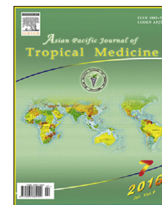
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm



Original research http://dx.doi.org/10.1016/j.apjtm.2016.05.006

Study on the effect and mechanism of the dysfunction of $CD4^{+}$ T cells in the disease process of chronic cardiac failure

Yin-Hao Cai[1], Zi-Jian Ma[1], Xiu-Ying Lu[1], En-Le He[1], Ming-Yao You[2*]

[1]*Emergency Department, The Affiliated Hospital of Guizhou Medical University, Guiyang, 550004, Guizhou, China*

[2]*Neurology Department, The Affiliated Hospital of Guizhou Medical University, Guiyang, 550004, Guizhou, China*

ARTICLE INFO

Article history:
Received 15 Apr 2016
Received in revised form 16 May 2016
Accepted 23 May 2016
Available online 31 May 2016

Keywords:
CD4 T cells
Chronic cardiac failure
Heart function
Prognosis

ABSTRACT

Objective: To study the effect and mechanism of the dysfunction of $CD4^{+}$ T cells in the disease process of chronic cardiac failure (CHF).

Methods: According to different group technologies, 100 CHF patients were divided into the following groups: ischemia group and non-ischemia group, heart function III–IV group and heart function I–II group, event group and non-event group, and 50 healthy volunteers were included in the control group. Real-time PCR was used to detect transcription factors T-bet and GATA-3 of Th1 and Th2; flow cytometry was applied to determine the ratio of Th17 and Treg cells; ELISA was employed to test cytokines IFN-γ, IL-4, IL-17 and IL-10 of peripheral blood Th1, Th2, Th17 and Treg cells, respectively; ultrasonic cardiogram was used to exploit to LVEF and LVEDd; and electrochemilu minescene immunoassay was used to examine plasma BNP. The differences of all indexes of all groups were analyzed and the correlation between CD4 T cells and clinical indexes was analyzed by Pearson correlation analysis.

Results: As compared to the control group, the transcription factors T-bet and GATA-3 of Th1 and Th2, the ratio of cytokines Th17 and IFN-γ, cytokines IL-17, T-bet/GATA-3, IFN-γ/IL-4, Th17 cells/Treg cells, IL-17/IL-10 of the ischemia group and non-ischemia group, heart function III–IV group and heart function I–II group, event group and non-event group were all increased significantly, while their transcription factor GATA-3 of Th2, cytokines IL-4, Treg cells ratio, cytokines IL-10 were decreased obviously. The differences showed statistical significance ($P < 0.05$). The increase or decrease of the partial CD4+ T cells of the ischemia group, heart function III–IV group and event group was more distinctly. The results of Pearson correlation analysis showed that IFN-γ and IL-17 were significantly positively correlated with LVEDd and BNP, IL-4 and IL-10 were also significantly positively correlated with LVEF, but correlated negatively with BNP, and IL-17 was negatively correlative with LVEF.

Conclusions: There was a correlation between CHF and the dysfunction of $CD4^{+}$ T cells showing immune activation phenomenons of deviations from the Th1/Th2 balance towards Th1 and from the Th17/Treg balance towards Th17, which was also related to the types, severity and prognosis of the disease.

1. Introduction

Chronic cardiac failure (CHF) is a clinical syndrome with a complicated pathogenesis, which is the severe stage of all kinds of cardiovascular diseases [1,2]. In recent years, researchers have found pathological immune responses against myocardial cells in CHF patients. After myocardial injury, auto-antigen activated immune cells and inflammatory cytokines are released and participate in the incidence and development of heart failure [3–5]. As an important part of the immune system, cell immune plays an important role in eliminate pathogenic bacteria and cancer cells [6]. T cells play a critical role in cell immune including the helper T cells of CD4 and cytotoxic T cells of CD8 [7]. Helper T cells have attracted much attention in recent years due to their anti-tumor effect which has been proved to be as strong as that of cytotoxic T cells of CD8 [8].

*Corresponding author: Ming-Yao You, Neurology Department, The Affiliated Hospital of Guizhou Medical University, Guiyang, 550004, Guizhou, China.
Tel: +86 851 86773370
E-mail: youmingyao322@163.com

Peer review under the responsibility of Hainan Medical College.
Foundation project: This study was supported by the Brainstorm Project of Guizhou Science and Technology Office (Grant No. SY 20133016) and Guiyang Science and Technology Planning Project (Grant No. 20151001).

