Original Article
Dynamic changes in CD4+CD25+ regulatory T cell activity in patients with acute decompensated heart failure

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Abstract: Previous studies demonstrated that circulating regulatory T (Treg) cell levels decreased in patients with stable heart failure. However, whether and how the levels of Treg cells change in patients with acute decompensated heart failure (ADHF) has not yet been investigated. The present study was designed to investigate the dynamic changes in CD4+CD25+ Treg cell activity that occur in ADHF patients. Forty patients with ADHF, 20 patients with chronic stable heart failure (CSHF) and 20 healthy subjects were enrolled in the present study. The frequencies of CD4+CD25+ Treg cells were detected using flow cytometry analysis, and plasma IL-10 and TGF-β1 levels were measured using ELISA. The results revealed a significant decrease in CD4+CD25+ Treg cell frequencies and IL-10 and TGF-β1 levels in ADHF patients at admission in comparison with the CSHF and control groups. The CD4+CD25+ Treg cell frequencies and IL-10 and TGF-β1 levels were higher in the ADHF patients at discharge than at admission. No differences were observed between the ADHF patients at discharge and the CSHF patients. In addition, the CD4+CD25+FOXP3+ T cell frequencies and IL-10 and TGF-β1 levels were significantly decreased in class III patients relative to class II patients at discharge. Our findings indicate that ADHF is associated with low CD4+CD25+ Treg cell activity at admission. Although these abnormal levels are improved after treatment, the values remained lower in ADHF patients at discharge than in healthy subjects.

Keywords: CD4+CD25+ regulatory T cell, acute decompensated heart failure, inflammation

Introduction

Heart failure (HF) is a clinical syndrome that results from impaired ventricular filling and ejection caused by structural or functional disorders of the cardiac tissue. The main clinical manifestations of heart failure caused by exercise tolerance are limited breathing difficulties and fatigue, as well as pulmonary congestion and edema of the limbs related to fluid retention [1]. Epidemiological data revealed that coronary artery disease, hypertension and dilated cardiomyopathy (DCM) are the main causes of heart failure [1]. Acute decompensated heart failure (ADHF) is a severe form of heart failure that is caused by deterioration. This condition is observed primarily in patients with a previous diagnosis of HF. ADHF is a life-threatening condition that requires immediate medical attention and usually leads to emergency hospital admission.

Recent studies have shown that HF is a low grade chronic inflammatory disease in which T lymphocytes, macrophages and other inflammatory cells and a large number of inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, constitute a large and complex network system of inflammation that regulates the development of HF [2-4]. Accumulating evidence demonstrated that circulating CD4+CD25+ regulatory T (Treg) cell leels are significantly reduced in patients with chronic stable heart failure (CSHF) [5-10] and

