*

Table 7
Correlations between Tregs and CK was assessed by Pearson's correlation analysis in LVH patients.

Characteristic	R	P value
CK (pg/ml)	-0.2587	0.014*

4. Discussion

In the present study, we observed for the first time that circulating Treg levels were decreased in patients with hypertensive myocardial hypertrophy compared with hypertensive patients without LVH. The Treg level was independent of the blood pressure control status. Among hypertensive patients, the circulating Treg level in postmenopausal females was lower than that in males. We also are the first to observe that serum IL-6 levels were increased in hypertensive patients with LVH compared with those without LVH. In addition, the serum levels of the anti-inflammatory cytokines IL-10 and TGF-β1 were decreased in hypertensive patients. In addition, circulating Tregs were positively correlated with the levels of TGF-β1 and negatively correlated with those of CK, hs-CRP, LVMI, LDL and apoB.

Endothelial cells are a vital regulator of vascular tone, and endothelial dysfunction serves as an initiating factor of hypertension. Impaired endothelium-dependent relaxation of the arteries has been found in both hypertensive patients and animal models [25]. Endothelial function can be protected by Tregs [26], whereas a decreased proportion of Tregs can induce endothelial dysfunction and initiate the onset of hypertension [27, 28]. Mice infused with aldosterone or Ang II were found to show endothelial dysfunction, adverse vascular remodeling and elevated SBP, whereas these pathologic processes could be effectively attenuated by adoptive transfer of Tregs [7, 29].

Tregs comprise 5–10% of all peripheral $CD4^+$ T cells in adults. Although $CD4^+$ T cells have no obvious effect on Ang II-induced blood pressure elevation [30], Treg depletion or dysfunction can exacerbate endothelial dysfunction and promote the occurrence of hypertension [8, 27, 28]. Similarly, no significant differences in circulating $CD4^+$ T cells were found among the CG, EH and LVH groups in our study, while

the proportion of circulating Tregs was significantly decreased in patients with hypertension, indicating that the reduced proportion of circulating Tregs might promote the progression of hypertension. Another study revealed that mice treated with Ang II or Ang II + Tregs upregulated $Foxp3^+$ cell infiltration in the heart [12], indicating that circulating Tregs might be recruited to the heart to inhibit the onset of hypertension. It is possible that the renin-angiotensin system is activated in patients with hypertension and that Ang II activates chemoattractant signaling in the recruitment of Tregs from the circulation to cardiac tissue for accumulation in the early stage of hypertension; therefore, circulating Treg levels are decreased in patients with hypertension.

Hypertension increases the workload of the heart and causes structural or functional changes in the myocardium. These changes include LVH, which can cause an electric remodeling process and further contribute to increased incidences of ventricular arrhythmias and sudden cardiac death. Previous studies have revealed that a decreased proportion of Tregs is crucial for the onset of hypertension and LVH in mice and rats [8, 11, 12, 28]. Hypertensive mice treated with Treg transfer develop less LVH and exhibit reduced susceptibility to inducible ventricular tachycardia, accompanied by diminished cardiac cell infiltration and a reduced proportion of activated splenic $CD4^+$ cells [12]. Therefore, Tregs might inhibit LVH and electrical remodeling by inhibiting the activation of immune cells and their migration from peripheral immune organs into the heart. However, these results were all generated with animal models and do not reveal whether the proportion or function of Tregs is altered between LVH and EH patients. In this study, we found that circulating Treg levels were significantly lower in hypertensive patients with LVH than in those without LVH, indicating that decreased circulating Treg levels further facilitate cardiac injury in hypertension.

To explore the correlation between Treg levels and blood pressure regulation, we compared the proportion of Tregs in hypertensive patients according to their blood pressure control condition. The results showed no difference in circulating Tregs between the well-controlled and poorly controlled subgroups. Similarly, mice treated with Ang II + Tregs were found to be as hypertensive as Ang II-treated controls, but cardiac hypertrophy and fibrosis were significantly ameliorated [12]. These findings underscore the notion that Treg-mediated cardiac protection is independent of modulatory effects on blood pressure.

We also explored the sex difference in Tregs in hypertensive patients. We found lower Treg levels in females than in males among hypertensive patients over 49 years old, while no sex difference was found between males and females of all ages. In previous studies, administration of 17β-estradiol, the main circulating form of estrogen in premenopausal females, limited hypertension and LVH in ovariectomized female mice [22, 23], indicating that estrogen may protect premenopausal females from hypertension and LVH. These results indicate that Tregs may mediate the modulatory effect of estrogen in attenuating EH and LVH.