1995-7645/Copyright © 2016 Hainan Medical College. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

CD4 T cells include subsets such as Th1, Th2, Th17, Treg and Tfh. Th1 cells secrete IFN-γ, TNF-α, IL-2 and so on and mediate immune responses of antiviral cells and intracellular bacteria [9]. Th2 cells secrete IL-4, IL-5, IL-13 and so on and mediate immune responses of anti-parasitism extracellular microbial [10]. Th17 cells secrete IL-17 and mediate inflammatory reactions such as rheumatoid arthritis [11]. Treg cells secrete IL-10 and TGF-β and also mediate immunosuppression [12]. Tfh cells secrete IL-21 and mediate humoral immune responses by assisting B cells [13]. All cell subsets maintain a balance stage. The unbalance of $CD4^+$ T cells participates in the occurrences and developments of various autoimmunity diseases such as systemic lupus erythematosus [14], cancers [15], infections [16] and so on. Over the years, the correlations between immune responses with the pathogenesis of CHF have attracted extensive attention, but there are a few studies analyzing CHF patients and $CD4^+$ T cells systematically. This study aimed to analyze the relationship between the dysfunction of $CD4^+$ T cells with the pathogenesis of CHF by analyzing the $CD4^+$ T cells in CHF patients mainly including the Th1/Th2 balance and Th17/Treg balance and their cytokines levels and collecting the clinical data of those patients.

2. Materials and methods

2.1. Clinical data

A total of 100 cases treated in the Affiliated Hospital of Guizhou Medical University from July 2014 to February 2015 were chosen as the study subjects. All patients were all definitely diagnosed in accordance with symptoms, signs, medical histories, experimental examinations and imaging tests. Patients with combined immune system diseases, endocrine system diseases such as thyroid diseases, acute or chronic infections, acute or chronic inflammatory diseases, rheumatic diseases, acute myocardium infarction and unstable angina pectoris in recent period, recent histories of using drug such as corticosteroids which affected immune responses or hepatic and renal dysfunction were excluded. Among those 100 cases, 63 of them were males and 37 were females. Their ages range from 56 to 80 years. The average age was (67.82 ± 12.27) years. Patients were divided into different subgroups by different group technologies. Based on the different protopathy, they were divided into the ischemia group and non-ischemia group. There were 75 cases in the ischemia group in which 48 cases were males and 27 were females aging from 56 to 78 years with an average age of (67.12 ± 12.41) years. There were 25 cases in the non-ischemia group in which 15 cases were males and 10 were females aging from 58 to 80 years with an average age of (68.03 ± 12.30) years. According to the cardiac function grade of New York Heart Academy (NYHA), patients were divided into the heart function III–IV group and heart function I–II group. There were 62 cases in the heart function III–IV group in which 41 were males and 21 were females aging from 58 to 79 years with an average age of (68.21 ± 11.69) years. There were 38 cases in the heart function I–II group in which 22 were males and 16 were females aging from 56 to 80 years with an average age of (67.09 ± 13.15) years. After a year of follow-up, according to the presence or absence of cardiovascular event, patients were divided into the event group and non-event group. There were 18 cases in the event group in which 11 were males and 7 were females aging from 60 to 80 years with an average age of (68.87 ± 12.08) years, while there were 82 cases in the non-event group in which 52 were males and 30 were females aging from 56 to 77 years with an average age of (67.31 ± 12.26) years. Meanwhile, 50 healthy volunteers were regarded as the control group. In the control group, 28 of them were males and 22 were females whose ages ranged from 55 to 80 years. The average age was (67.29 ± 13.30) years. Differences of ages and genders showed no statistical significance. The study was approved by the Hospital's Ethics Committee. All chosen subjects signed a informed consent.

2.2. Detection methods

2.2.1. Transcriptional factors Th1 and Th2 detected by real-time PCR

PCR primers were referred to the related study [17], and reverse transcription kits were purchased from Promega (USA). Trizol reagent produced by Invitrogen was used to extract RNA. Heat-start fluorescence PCR kits were bought from Gibco-BRL (USA). 7300 Real-time PCR System was provided by ABI Cooperation (USA). Five milliliters peripheral venous blood of each chosen cases were drawn and ethylenediaminetetraacetic acid (EDTA) was used for anti-coagulation. Peripheral blood mononuclear cell (PBMC) was separated routinely and then the total mRNA was extracted and stored at low temperature in accordance with the instruction of the kits. The condition for the synthesis of DNA was 50 min at 42 °C, 5 min at 95 °C. Fluorescence signals were collected by three-step reaction. The relative expression quantities of the transcription factors T-bet and GATA-3 of Th1 and Th2 could be obtained with 7300 Real-time PCR System.