Table 1. Clinical characteristics of patients

Characteristics	Control (n=20)	CSHF (n=20)	ADHF at admission (n=40)	ADHF at discharge (n=40)
Age (years)	60.0±11.3	62.9±10.7	64.7±10.1	
Male gender, n (%)	11 (55)	12 (60)	23 (57.5)	
Ischemic heart disease	-	12 (60)	22 (55)	
Dilated cardiomyopathy	-	4 (20)	8 (20)	
Hypertensive heart disease	-	2 (10)	4 (10)	
Valvular heart disease	-	2 (10)	6 (15)	
SBP, mmHg	112±9	117±22	133±25[*]	117±13[#]
DBP, mmHg	72±6	71±15	82±23[*]	70±9[#]
HR, bpm	63±8	73±19[*]	88±21[*]	76±15[*,#]
Laboratory examination				
TC (mmol/L)	3.7±0.7	3.6±0.7	3.7±0.9	3.7±0.8
TG (mmol/L)	1.6±0.5	1.4±0.4	1.3±0.7	1.2±0.6
LDL-C (mmol/L)	2.1±0.5	2.1±0.5	2.2±0.7	2.2±0.6
HDL-C (mmol/L)	0.9±0.2	0.9±0.2	0.9±0.3	1.0±0.3
Creatinine (mmol/L)	64.6±7.6	70.0±14.4	71.3±23.5	71.6±21.0
Uric acid (mmol/L)	349.4±87.0	383.4±108.5	398.2±112.3	386.5±113.4
GLU (mmol/L)	4.9±0.6	5.9±0.7	6.1±1.4[*]	6.1±1.3[*]
NT-proBNP (pg/ml)	112.0±57.4	392.4±104.6[*]	3328.2±2366.0[*]	1029.4±926.6[*,#]
CRP (mg/L)	1.2±0.4	4.2±2.1[*]	8.0±5.8[*]	4.8±4.3[*,#]
Echocardiographic data				
LVEDD (mm)	46.8±2.7	56.4±4.8[*]	59.5±8.4[*]	59.4±8.1[*]
LVEF (%)	62.2±4.0	40.8±5.6[*]	38.3±8.3[*]	39.9±7.6[*]
Medications, n (%)				
ACEI/ARB	-	19 (95)	24 (60)	35 (87.5)
Beta-blocker	-	18 (90)	18 (45)	28 (70)
Diuretics	-	12 (60)	28 (70)	32 (80)
Aldosterone blocker	-	20 (100)	22 (55)	40 (100)
Digoxin	-	4 (20)	8 (20)	8 (20)
Nitrate	-	9 (45)	17 (43)	17 (43)
Acute phase treatment, n (%)				
hBNP	18 (45)			
Phosphodiesterase inhibitor	15 (38)			
Diuretics	40 (100)			

Note: [*]P<0.01 vs. Control, [#]P<0.01 vs. Admission. The data are shown as mean ± SD or number of patients. ADHF, acute decompensated heart failure; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NT-proBNP, Amino-terminal pro-B-type natriuretic peptide; CRP, C reactive protein; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; hBNP, human brain natriuretic peptide; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction.

represent an independent predictor of recurrent hospitalization within 12 months in CSHF patients [11]. However, whether circulating Treg levels decrease significantly in ADHF patients and recover after treatment have not yet been investigated. Therefore, we measured circulating Treg levels and levels of the related cytokines IL-10 and transforming growth factor beta (TGF-β)-1 at admission and discharge in ADHF patients.

Materials and methods

Patients

In total, forty consecutive patients who were diagnosed with ADHF and 20 patients with

CSHF were enrolled in the present study. The ADHF diagnoses were established by experienced cardiologists based on contemporary guidelines. ADHF was defined as a rapid or gradual onset of signs and symptoms of HF, including significant peripheral edema and dyspnea. The CSHF patients had New York Heart Association (NYHA) class II or III symptoms and no alterations in medical therapy or changes in symptoms of heart failure within 1 month. In addition, the CSHF patients had no myocardial infarction within 3 months of inclusion. The control group consisted of 20 age-matched healthy subjects. The clinical profiles of the patients and healthy controls are provided in **Table 1**.

Patients with advanced liver disease, renal failure, malignant disease, septicemia, current steroid therapy, and other inflammatory diseases were excluded from the study. Because of high mortality in ADHF patients treated with mechanical ventilation, those patients were also excluded.

Written informed consent was obtained from each patient. The study was approved by the Ethics Committee of Beijing Anzhen Hospital.

Blood samples

The first blood samples from ADHF patients were obtained as soon as the patients arrived on the day of admission, and the second blood samples were obtained at discharge. The blood samples from CSHF patients and the control group were obtained in the cardiovascular outpatient department and the health examination center, respectively. Blood samples were obtained in the recumbent position with a 21-gauge needle, with clean venipuncture of an antecubital vein. The samples were collected into sodium heparin vacutainers (Becton-Dickinson). The peripheral blood mononuclear cells (PBMCs) were prepared for flow cytometric analysis using a Ficoll density gradient. The plasma obtained after centrifugation was stored at -80°C until further use.