The protective effect of Tregs on microvascular endothelial function in hypertension may be attributed to the release of Treg-associated anti-inflammatory cytokines, such as TGF-β and IL-10, thereby inhibiting NADPH oxidase activity and improving endothelium-dependent relaxation of the arteries [31–33]. We

found for the first time that serum IL-10 and TGF-β1 levels were decreased in hypertensive patients with or without LVH, indicating a role for anti-inflammatory cytokines in the development of EH and LVH. In addition, circulating Tregs were positively correlated with the levels of TGF-β1 in our study. There were two reasons. First, Tregs can secret TGF-β1 [34]. Second, TGF-β1 can induce the differentiation of naive T cells into Tregs and thus increase the number of Tregs [35]. TGF-β1-deficient mice exhibit a significantly decreased proportion of peripheral Tregs [36]. Therefore, the positive correlation between Tregs and TGF-β1 is undeniable.

Surprisingly, we found that circulating Tregs were negatively correlated with the serum levels of IL-10. Similarly, Tlili et al. [29] found that adoptive transfer of Tregs normalized the increased level of IL-10 in Ang II-induced hypertensive mice. They speculated that both Tregs and IL-10 could downregulate the levels of proinflammatory cytokines. After Treg adoptive transfer, feedback loops that modulate proinflammatory cytokines by increasing IL-10 were inhibited, and therefore, the level of IL-10 was decreased. In fact, IL-10 can be produced by multiple cell types, such as neutrophils, dendritic cells (DCs), mast cells, monocytes, macrophages, eosinophils, and natural killer cells, in addition to B cells and $CD8^+$ and $CD4^+$ T cells [37]. We could not rule out the secretory function of other cells in terms of IL-10. In addition, the infiltration of proinflammatory cytokines may act in different ways between the serum and heart tissue. In our study, circulating Tregs were negatively correlated with serum IL-10, while in another study, cardiac IL-10 was elevated after adoptive transfer of Tregs [38], so serum and cardiac IL-10 may have different correlations with circulating Tregs.

IL-6 is produced by a variety of cell types, including monocytes, T cells, B cells and endothelial cells. IL-6 signaling is critical for cardiomyocyte hypertrophy and Th17/Treg balance regulation [16]. Cardiac IL-6 expression is increased in pressure-overloaded hearts [39]. Similarly, serum IL-6 was increased in LVH patients in our study. Studies targeting IL-6 further support an essential role for this cytokine as a driver of inflammatory disease mechanisms in animal models of hypertension, preeclampsia, and LVH. In previous studies, IL-$6^{-/-}$ mice were protected from both AngII-induced hypertension [40, 41] and LVH [42]. Pressure overload-induced LV cardiac remodeling and functional deterioration were shown to be attenuated in the absence of IL-6, indicating an important role for this cytokine in hypertensive myocardial remodeling [42]. In addition, bazedoxifene, a drug for anti-inflammatory therapy, can protect the heart from cardiac remodeling by inhibiting IL-6/gp130 signaling in TAC mice [43]. Thus, IL-6 may be a potential therapeutic target in LVH.

An increased level of CRP or hs-CRP is closely related to the onset of LVH in patients with hypertension and can be an independent predictor of LVH [44, 45]. We also found elevated hs-CRP levels in hypertensive patients with LVH compared with hypertensive patients without LVH and healthy individuals, indicating the hyperinflammatory state in patients with LVH and a possible application for hs-CRP in predicting the occurrence of LVH. In addition, the plasma NT-proBNP level was significantly elevated in patients with LVH in our study. Similarly, plasma NT-proBNP has been found to rise progressively with increasing hypertension severity, particularly when LVH is present [46]. In a previous study on Black patients with hypertension, however, even though NT-proBNP was effective in differentiating hypertensive

subjects with or without LVH from those with hypertensive heart failure, it could not differentiate hypertensive patients with LVH from those without LVH [47]. Therefore, whether NT-proBNP is an ideal marker for LVH is still controversial. The LVMI is commonly used to identify patients with cardiac hypertrophy. We found a negative correlation between circulating Tregs and LVMI values in all 243 subjects, indicating that circulating Tregs may be combined with the LVMI to predict LVH in hypertensive patients. In patients with rheumatic heart disease, Tregs were found to have negative relationship with CK-MB [48]. In this study, we also found a negative correlation between circulating Tregs and serum CK. However, the detailed mechanism of their negative relationship needs to be further explored.