2.2.2. The ratio of Th17 and Treg cells tested by flow cytometry

Anti-human CD8 antibody marked by FITC and anti-human CD3 antibody marked by PC5 were added into the stand-by cells and incubated for 30 min at normal atmospheric temperature without sunlight and then washed by PBS. After that, they were involved in a fixation reaction with stationary liquid for 20 min at normal atmospheric temperature without sunlight. Then, the supernate was cleared out and they were washed with PBS again. After adding membrane rupture reagent, the supernate was eliminated by centrifugation and cytokines were dyed. Next, IL-17 marked by PE was added. The same control antibodies were added into the control group for incubation for 30 min at normal atmospheric temperature without sunlight and then it was washed by PBS. Finally, they were ready for detection. The ratio of Th17 was tested by FACS-CALIBUR flow cytometry produced by BD Compang (USA).

Anti-human CD127 monoclonal antibody marked by PE, anti-human CD4 monoclonal antibody marked by FITC and anti-human CD25 monoclonal antibody marked by PC5 were added into the stand-by cells, while the same antibodies were added into the control tubes. They were incubated for 20 min at normal atmospheric temperature without sunlight and then washed by PBS. Then, red blood cell lysate was added and they were incubated again at normal atmospheric temperature without sunlight for 10 min. After centrifugation, the supernate was removed and they were fixed with stationary liquid and punched by membrane rupture reagent. Foxp3 antibody marked by PE was added in them. Also, the same antibody was added into the

control tubes. All of them were incubated for 30 min at normal atmospheric temperature without sunlight and then washed by PBS. Eventually, they were ready for detection. The ratio of Treg cells was examined by FACS-CALIBUR flow cytometry produced by BD Compang (USA).

2.2.3. Peripheral blood cytokines determined by ELISA

Six milliliters peripheral blood was extracted and centrifuged to separate serum. ELISA was used to determine peripheral blood IFN-γ, IL-4, IL-17 and IL-10. All kits were bought from BD Company (USA).

2.2.4. Clinical indexes

PHILIPS IE 5500 color ultrasonic tester was implied to test the left ventricular ejection fractions (LVEF) and left ventricular end-diastolic diameter (LVEDd) with the long axis view of the left heart near the sternum as the section and a transducer frequency of 2–4 MHz. Electrochemilu minescene immunoassay method was employed to test the plasma brain natriuretic peptide (BNP).

2.3. Statistical management

SPSS19.0 statistic software was used for analysis. Comparisons in multiple groups were analyzed by variance analysis. Comparisons between two groups were tested by SNK-q test. Pearson correlation analysis was also used. The inspection level α was 0.05.

3. Results

3.1. Comparison of the CD4 T cell subsets between CHF ischemia group and non-ischemia group and the control group

The transcription factor T-bet of Th1, cytokines IFN-γ, the ratio of Th17, cytokines IL-17, T-bet/GATA-3, IFN-γ/IL-4, Th17 cells/Treg cells, IL-17/IL-10 of the ischemia group and non-ischemia group were all significantly higher than those of the control group, while their transcription factor GATA-3 of Th2, cytokines IL-4, Treg cells ratio, cytokines IL-10 were obviously lower. The differences were statistically significant ($P < 0.05$). Besides, the cytokines IFN-γ of Th1, cytokines IL-17 of Th17, T-bet/GATA-3, Th17 cells/Treg cells, IL-17/IL-10 of the ischemia group were all distinctly higher than those of the non-ischemia group, while its cytokines IL-4 of Th2 and cytokines IL-10 of Treg cells were evidently lower than those of the non-ischemia group. The differences were also statistically significant ($P < 0.05$) (Table 1).

3.2. Comparison of the CD4 T cell subsets between CHF heart function Ⅲ–Ⅳ group and heart functionⅠ–Ⅱ group and the control group

The transcription factor T-bet of Th1, cytokines IFN-γ, the ratio of Th17, cytokines IL-17, T-bet/GATA-3, IFN-γ/IL-4, Th17 cells/Treg cells, IL-17/IL-10 of the heart function Ⅲ–Ⅳ group and heart functionⅠ–Ⅱ group were all significantly higher than those of the control group, while their transcription factor GATA-3 of Th2, cytokines IL-4, Treg cells ratio, cytokines IL-10 were obviously lower. The differences were statistically significant ($P < 0.05$). Moreover, the cytokines IFN-γ of Th1, cytokines IL-17 of Th17, T-bet/GATA-3 and Th17 cells/Treg cells of the heart function Ⅲ–Ⅳ group were all distinctly higher than those of the heart functionⅠ–Ⅱ group, while its cytokines IL-4 of Th2 and the ratio of cytokines Treg cells were evidently lower. The differences were also statistically significant ($P < 0.05$) (Table 2).