Flow cytometry analysis

For the T regulatory cell analysis (CD4+CD25+FOXP3+), the PBMCs were first stained with anti-CD4-FITC (eBioscience) and anti-CD25-APC (eBioscience) antibodies after fixation and permeabilization according to the manufacturer's instructions. The cells were then stained with an anti-FOXP3-PE antibody (eBioscience). Isotype controls were used to correct for compensation and confirm the antibody specificity. Data were collected using a FACS Caliburflow cytometer (BD Biosciences) and analyzed using the FlowJo software (Treestar, Inc).

ELISA detection of the levels of TGF-β1 and IL-10

The levels of TGF-β1 and IL-10 were measured using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Neobioscience, Shenzhen, China). The minimal detectable concentrations were 15 pg/ml for TGF-β1 and 0.2 pg/ml for IL-10. The intra-assay and inter-assay coefficients of variation for all ELISA were <5% and <10%, respectively. All samples were measured in duplicate.
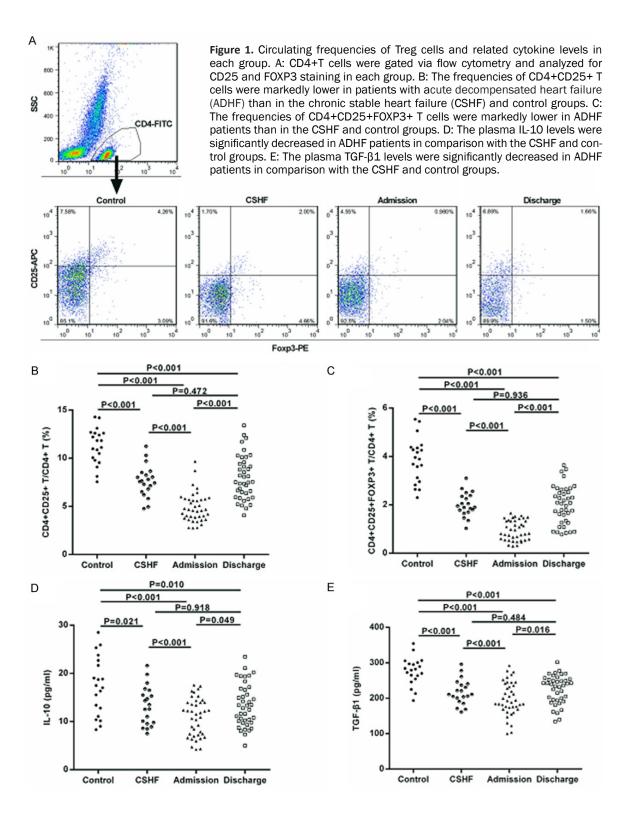
Statistical analysis

Data were presented as the mean ± SD. When comparing only 2 groups, the Student's *t*-test was used. For comparisons involving 3 or more groups, one-way ANOVA followed by the Neuman-Keuls post-hoc test was used. Spearman's correlation was used to calculate the correlations between two continuous variables. In all tests, a value of $P<0.05$ was considered to be statistically significant.
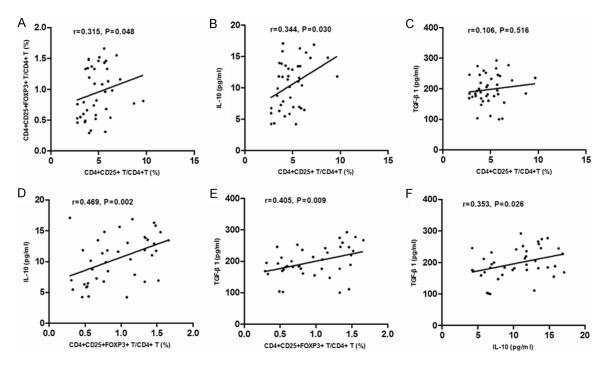
Results

