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CLINICAL SIGNIFICANCE OF PERIPHERAL BLOOD CD4/CD8 T CELL RATIO IN PATIENTS WITH ISCHEMIC HEART FAILURE

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ABSTRACT

Aims: To analyze the clinical significance of peripheral blood CD4/CD8 T cell ratio in patients with ischemic heart failure (IHF).

Methods: This prospective study was conducted in Yanbian Hospital, Yanji city, China. A total of 100 IHF patients admitted in the department of cardiology were included as the case group. According to the New York heart association (NYHA) functional class, these patients were further divided into NYHA II (17), III (31), and IV (52) sub-groups. For comparison 50 normal patients without any history or evidence of heart disease were included as a control group. Comparisons between these groups were done to observe the changes in peripheral blood $CD4^+$ T cell, $CD8^+$ T cell, and the CD4/CD8 ratio.

Results: There was a significant decrease in peripheral blood level of $CD4^+$ T cell (468.0(297.0-657.0) vs 702.0(600.0-912.0); $P < 0.001$), and $CD8^+$ T cell (312.0(193.8-460.8) vs 624.0(479.0-842.0); $P < 0.001$) in IHF group compared with the control group. The CD4/CD8 ratio was significantly higher in IHF compared to Control (1.67±0.73 vs 1.15±0.30; $P < 0.001$). Furthermore, multivariable regression analysis showed that CD4/CD8 ratio (≥1.68) (OR 49.920; CI 4.425-497.510; $P < 0.01$), were independently linked with IHF.

Conclusions:

1. There is a significant decline in the peripheral $CD4^+$ T cell and $CD8^+$ T cell level in the patient with IHF, while the ratio of CD4/CD8 increased, indicating the presence of immune system regulation defect in the pathogenesis of IHF.
2. The CD4/CD8 ratio (≥1.68) is an independent predictor of IHF, and it could be used as a clinical bio-marker for the diagnosis of IHF.

Keywords: T lymphocyte subsets, CD4/CD8 ratio, ischemic heart failure, HF functional class.

INTRODUCTION

HF tends to affect the elderly, with a prevalence of over 26 million people worldwide, is one of the principle reasons for patients morbidity and mortality [1]. Several conditions like myocardial infarction (MI), hypertension, viral infections, and endocrine disorders can initiate the pathogenesis and progression to chronic HF. IHF is the most common phenotype of HF. Neuro-hormonal activation and persistent inflammation are one among the multiple pathophysiological factors of IHF. Previous clinical and evidence-based studies suggest that activation and expansion of T lymphocytes can be involved in the pathogenesis of HF, independent of etiology [2-5]. According to a study, resident T lymphocytes are in fact, present in steady state heart tissue, implying under healthy conditions heart tissue harboring T lymphocytes are harmless [6].

After a myocardial injury, cardiac auto-antigens which are yet unidentified stimulate mononuclear phagocytic networks and dendritic cells in heart and spleen. In the early stage of acute myocardial infarction (AMI), a primary wave of neutrophils and insurgent pro-inflammatory macrophages (M1) follows through that promote tissue digestion and subsequent timely polarization into regulatory anti-inflammatory macrophages (M2) that resolve inflammation and promote healing in heart overtime [7]. $CD4^+$ T cells including $Foxp3^+$ Tregs homing to heart from spleen and heart draining lymph nodes promote wound healing in the early stage of AMI by favorably influencing monocyte/macrophage differentiation and by promoting collagen matrix formation, angiogenesis, wound repair and stable scar formation [8-9]. However, under repeated exposures of autoantigens and persistence of inflammation antigen-experienced effector and memory T cells which were stored in splenic tissue, causes reactivation of heart directed adaptive immunity. In chronic pressure overload induced-MI $CD4^+$ T cells activation and infiltration to left ventricle in experimental mice contributed to HF progression through secretion of cytokines, myocardial fibrosis, and heart remodeling. T cell depletion in the same model preserved contractile function, reduced fibrosis, and attenuated adverse remodeling [3,4]. These suggest that even though activation of $CD4^+$ T cells in the early stage of AMI is beneficiary in terms of angiogenesis, wound healing and stable scar formation, the persistence of inflammatory processes is detrimental to cardiac muscle as severe myocardial healing defects including dysfunctional extracellular matrix deposition, alteration in cardiac structural composition, ventricular hypertrophy, and deleterious cardiac remodeling follows through [10].