3.3. Comparison of the CD4 T cell subsets between CHF event group and non-event group and the control group

The transcription factor T-bet of Th1, cytokines IFN-γ, the ratio of Th17 cells, cytokines IL-17, T-bet/GATA-3, IFN-γ/IL-4, Th17 cells/Treg cells, IL-17/IL-10 of the event group and non-event group were all significantly higher than those of the control group, while their transcription factor GATA-3 of Th2, cytokines IL-4, Treg cells ratio, cytokines IL-10 were all obviously lower. The differences were statistically significant ($P < 0.05$). In addition, the cytokines IFN-γ of Th1, cytokines IL-17 of Th17, T-bet/GATA-3, Th17 cells/Treg cells and IL-17/IL-10 of the event group were all distinctly higher than those of the non-event group, while its transcription factor GATA-3 of Th2, cytokines IL-4, the ratio of cytokines Treg cells and cytokines IL-10 of Treg were evidently lower. The differences were also statistically significant ($P < 0.05$) (Table 3).

3.4. The correlation analysis between the CD4 T cell subsets of CHF patients and clinical indexes

Pearson correlation analysis revealed that there was a strong positive correlation among IFN-γ and LVEDd BNP, IL-4 was significantly positively correlated with LVEF but negatively correlated with BNP, IL-17 was positively correlative with LVEDd and BNP but negatively correlated with LVEF, and IL-10 was also significantly positively correlated with LVEF but negatively correlated with BNP (Table 4).

4. Discussion

CHF can be caused by various heart diseases such as coronary heart disease and hypertension. CHF appears in the later stage of heart diseases which was mainly characterized by heart failure. Due to the weak myocardial contractility of CHF patients, the cardiac output reduces and the normal metabolism cannot get satisfied, which leads to the short of tissues and organs infusion [18]. The incidence rate of CHF maintains 0.9%–2%, and the incidence rate could increase to over 10% among patients over 70 years old. With the improvement of the first and second levels of heart diseases prevention, aggravation of astogeny and the perfection of the treatment of coronary events, the incidence rate of CHF increases year by year [19]. Even though there is no new myocardial injury, CHF can still develop by myocardial reconstitution. Studies suggest that the prognosis of heart failure in its stable stage is similar to common malignant tumors, but the survival rate of patients with developing heart failure is lower than that of patients with malignant tumors [20].

The pathogenesis of CHF is quite complicated which is related to many factors such as heredity, immunity and biochemistry. It has been confirmed that the incidence and development of CHF is related to the neuroendocrine activation. The neuroendocrine activation of angiotensin converting

Table 1

Comparison of the CD4 T cell subsets between CHF ischemia group and non-ischemia group and the control group.

CD4 T cell subset		Ischemia group (n = 75)	Non-ischemia group (n = 25)	The control group (n = 50)
Th1	Transcription factor T-bet	1.89 ± 0.45*	1.34 ± 0.26*	0.62 ± 0.18
	Cytokines IFN-γ (pg/mL)	76.21 ± 10.23*#	70.25 ± 7.78*	45.46 ± 5.20
Th2	Transcription factor GATA-3	0.35 ± 0.04*	0.50 ± 0.09*	1.21 ± 0.13
	Cytokines IL-4 (pg/mL)	29.18 ± 3.89*#	35.72 ± 4.24*	44.30 ± 4.75
Th1/Th2	Transcription factor T-bet/GATA-3	5.40 ± 0.78*#	2.68 ± 0.54*	0.51 ± 0.06
	Cytokines IFN-γ/IL-4	2.61 ± 0.34*	1.97 ± 0.28*	1.03 ± 0.11
Th17	Th17 cells ratio (%)	2.46 ± 0.45*	2.08 ± 0.24*	0.47 ± 0.21
	Cytokines IL-17 (pg/mL)	25.58 ± 3.09*#	21.05 ± 2.35*	14.28 ± 2.14
Treg	Treg cells ratio (%)	4.01 ± 0.40*	5.12 ± 1.12*	8.55 ± 1.83
	Cytokines IL-10 (pg/mL)	10.37 ± 2.09*#	13.23 ± 3.06*	22.65 ± 3.63
Th17/Treg	Th17 cells/Treg cells	0.61 ± 0.06*#	0.41 ± 0.04*	0.05 ± 0.01
	IL-17/IL-10	2.47 ± 0.25*#	1.59 ± 0.20*	0.63 ± 0.08

Compared with the control group, *$P < 0.05$; compared with the non-ischemia group, #$P < 0.05$.