No significant differences in age, gender, systolic blood pressure (SBP), diastolic bold pressure (DBP), lipid and lipoprotein fractions, fasting glucose, creatinineor uric acid (UA) were observed in the ADHF, CSHF and control groups. Heart rate (HR), NT-proBNP, C-reactive protein (CRP) and the left ventricular end-diastolic dimension (LVEDD) were significantly higher in the ADHF group than in the control group. In contrast, the left ventricular ejection fraction (LVEF) was lower in the ADHF group than in the control group (**Table 1**).

As shown in **Figure 1**, the CD4+CD25+ T cell and CD4+CD25+FOXP3+ T cell frequencies were significantly decreased in the patients with ADHF at the time of admission in comparison to those of the frequencies observed in the CSHF and control groups. The plasma IL-10 and TGF-β1 levels at admission were also lower in the ADHF group. The CD4+CD25+ T cell and CD4+CD25+FOXP3+ T cell frequencies were sig-

Figure 1. Circulating frequencies of Treg cells and related cytokine levels in each group. A: CD4+T cells were gated via flow cytometry and analyzed for CD25 and FOXP3 staining in each group. B: The frequencies of CD4+CD25+ T cells were markedly lower in patients with acute decompensated heart failure (ADHF) than in the chronic stable heart failure (CSHF) and control groups. C: The frequencies of CD4+CD25+FOXP3+ T cells were markedly lower in ADHF patients than in the CSHF and control groups. D: The plasma IL-10 levels were significantly decreased in ADHF patients in comparison with the CSHF and control groups. E: The plasma TGF-β1 levels were significantly decreased in ADHF patients in comparison with the CSHF and control groups.

nificantly increased in the ADHF patients at discharge in comparison with the frequencies observed in the ADHF patients at admission, and the plasma IL-10 and TGF-β1 levels at discharge were also higher than the levels observed at admission. No differences in CD4+CD25+ T cell and CD4+CD25+FOXP3+ T cell frequencies and IL-10 and TGF-β1 levels were observed between the ADHF patients at discharge and the CSHF patients. However, the

Figure 2. Correlation analysis of Treg cells and related cytokine levels in ADHF patients at admission. A: CD4+CD25+ T cells correlated positively with CD4+CD25+FOXP3+ T cells in ADHF patients at admission. B: CD4+CD25+ T cells correlated positively with IL-10 levels in ADHF patients at admission. C: CD4+CD25+ T cells correlated positively with TGF-β1 in ADHF patients at admission. D: CD4+CD25+FOXP3+ T cells correlated positively with IL-10 levels in ADHF patients at admission. E: CD4+CD25+FOXP3+ T cells correlated positively with TGF-β1 levels in ADHF patients at admission. F: Plasma IL-10 levels correlated negatively with TGF-β1 levels in ADHF patients at admission.

$CD4^+CD25^+$ T cell and $CD4^+CD25^+FOXP3^+$ T cell frequencies and the IL-10 and TGF-β1 levels were still lower in the ADHF patients at discharge than in the control group.
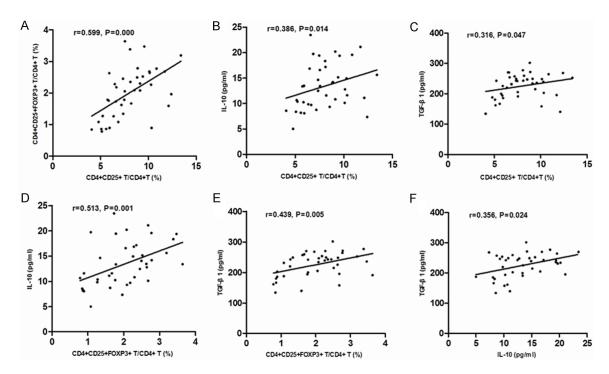
A correlation analysis revealed that the CD4$^+$CD25$^+$ T cell frequencies correlated positively with the CD4$^+$CD25$^+$FOXP3$^+$ T cell frequencies (r=0.315, P=0.048) and with the plasma IL-10 levels (r=0.344, P=0.030), although no correlation was observed with the TGF-β1 levels (r=0.106, P=0.516). The CD4$^+$CD25$^+$FOXP3$^+$ T cell frequencies correlated positively with the plasma IL-10 (r=0.469, P=0.002) and TGF-β1 (r=0.405, P=0.009) levels, and the plasma IL-10 levels correlated positively with the plasma TGF-β1 levels (r=0.353, P=0.026) in the ADHF patients at admission (**Figure 2**).