Moreover, dyslipidemia and abnormal blood glucose levels were previously found in hypertensive patients with or without LVH [49, 50]. Increased body weight, BMI, HbA1c and Lp(a) values as well as a decreased HDL-C level were also found in hypertensive patients in our study. We also found that the proportion of circulating Tregs was negatively related to LDL-C and apoB in patients with hypertension. LDL, the shell of which contains apoB-100, can carry cholesterol to form LDL-C. However, oxidized LDL (ox-LDL), not native LDL, can promote cholesterol accumulation in monocytes and macrophages [51]. The Fas/Fas ligand (FasL) pathway in activation-induced cell death serves as the major mechanism of peripheral Treg apoptosis [52], and increased ox-LDL levels may activate Fas/FasL/Caspase-3-mediated Treg apoptosis [52, 53]. These results may provide clues about the negative correlation between Tregs and LDL-C in hypertension. A negative correlation was previously found between the levels of circulating Tregs and HbA1c in obese patients [54], while no correlation was found in our study. This may be related to the small number of diabetic patients in our study.

The present study had several limitations. First, macrophages, neutrophils, and other subsets of $CD4^+$ T cells were significant inflammatory cells, but we did not detect their proportions. In addition, the 243 patients were all from the Second Xiangya Hospital, so the conclusion has to be verified in a large, multicenter study.

5. Conclusion

In conclusion, altered levels of circulating Tregs, IL-10, TGF-β1, and IL-6 were found in EH and LVH patients. The morbidity and mortality in patients with LVH were significantly increased, but the current treatment approach for LVH still follows standard hypertension guidelines and has unclear benefits [55]. Our study provides possible molecular targets for immunotherapy for hypertension and hypertensive myocardial hypertrophy. In contrast to the administration of traditional antihypertensive drugs, increasing the proportion of functional Tregs and supplementing anti-inflammatory cytokines or reducing proinflammatory cytokine levels may be new approaches for the treatment of hypertension and LVH.

Declarations

Availability of data and materials

The data used in the current study are available upon reasonable request to the corresponding author.

Contributions

Ying Tang and Li Shen carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. Jing-hui Bao carried out the immunoassays. Ying Tang participated in the sequence alignment. Ying Tang participated in the design of the study and performed the statistical analysis. Dan-yan Xu conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study protocol was approved by the Institutional Medical Ethics Committee of the Second Xiangya Hospital of Central South University (2018/No.046). Written informed consent to participate was acquired from patients themselves or their families.

Consent for publication

Not applicable.

Competing Interests

The authors declare that they have no conflicts of interest.

Acknowledgements

Not applicable.

Funding

This work was supported by the National Nature Scientific Funding of China (No. 81871858, 82072555, 82172550) and Chinese Cardiovascular Association-Access fund (2019-CCA-ACCESS-023).