Table 2

Comparison of the CD4 T cell subsets between CHF heart function Ⅲ–Ⅳ group and heart functionⅠ–Ⅱ group and the control group.

CD4 T cell subset		Heart function Ⅲ–Ⅳ group (n = 62)	Heart function Ⅰ–Ⅱ group (n = 38)	The control group (n = 50)
Th1	Transcription factor T-bet	1.76 ± 0.42*	1.45 ± 0.25*	0.62 ± 0.18
	Cytokines IFN-γ	75.16 ± 8.76*#	69.26 ± 7.44*	45.46 ± 5.20
Th2	Transcription factor GATA-3	0.37 ± 0.04*	0.46 ± 0.06*	1.21 ± 0.13
	Cytokines IL-4	30.31 ± 3.26*#	36.18 ± 4.18*	44.30 ± 4.75
Th1/Th2	Transcription factor T-bet/GATA-3	4.76 ± 0.66*#	3.15 ± 0.46*	0.51 ± 0.06
	Transcription factor IFN-γ/IL-4	2.48 ± 0.30*	1.91 ± 0.25*	1.03 ± 0.11
Th17	Th17 cell ratio	2.38 ± 0.38*	2.12 ± 0.30*	0.47 ± 0.21
	Cytokines IL-17	25.16 ± 3.24*#	22.21 ± 2.64*	14.28 ± 2.14
Treg	Treg cell ratio	3.62 ± 0.45*#	5.43 ± 1.24*	8.55 ± 1.83
	Cytokines IL-10	11.09 ± 2.21*	12.15 ± 3.14*	22.65 ± 3.63
Th17/	Th17 cells/Treg cells	0.66 ± 0.08*#	0.40 ± 0.05*	0.05 ± 0.01
Treg	IL-17/IL-10	2.27 ± 0.31*	1.83 ± 0.22*	0.63 ± 0.08

Compared with the control group, *$P < 0.05$; compared with the heart functionⅠ–Ⅱ group, #$P < 0.05$.

Table 3

Comparison of the CD4 T cell subsets between CHF event group and non-event group and the control group.

CD4 T cell subset		Event group (n = 18)	Non-event group (n = 82)	The control group (n = 50)
Th1	Transcription factor T-bet	1.80 ± 0.52*	1.40 ± 0.32*	0.62 ± 0.18
	Cytokines IFN-γ	79.89 ± 6.12*#	68.82 ± 9.16*	45.46 ± 5.20
Th2	Transcription factor GATA-3	0.33 ± 0.05*#	0.45 ± 0.05*	1.21 ± 0.13
	Cytokines IL-4	26.28 ± 3.89*#	35.27 ± 4.20*	44.30 ± 4.75
Th1/Th2	Transcription factor T-bet/GATA-3	5.45 ± 0.69*#	3.11 ± 0.48*	0.51 ± 0.06
	Cytokines IFN-γ/IL-4	3.04 ± 0.34*#	1.95 ± 0.27*	1.03 ± 0.11
Th17	Th17 cell ratio	2.54 ± 0.42*	2.09 ± 0.34*	0.47 ± 0.21
	Cytokines IL-17	25.89 ± 3.87*#	21.78 ± 2.45*	14.28 ± 2.14
Treg	Treg cell ratio	3.21 ± 0.43*#	4.79 ± 1.35*	8.55 ± 1.83
	Cytokines IL-10	10.02 ± 1.76*#	13.04 ± 2.36*	22.65 ± 3.63
Th17/Treg	Th17 cells/Treg cells	0.79 ± 0.90*#	0.44 ± 0.05*	0.05 ± 0.01
	IL-17/IL-10	2.58 ± 0.25*#	1.67 ± 0.21*	0.63 ± 0.08

Compared with the control group, *$P < 0.05$; compared with the non-event group, #$P < 0.05$.

Table 4

The correlation analysis between the CD4 T cell subsets and clinical indexes.

CD4T cell subset	LVEDd		LVEF		BNP	
	r	P	r	P	r	P
IFN-γ	0.332	0.000	−0.119	0.453	0.289	0.002
IL-4	−0.165	0.318	0.274	0.009	−0.238	0.049
IL-17	0.309	0.000	−0.346	0.000	0.260	0.018
IL-10	−0.098	0.761	0.253	0.032	−0.247	0.042

enzyme inhibitor and β-receptor inhibitor is used for symptomatic treatment of CHF in clinical practice. Although it works to some degree, the mortality of CHF still remains high. It has been found in new studies that the activation of immune system and the continuous inflammatory responses are related to the occurrence of CHF [21,22]. The immune activation of CHF mainly featured by the direct stimulation by cardiac antigens or the exposure of cardiac antigens caused by cardiac antigens, which launches the pathological immune responses and

aggravated the cardiac injury and causes the decrease of heart function.