Similar to the results obtained at admission, at discharge, the ADHF patients presented CD4$^+$CD25$^+$ T cell frequencies that correlated positively with the CD4$^+$CD25$^+$FOXP3$^+$ T cell frequencies (r=0.599, P=0.000), plasma IL-10 levels (r=0.386, P=0.014) and TGF-β1 levels
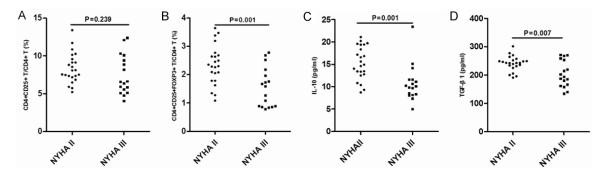
(r=0.316, P=0.047). These patients also presented CD4$^+$CD25$^+$FOXP3$^+$ T cell frequencies that correlated positively with the plasma IL-10 levels (r=0.513, P=0.001) and TGF-β1 levels (r=0.439, P=0.005). Moreover, the plasma IL-10 levels correlated positively with the plasma TGF-β1 levels (r=0.356, P=0.024) (**Figure 3**).

Based on NYHA classification standards, 23 ADHF patients were classified as functional class II and 17 patients were classified as functional class III at discharge. The results showed that the CD4$^+$CD25$^+$FOXP3$^+$ T cell frequencies and IL-10 and TGF-β1 levels were significantly decreased in the class III patients relative to the class II patients (**Figure 4**). Although the CD4$^+$CD25$^+$ T cell frequencies were lower in the class III patients than in the class II patients, significant differences in the CD4$^+$CD25$^+$ T cell frequencies were not observed between the two groups (**Figure 4**).

We assessed whether the CD4$^+$CD25$^+$ Treg cell response was associated with age, BMI, blood pressure, HR, liver and renal function,

Figure 3. Correlation analysis of Treg cells and related cytokine levels in ADHF patients at discharge. A: CD4+CD25+ T cells correlated positively with CD4+CD25+FOXP3+ T cells in ADHF patients at discharge. B: CD4+CD25+ T cells correlated positively with IL-10 levels in ADHF patients at discharge. C: CD4+CD25+ T cells correlated positively with TGF-β1 in ADHF patients at discharge. D: CD4+CD25+FOXP3+ T cells correlated positively with IL-10 levels in ADHF patients at discharge. E: CD4+CD25+FOXP3+ T cells correlated positively with TGF-β1 levels in ADHF patients at discharge. F: Plasma IL-10 levels correlated negatively with TGF-β1 levels in ADHF patients at discharge.