Cytotoxic T lymphocytes generally have a pre-established cytotoxic role in the adaptive immune system. They have a certain predilection in identifying to kill tumor cells [11] and virus-infected cells [12]. Experimental studies showed that $CD8^+$ T cells derived from rats with MI are cytotoxic to healthy cardiac cells, and upon adoptive transfer of activated splenocytes from post-MI rat resulted in $CD8^+$ cell-mediated cardiac injury in the healthy recipient rat [13]. Elevated peripheral levels of $CD8^+$ T cells are found in both early and advanced post-MI mice than in sham mice indicating the prominent role of activated $CD8^+$ T cells in IHF [5]. These accumulated reports suggest the orchestrated infiltration and activation of $CD8^+$ T cells, its cytotoxic effect on the myocardiocytes and activity of inflammatory macrophages is the culprit for the formation of IHF.

The number of T lymphocytes in the surrounding tissues is relatively stable, and the proportion of

$CD4^+$ and $CD8^+$ T lymphocytes is roughly the same in all tissues. Any Imbalance in lymphocyte subsets and CD4/CD8 ratio could be linked with systemic lupus erythematosus [14], generalized vitiligo [15] and type-1 diabetes mellitus [16]. It is increasingly evident that T lymphocyte subsets are involved in disease formation of IHF. Our primary objective was to determine the change in the value of circulating T cell subsets by evaluating the peripheral CD3, CD4, CD8 T cells and CD4/CD8 ratio in the interest to analyze the immune-regulatory dysfunction present in the patients with IHF.

MATERIALS AND METHODS

Ethics statement:

Our study concept and approach were approved by the Institutional Ethics Review Committee of Yanbian university hospital. All methods were implemented according to the relevant guidelines and regulations and informed written consent was obtained from all of the patient prior collection of blood for the required investigations.

Study population and definition:

In this prospective study, a total of 150 subjects with completed clinical data were screened for group selections. 100 IHF patients admitted to our department of cardiology in Yanbian University Hospital (Yanji, China) from January 2018 to January 2019 were included as the case group. These patients had HF of ischemic etiology, reduced ejection fraction and New York Heart Association (NYHA) functional class II-IV. IHF patients were definitely diagnosed in accordance with clinical presentation, cardinal features, blood examinations, and imaging tests. All IHF patients were taking standard medical therapies with statins, angiotensin-converting enzyme inhibitors (ACEI) and/or angiotensin type 1 receptor blockers (ARBs), β-blocker and diuretics. Among those 100 IHF subjects, 62 of them were males and 38 were females. The median age was 71.5(61.0-77.0) years. Patients were further divided into subgroups according to NYHA functional classification grade II (17), III (31) and IV (52). Meanwhile, 50 patients without any evidence of heart disease were selected as the control group. Among the control group, 20 were male and 30 were female, and their median age was 64.0(53.8-67.0) years. Patients with congenital heart disease, dilated cardiomyopathy, hypertensive cardiomyopathy, valvular heart disease, acute myocarditis, cardiac muscle disease secondary to a systemic condition, acute MI, endocrine diseases such as thyroid dysfunction, type 1 diabetes mellitus, rheumatic diseases, severe renal and hepatic dysfunction, acute and chronic infection, recent history of corticosteroid usage, and cancer were excluded to prevent any overlapping immune responses.