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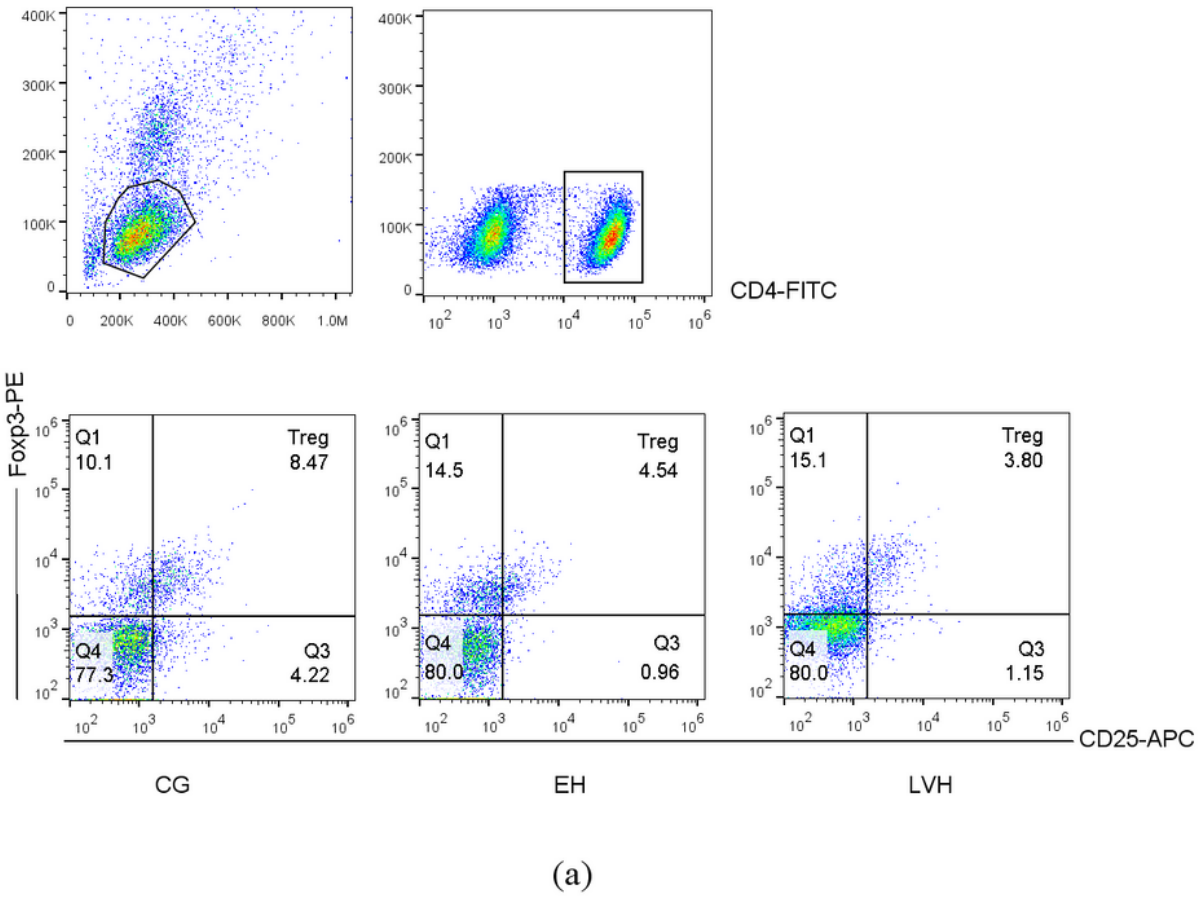
Figures



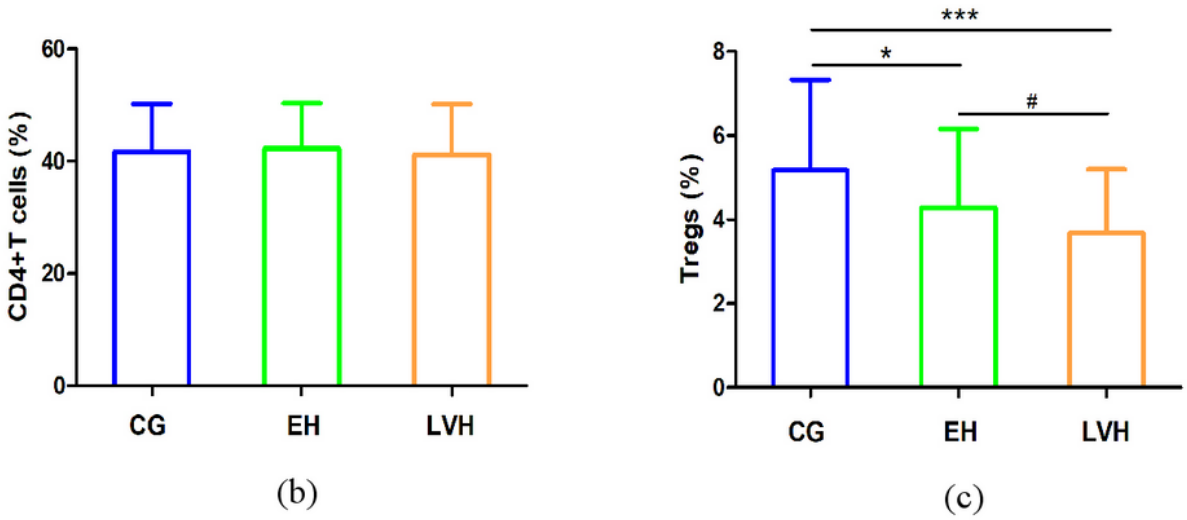


Figure 1

Circulating CD4+ T cells and Tregs in CG, EH and LVH patients.