Figure 4. Treg cells and related cytokine levels in ADHF patients at discharge according to NYHA classification. A: No differences in the CD4$^+$CD25$^+$ T cell frequencies were observed between the class III and II groups. B: CD4$^+$CD25$^+$FOXP3$^+$ T cell frequencies were significantly decreased in the class III patients relative to the class II patients. C: IL-10 levels were significantly decreased in the class III patients relative to the class II patients. D: TGF-β1 levels were significantly decreased in the class III patients relative to the class II patients.

lipid and lipoprotein fractions, fasting glucose, CRP, NT-proBNP and impaired ventricular function in ADHF patients at admission and discharge. As shown in **Table 2**, the frequencies of CD4$^+$CD25$^+$ T cells correlated positively with the LVEF but negatively with age, CRP and NT-proBNP. The frequencies of CD4$^+$CD25$^+$FOXP3$^+$ T cells correlated positively with the LVEF but negatively with CRP, NT-proBNP

and LVEDD. IL-10 levels correlated positively with LVEF but negatively with CRP and LVEDD. In addition, TGF-β1 levels correlated positively with LVEF in the ADHF patients at admission. As shown in **Table 3**, the frequencies of CD4$^+$CD25$^+$ T cells correlated positively with the LVEF but negatively with CRP. The frequencies of CD4$^+$CD25$^+$FOXP3$^+$ T cells correlated positively with the LVEF but negatively

Table 2. Spearman's correlation analysis in ADHF patients at admission

	CD4$^+$CD25$^+$ T cells (%)	CD4$^+$CD25$^+$FOXP3$^+$ T cells (%)	IL-10 (pg/ml)	TGF-β1 (pg/ml)
Age (years)	-0.361*	-0.076	-0.173	-0.037
SBP (mmHg)	-0.103	0.022	-0.184	0.017
DBP (mmHg)	0.169	0.064	0.049	0.105
HR (bpm)	0.176	-0.166	-0.166	-0.173
Creatinine (μmol/L)	0.087	-0.045	-0.058	0.174
UA (μmol/L)	-0.007	-0.145	-0.144	-0.159
GLU (mmol/L)	0.148	0.130	-0.035	0.069
TC (mmol/L)	0.000	0.007	0.263	-0.038
TG (mmol/L)	0.004	0.130	0.288	-0.081
LDL-C (mmol/L)	0.005	-0.047	0.091	-0.091
HDL-C (mmol/L)	-0.018	-0.081	0.277	0.309
NT-proBNP(pg/ml)	-0.479**	-0.471**	-0.239	-0.262
CRP (mg/L)	-0.326*	-0.500**	-0.388*	-0.250
LVEDD (mm)	-0.178	-0.404**	-0.501**	-0.194
LVEF (%)	0.443**	0.600**	0.550**	0.337**

Note: *P<0.05, **P<0.01.

Table 3. Spearman's correlation analysis in ADHF patients atdischarge

	CD4$^+$CD25$^+$ T cells (%)	CD4$^+$CD25$^+$FOXP3$^+$ T cells (%)	IL-10 (pg/ml)	TGF-β1 (pg/ml)
Age (years)	-0.191	-0.048	-0.159	-0.099
SBP (mmHg)	0.107	0.050	0.111	0.110
DBP (mmHg)	0.052	-0.223	-0.014	-0.113
HR (bpm)	0.108	0.134	0.324	0.115
Creatinine (μmol/L)	0.167	-0.227	-0.042	-0.266
UA (μmol/L)	0.136	-0.137	0.170	0.027
GLU (mmol/L)	0.255	-0.169	-0.140	-0.160
TC (mmol/L)	-0.126	-0.171	0.007	-0.261
TG (mmol/L)	0.294	0.099	0.081	0.079
LDL-C (mmol/L)	-0.135	-0.223	-0.080	-0.278
HDL-C (mmol/L)	-0.166	-0.079	0.083	-0.262
NT-proBNP (pg/ml)	-0.041	-0.089	-0.195	-0.085
CRP (mg/L)	-0.375*	-0.371*	-0.390*	-0.398*
LVEDD (mm)	-0.225	-0.360**	-0.122	-0.134
LVEF (%)	0.613**	0.529**	0.332*	0.321*

Note: *P<0.05, **P<0.01.

with CRP and the LVEDD. IL-10 levels correlated positively with LVEF but negatively correlated with CRP. In addition, TGF-β1 levels correlated positively with the LVEF but negatively with CRP in the ADHF patients at discharge.