The role CD4 T cells playing in the cellular immunity has been a hot spot of research in recent years. By far, many subsets of CD4 have been discovered such as Th1, Th2, Th17, Treg, Tfh, etc. Th1 and Th2 were discovered earlier so that researches about them were more mature. Th1 cells secrete IFN-γ, TNF-α and IL-2 and mediate the cellular immune responses of antiviral or intracellular bacteria and pro-inflammatory cellular immune responses; Th2 cells secrete IL-4, IL-5, IL-13 and others, mediate immune responses and humoral immune responses of anti-parasitic extracellular microbial and inhibit inflammatory response. In normal healthy bodies, the ratio Th1/Th2 keeps relative balance, so that the bodies' immune homeostasis can also be maintained. The unbalance of Th1/Th2 can lead to a series of diseases. The results of this study showed that as compared to the control group the transcription factor T-bet of Th1 and cytokines IFN-γ of the ischemia group, non-ischemia group, heart function Ⅲ–Ⅳ group, heart function Ⅰ–Ⅱ group, event group and non-event group increased significantly while their transcription factor GATA-3 of Th2, cytokines IL-4 decreased distinctly. The evident increase of IFN-γ/IL-4 and T-bet/GATA-3 indicated that in CHF the balance of Th1/Th2 shifted towards Th1. The pro-inflammatory cellular immunity caused by hyperfunction of Th1 could possibly be a mechanism of pathologic autoimmune response of CHF. It is also found in clinical researches that serum inflammatory factors in CHF patients present high expressions and the common-used drugs of CHF such as β receptor inhibitor and statin work in the immune system and adjust the Th1/Th2 balance [23]. The more significant the shift of Th1 is, the long the pathologic autoimmune response of CHF patients persists, the more severe the degree is, the obvious the decrease of the heart function shows and the poorer the prognosis becomes.

Th17 is also a kind of subset of CD4 T cells, which is different with Th1 and Th2. It mediates inflammatory responses such as rheumatoid arthritis and immune system diseases by secreting pro-inflammatory cytokines such as IL-17 [24]. IL-17 possesses strong pro-inflammatory effect which can stimulate epithelial cells, endothelial cells, fibroblasts and other cells to release massive cytokines such as metalloproteinase-1, granulocyte colony-stimulating factor and IL-6 causing tissue injuries and inflammatory infiltration [25]. It is found that the high expression of IL-17 in plaque or plasma of patients with coronary heart disease is the main reason accounting for the unstable plague and rupture [26]. Treg cells can inhibit the activation of immune cells with the immunosuppressive and anergic effects which are used to maintain immune tolerance. It was found that the decrease of Treg cells played an important role in the occurrence of autoimmune diseases and diseased related to impaired immune balance [3]. Researchers on animals revealed that the decrease of Treg cells would aggravate the inflammatory responses of local injuries and increased the risk of atherosclerosis [27]. Th17 and Treg cells have mutually exclusive signal pathways in differentiations since they are all from naive T cells. They inhibit each other. Under normal circumstances, the immune system of the body is not activated and the differentiation of Treg cells is normal and plays the inhibitory role by different mechanisms, which can avoid body injuries under the premise of the maintenance of immunity homeostasis. The balance of Th17/Treg plays an important role in the maintenance of immunity homeostasis, and the unbalance of Th17/Treg participates in the occurrences of diseases such as infections, cancers and autoimmune diseases [28,29]. It is found that the unbalance of Th17/Treg also plays an important role in the incidence of ACS and the stable plague [30]. The study results of this study showed that as compared to the control group, the ratio of Th17 cells and cytokines IL-17 of the ischemia group, non-ischemia group, heart function Ⅲ–Ⅳ group, heart function Ⅰ–Ⅱ group, event group and non-event group all increased significantly while their ratio of Treg cells and cytokines IL-10 decreased distinctly. Besides, Th17/Treg and IL-17/IL-10 also increased obviously. All differences showed statistical significance ($P < 0.05$). The results demonstrated that the balance of Th17/Treg shifted towards Th17 in CHF. It is considered that the decrease of Treg cells aggravates the ventricular remodeling and the impaired immune tolerance causes pathological immune responses, which eventually make the myocardial cells become attack targets. In addition, the increase of Th17 exacerbates the severity of inflammatory response, rejects the pathway of Treg cells, inhibits the production of Treg cells and causes the decrease of Treg cells [29].