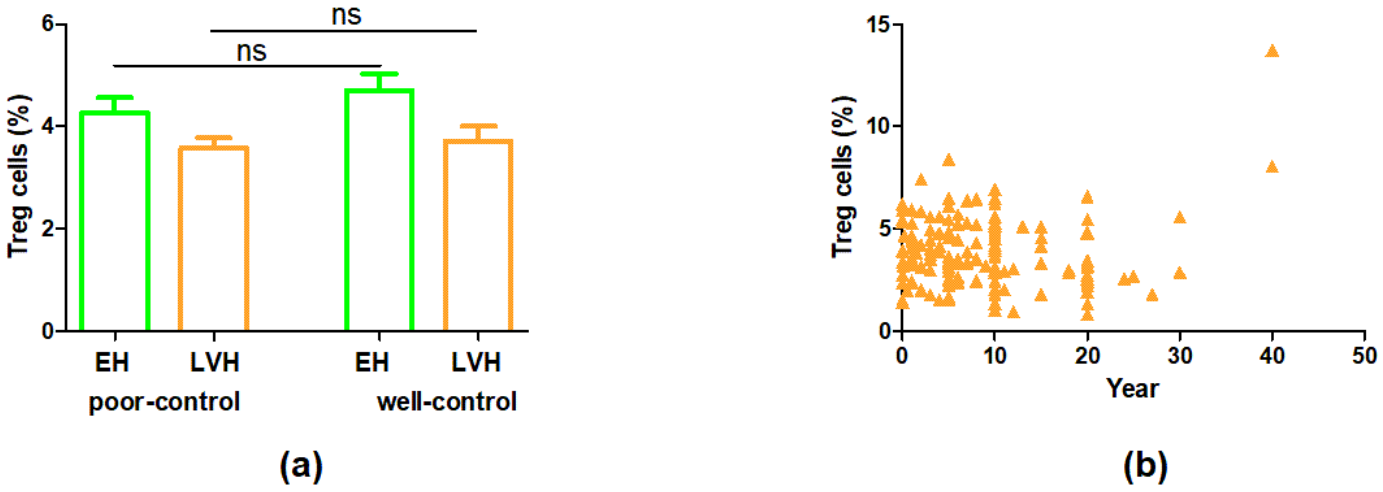


Figure 2

Circulating Tregs and blood pressure control in hypertensive patients.