In addition, we also assessed whether acute phase treatment affected the activity of the CD4$^+$CD25$^+$ Treg cells in ADHF patients. However, the results showed that acute phase treatment had no significant effects on the activity of the CD4$^+$CD25$^+$ Treg cells at discharge (data not shown).

Discussion

Treg cells, which include CD4$^+$CD25$^+$ Treg cells (also called natural regulatory T cells, nTreg), Th3 cells and Treg1 cells, were discovered in the 1990s [12-14]. CD4$^+$CD25$^+$ Treg cells are

the most important regulatory T cell subset. These cells express CD4 and CD25 (IL-2 receptor-α chain) on their cell membrane and have suppressive functions that are dependent on the transcription factor fork head/winged-helix transcription factor box P3 (FOXP3) [15]. CD4$^+$CD25$^+$ Treg cells have power fulanti-inflammatory properties that manifest through cell-to-cell contact, as well as through the secretion of anti-inflammatory cytokines, such as IL-10 and TGF-β; thus, these cells also maintain immune homeostasis and immune tolerance. Additionally, the protective role of CD4$^+$CD25$^+$ Treg cells in certain cardiovascular diseases, such as atherosclerosis and hypertension, has been widely studied over the past ten years [16-18].

Tang et al. first found that CD4$^+$CD25$^+$ T cell frequencies and FOXP3 expression were significantly decreased in DCM patients with heart failure [5]. The cellular experiments performed in that study also demonstrated that the suppressive capacity of the CD4$^+$CD25$^+$ T cells was more impaired in the DCM patients than in the healthy controls [5]. According to the etiology of heart failure, 99 stable heart failure patients were divided into an ischemic heart failure group and a non-ischemic heart failure group. The results revealed that regardless of etiology, CD4$^+$CD25$^+$ T cell frequencies and suppressive capacity were reduced in patients with heart failure [6]. Another study found that in subjects with both normal and abnormal LVEF values, the CD4$^+$CD25$^+$ T cell frequencies were significantly decreased in patients with heart failure compared with individuals in the control group [9]. Therefore, the results of these studies suggest that the observed decrease in Treg cell levels is a substantive characteristic of heart failure; however, these studies only examined the Treg cell levels at a single time point in patients with chronic heart failure.

In this study, we present the first evidence that the levels of circulating Treg cells and their associated cytokines are significantly decreased in ADHF patients at admission. In addition, the decreasing tendency in the levels of circulating Treg cells and their associated cytokines significantly improves after comprehensive treatment with anti-heart failure

drugs. However, our results show that the Treg cell levels in the ADHF patients at discharge failed to reach normal levels. Therefore, this study indicates that abnormal Treg cells levels were correlated with the onset of chronic heart failure and associated with the presence of ADHF. However, the underlying mechanisms responsible for the decreased Treg cell levels observed in patients with heart failure remain uncertain. Tang et al. demonstrated that in patients with heart failure, a decrease in circulating Treg cell levels may be the result of decreased thymic output and increased susceptibility to apoptosis in the periphery [7]. Another study found that decreased vitamin Dcorrelates positively with low levels of circulating Treg cells in patients with chronic heart failure and that vitamin D treatment promotes the generation of Treg cells. These findings demonstrated another method by which Treg cell deficiency can occur in heart failure patients [10].

A previous study demonstrated that circulating Treg cell levels correlate positively with the LVEF but negatively with the LVEDD in patients with chronic heart failure [6]. Another study found that circulating Treg cell levels are associated with impaired left ventricular function in patients with coronary artery disease [19], which is the most important cause of heart failure. Therefore, we analyzed the correlation between Treg cell levels and impaired left ventricular function in ADHF patients. The results showed that Treg cell levels are associated with left ventricular function in ADHF patients at admission and discharge, and the associated cytokines also exhibited similar trends. Collectively, these studies demonstrate the close relationship between Treg cells and left ventricular function in patients with cardiovascular disease. This relationship is also supported by evidence from a number of animal experiments, in which the depletion of Treg cells significantly exacerbated left ventricular dysfunction and the adoptive transfer of Treg cells effectively improved left ventricular function during myocardial infarction, myocarditis and dilated cardiomyopathy [20-22].