The results of a further correlation analysis of the cytokines secreted by various subsets of CD4 T cells, sensitive index of heart failure BNP and heart function indexes LVEDd and LVEF showed that IFN-γ and IL-17 were significantly positively correlated with LVEDd and BNP, IL-4 and IL-10 were also significantly positively correlated with LVEF, but correlated negatively with BNP, and IL-17 was negatively correlative with LVEF. These cytokines can, to some degree, reflect the development of the disease, which may have potential values on the treatment and estimation of prognosis.

To sum up, after systematically analyzed the CD4+ T cell subsets and their cytokines levels, it is concluded that there was a correlation between CHF and the dysfunction of CD4+ T cells showing immune activation phenomena of shifting from the Th1/Th2 balance towards Th1 and from the Th17/Treg balance towards Th17, and meanwhile the types, severity and prognosis of the disease are involved.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Arutyunov AG, Dragunov DO, Arutyunov GP, Sokolova AV. [Impact of the dosing of basic drugs on the risk of rehospitalization in patients with chronic heart failure]. *Ter Arkh* 2016; **88**(1): 29-34. Russian.

[2] Rinne ST, Liu CF, Wong ES, Hebert PL, Heidenreich P, Bastian LA, et al. Organizational structure for chronic heart failure and chronic obstructive pulmonary disease. *Am J Manag Care* 2016; **22**(3): e82-e87.

[3] Barsotti NS, Almeida RR, Costa PR, Barros MT, Kalil J, Kokron CM. IL-10-producing regulatory B cells are decreased in patients with common variable immunodeficiency. *PLoS One* 2016; **11**(3): e0151761.

[4] Gu M, Ma Y, Zhou T, Xia Y. Evaluation of a community health service center-based intervention program for managing chronic heart failure. *Balkan Med J* 2016; **33**(1): 45-51.

[5] Harjola VP, Mebazaa A, Čelutkienė J, Bettex D, Bueno H, Chioncel O, et al. Contemporary management of acute right ventricular failure: a statement from the Heart Failure Association and the Working Group on Pulmonary Circulation and Right

Ventricular Function of the European Society of Cardiology. *Eur J Heart Fail* 2016; **18**(3): 226-241.

[6] Noriega V, Martínez-Laperche C, Buces E, Pion M, Sánchez-Hernández N, Martín-Antonio B, et al. The genotype of the donor for the (GT)n polymorphism in the promoter/enhancer of FOXP3 is associated with the development of severe acute GVHD but does not affect the GVL effect after myeloablative HLA-identical allogeneic stem cell transplantation. *PLoS One* 2015; **10**(10): e0140454.

[7] Fontoura IC, Trombone AP, Almeida LP, Lorenzi JC, Rossetti RA, Malardo T, et al. B cells expressing IL-10 mRNA modulate memory T cells after DNA-Hsp65 immunization. *Braz J Med Biol Res* 2015; **48**(12). 1095–1100.

[8] Takeuchi A, Badr Mel S, Miyauchi K, Ishihara C, Onishi R, Guo Z, et al. CRTAM determines the CD4+ cytotoxic T lymphocyte lineage. *J Exp Med* 2016; **213**(1): 123-138.

[9] See JX, Samudi C, Saeidi A, Menon N, Choh LC, Vadivelu J, et al. Experimental persistent infection of BALB/c mice with small-colony variants of *Burkholderia pseudomallei* leads to concurrent upregulation of PD-1 on T cells and skewed Th1 and Th17 responses. *PLoS Negl Trop Dis* 2016; **10**(3): e0004503.

[10] van der Heide V, Möhnle P, Rink J, Briegel J, Kreth S. Down-regulation of MicroRNA-31 in CD4+ T cells contributes to immunosuppression in human sepsis by promoting TH2 skewing. *Anesthesiology* 2016; **124**(4): 908-922.

[11] Ciraci C, Janczy JR, Jain N, Haasken S, Pecli E, Silva C, et al. Immune complexes indirectly suppress the generation of Th17 responses in vivo. *PLoS One* 2016; **11**(3): e0151252.

[12] Dietze KK, Schimmer S, Kretzmer F, Wang J, Lin Y, Huang X, et al. Characterization of the Treg response in the hepatitis B virus hydrodynamic injection mouse model. *PLoS One* 2016; **11**(3): e0151717.

[13] Fehervari Z. Instructing TFH cells. *Nat Immunol* 2016; **17**(3): 229.