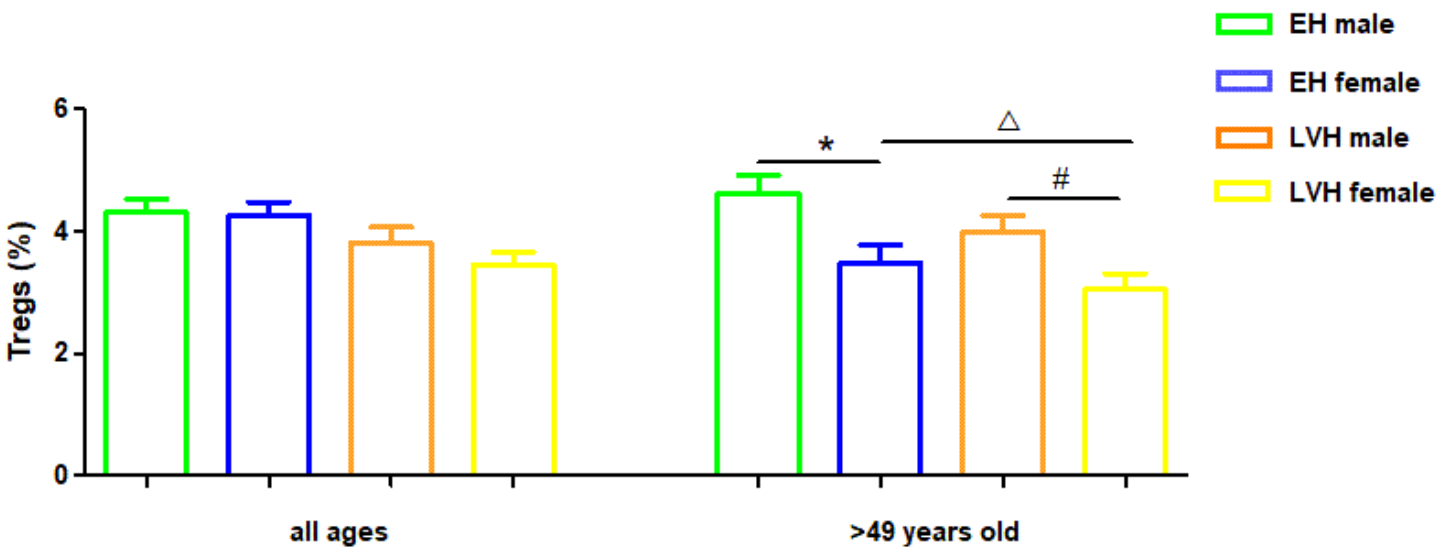


Figure 3

Sex difference of circulating Tregs in EH and LVH patients.

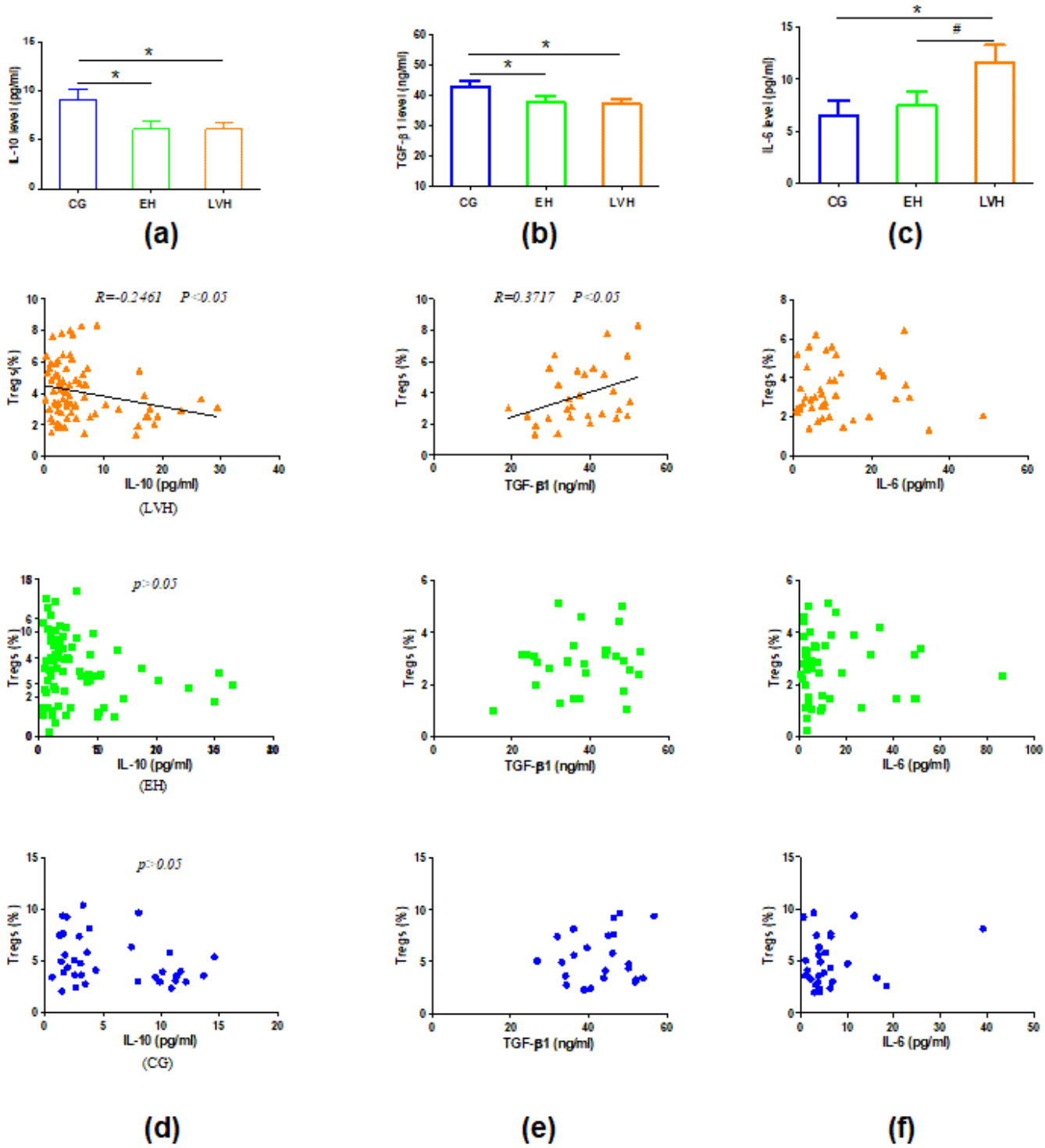


Figure 4

Serum cytokine levels in each group.