In the present study, we also investigated the correlations between Treg cell levels and age, blood pressure, heart rate, liver and renal function, lipid and lipoprotein fractions, fasting

glucose, NT-proBNP and CRP levels in ADHF patients. We found that the Treg cell levels correlated negatively with the CRP levels in the ADHF patients at admission and discharge, suggesting a close relationship between Treg cells and inflammation. However, although the Treg cell levels correlated negatively with age and NT-proBNP levels in the ADHF patients at admission, these correlations were not observed at discharge. The cause of this change remains unknown. In addition, no significant correlations were observed between the Treg cell levels and BMI, blood pressure, heart rate, liver and renal function, lipid and lipoprotein fractions or fasting glucose levels in the present study.

The predictive value of Treg cell levels for determining the incidence and prognosis of various conditions, including malignant diseases, transplants and infectious diseases, has been demonstrated in a number of studies [23-27]. The underlying causes of Treg cell level changes in patients with cardiovascular disease have also been studied in recent years [11, 28]. Subgroup analyses performed in the Malmö Diet and Cancer Study demonstrated that lower Treg cell levels at baseline are associated with the onset of myocardial infarction but not stroke [28]. Importantly, Okamoto et al. found that circulating Treg cell levels were not only significantly decreased in HF patients but also associated with one-year recurrent hospitalization rates, indicating that Treg cell levels may serve as a predictor of poor HF prognosis [11]. Although those results are interesting, the study sample size was too small and the follow-up time was too short; thus, the study failed to provide information about Treg cell levels and cardiac death. Cardiac death should be considered as the primary endpoint in any HF study because some studies have reported a fatality rate of nearly 22% in the first year after hospitalization for HF [1]. Collectively, prospective studies using large sample sizes should be performed to clarify the relationship between decreased Treg cell levels and HF prognoses, including ADHF.

In conclusion, decreased Treg cell levels were associated with the onset of ADHF symptoms and could be up-regulated after comprehensive treatment with anti-heart failure drugs. These findings suggest that circulating Treg cell levels may serve as a useful biomarker of ADHF and that monitoring dynamic changes in $CD4^+CD25^+$ regulatory T cell activity may be useful for determining the effect of anti-heart failure therapy. However, the present study had some limitations. First, the sample size was too small and should be increased in subsequent studies. Second, although we present the first investigation of the dynamic changes in $CD4^+CD25^+$ regulatory T cell activity that occur in ADHF patients, we failed to follow-up with these patients. Therefore, the exact meaning of the observed changes in $CD4^+CD25^+$ Treg cells in ADHF patients should be investigated further.

Disclosure of conflict of interest

None.

Abbreviations

ADHF, acute decompensated heart failure; CSHF, chronic stable heart failure; ACEI, angiotensin converting enzyme inhibitor; ALT, alanine aminotransferase; ARB, angiotensin receptor blocker; AST, aspartate aminotransferase; CRP, C reactive protein; DBP, diastolic blood pressure; DCM, dilated cardiomyopathy; hBNP, human brain natriuretic peptide; HDL, high-density lipoprotein; HF, heart failure; HR, heart rate; IL, interleukin; LDL, low-density lipoprotein; LVEDD, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; NT-proBNP, Amino-terminal proB-type natriuretic peptide; NYHA, New York Heart Association; PBMCs, peripheral blood mononuclear cells; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; TGF, transforming growth factor; TNF, tumor necrosis factor; UA, uric acid.

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