[14] Margiotta D, Navarini L, Vadacca M, Basta F, Lo Vullo M, Pignataro F, et al. Relationship between leptin and regulatory T cells in systemic lupus erythematosus: preliminary results. *Eur Rev Med Pharmacol Sci* 2016; **20**(4): 636-641.

[15] Haabeth OA, Lorvik KB, Yagita H, Bogen B3, Corthay A1. Interleukin-1 is required for cancer eradication mediated by tumor-specific Th1 cells. *Oncoimmunology* 2015; **5**(1): e1039763.

[16] Zaragoza O, Alvarez M, Telzak A, Rivera J, Casadevall A. The relative susceptibility of mouse strains to pulmonary *Cryptococcus neoformans* infection is associated with pleiotropic differences in the immune response. *Infect Immun* 2007; **75**(6): 2729-2739.

[17] Zhu M, Liang Z, Wang T, Chen R, Wang G, Ji Y. Th1/Th2/Th17 cells imbalance in patients with asthma with and without psychological symptoms. *Allergy Asthma Proc* 2016; **37**(2): 148-156.

[18] Kitai T, Kirsop J, Tang WH. Exploring the microbiome in heart failure. *Curr Heart Fail Rep* 2016; **13**(2): 103-109.

[19] van Riet EE, Hoes AW, Limburg A, Landman MA, Kemperman H, Rutten FH. Extended prediction rule to optimise early detection of heart failure in older persons with non-acute shortness of breath: a cross-sectional study. *BMJ Open* 2016; **6**(2): e008225.

[20] Bilbao A, Escobar A, García-Perez L, Navarro G, Quirós R. The Minnesota living with heart failure questionnaire: comparison of different factor structures. *Health Qual Life Outcomes* 2016; **14**(1): 23.

[21] Kay AB, Clark P, Maurer M, Ying S. Elevations in T-helper-2-initiating cytokines (interleukin-33, interleukin-25 and thymic stromal lymphopoietin) in lesional skin from chronic spontaneous (‘idiopathic’) urticaria. *Br J Dermatol* 2015; **172**(5): 1294-1302.

[22] Huang X, Ren L, Ye P, Cheng C, Wu J, Wang S, et al. Peroxisome proliferator-activated receptor γ deficiency in T cells accelerates chronic rejection by influencing the differentiation of CD4+ T cells and alternatively activated macrophages. *PLoS One* 2014; **9**(11): e112953.

[23] Földes G, Anker SD, von Haehling S. Atorvastatin modulates Th1/Th2 response in patients with chronic heart failure. *J Card Fail* 2009; **15**(6): 551.

[24] Thibaudin M, Chaix M, Boidot R, Végran F, Derangère V, Limagne E, et al. Human ectonucleotidase-expressing CD25high Th17 cells accumulate in breast cancer tumors and exert immunosuppressive functions. *Oncoimmunology* 2015; **5**(1): e1055444.

[25] McGill JL, Rusk RA, Guerra-Maupome M, Briggs RE, Sacco RE. Bovine gamma delta T cells contribute to exacerbated IL-17 production in response to Co-Infection with bovine RSV and Mannheimia haemolytica. *PLoS One* 2016; **11**(3): e0151083.

[26] Wen Y, Zeng Z, Gui C, Li L, Li W. Changes in the expression of Th17 cell-associated cytokines in the development of rheumatic heart disease. *Cardiovasc Pathol* 2015; **24**(6): 382-387.

[27] Alberti TB, Marcon R, Bicca MA, Raposo NR, Calixto JB, Dutra RC. Essential oil from *Pterodon emarginatus* seeds ameliorates experimental autoimmune encephalomyelitis by modulating Th1/Treg cell balance. *J Ethnopharmacol* 2014; **155**(1): 485-494.

[28] Wang X, Sun R, Chen Y, Lian ZX, Wei H, Tian Z. Regulatory T cells ameliorate acetaminophen-induced immune-mediated liver injury. *Int Immunopharmacol* 2015; **25**(2): 293-301.

[29] Singh A, Vajpayee M, Ali SA, Chauhan NK. Cellular interplay among Th17, Th1, and Treg cells in HIV-1 subtype “C” infection. *J Med Virol* 2014; **86**(3): 372-384.

[30] Björkbacka H, Berg KE, Manjer J, Engelbertsen D, Wigren M, Ljungcrantz I, et al. CD4($^{+}$) CD56($^{+}$) natural killer T-like cells secreting interferon-γ are associated with incident coronary events. *J Intern Med* 2016; **279**(1): 78-88.