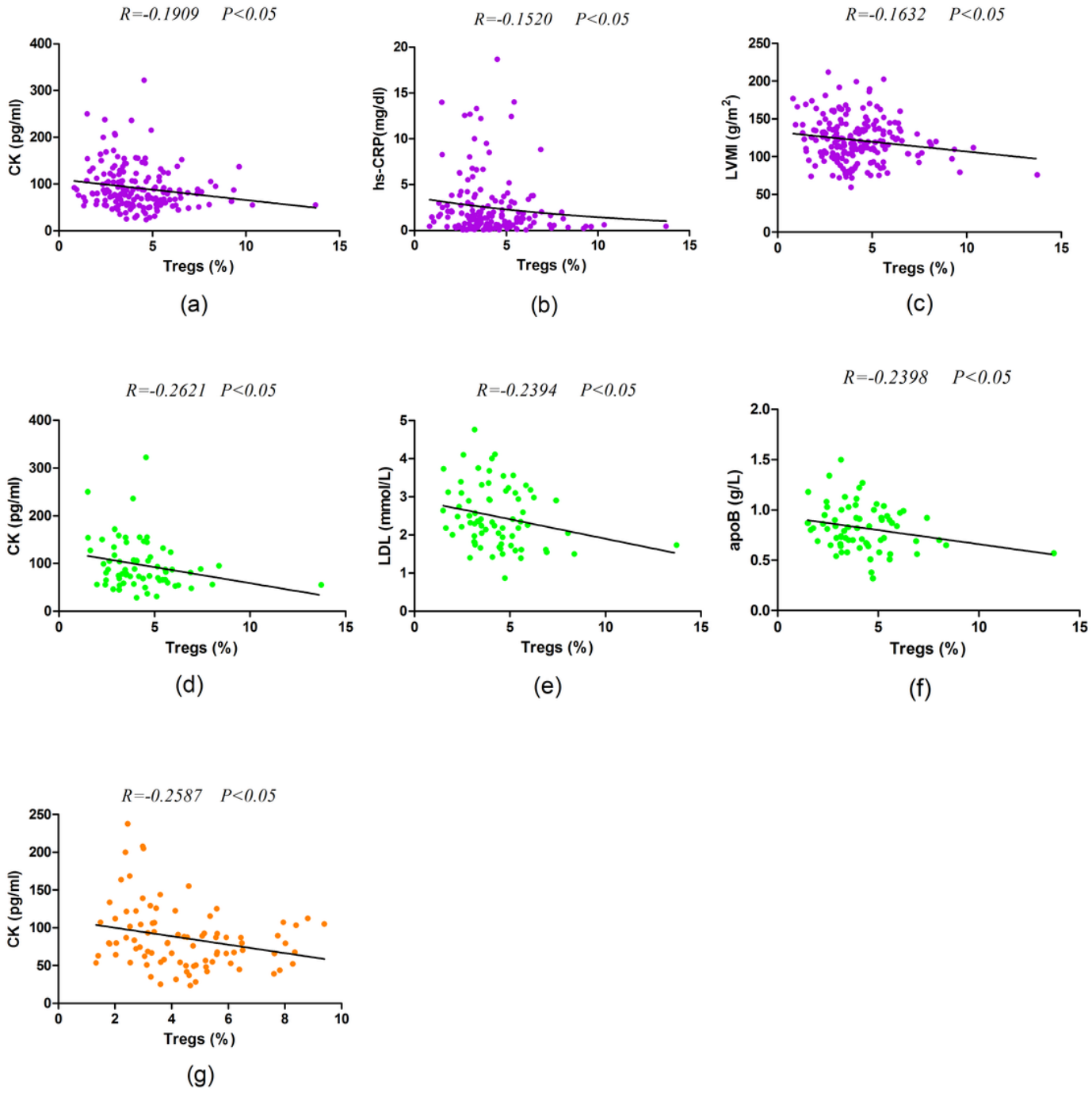


Figure 5

The correlation of Tregs and clinical characteristics in patients.