In this research, dyslipidemia was defined if patients had previously known dyslipidemia or low-density lipoprotein cholesterol (LDL) greater than or equal to 140 mg/dL or total cholesterol (TC) greater than or equal to 220 mg/dL. Hypertension was defined as a recording of systolic blood pressure > 140 mmHg or diastolic blood pressure > 90 mmHg on multiple occasions, and/or having a history of antihypertensive

medications. Diabetes mellitus was defined if patients had previously known diabetes, history of antihyperglycemic medications, and/or a fasting plasma glucose level >126 mg/dL, HbA1c level ≥ 6.5%. We implemented a standard questionnaire with all the patients, recording characteristics including age, gender, current smoking status, clinical history, and medication history. Measurements of body weight index and blood pressure were done. Assessment of HF patient's quality of life was done.

Laboratory examination:

On admission, we took about 2cc of peripheral venous blood from patients of both the IHF and control groups and collected in an ethylene diamine tetra-acetic acid salt tube. For the determination of absolute values of the peripheral blood $CD4^+$, $CD4^+$, and $CD8^+$ T lymphocytes, we used Cyto Counter flow cytometry (Semibio, China). For each type of T cell, 100 microliters (µL) of blood were placed into a test tube of 12x75 mm size and then 10 µL of a suitable monoclonal antibody solution was added. The monoclonal antibodies used in our study were T11-RD1/B1-FITC and T4-RD11/T8FITC. Later on, those samples were incubated with antiserum for about 10 minutes in room temperature. The samples were then placed inside the Coulter Multi-Q-Prep instrument. Thereafter, 600 µL of immuno-preparation A (Erythrocyte Lytic Agent), 265 µL of immuno-preparation B (leukocyte stabilizer) and 100 µL of immuno-preparation C (cell membrane fixative) were added to the samples. The preparations produced were in succession transferred to the flow cytometry machine and the absolute values of all the $CD3^+$, $CD4^+$, and $CD8^+$ T lymphocytes were determined. Calculation of the CD4/CD8 ratio was done. The levels of serum NT-proBNP, LDL, HDL, hemoglobin (HGB), hs-CRP and creatinine were analyzed using standard enzymatic methods.

Echocardiography:

Echocardiography was done using ultrasound system Acuson SC 2000 (Siemens Company, Germany). Calculation of the left ventricular end-systolic dimension (LVSd), left ventricular end-diastolic dimension (LVDd), left atrial diameter (LAD) and left ventricular ejection fraction (LVEF) were done following the criterion by American Society of Echocardiography. The images were carefully inspected and stored.

Statistical analysis:

In this study, SPSS version 25.0 (SSPS Inc., Chicago, IL) was used for the statistical analysis. Numerical variables were shown as median with interquartile range, and descriptive statistics were shown as mean values with standard deviation. Numbers with percentages were used for categorical variables. Kolmogorov-Smirnov test was used for the evaluation of normality. Student's t-test was used to calculate differences between mean values. Mann-Whitney *U*-test was used to determine differences between median values. Chi-square test was applied for tested differences in frequencies between the studied groups. ROC curves were plotted in order to calculate the area under the curve. The Youden index (YI) was calculated to determine the optimal cutoff value for age, hsCRP, NT-proBNP, CD4, CD8, CD3, CD4/CD8, LAD, LVDd, LVSd, LVEF, and CI, and then in accordance with the cut-off value, they were further divided into two groups. Multivariate logistic regression analysis was performed for the variables identified as statistically significant

in univariate analysis. Finally, since variables are inter-related, multivariate regression analysis, stepwise method, was performed to determine the independent predictors of HF. The probability of F was used to select the variables to be included in the model, the variables with p-values less than 0.05 were entered and variables with *P*-values larger than 0.10 were removed from the model. A $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Comparison of the clinical parameters between IHF and the control group:

The baseline characteristics and laboratory values of IHF and control patients are presented in table 1. There was a significant difference in age (71.5(61.0-77.0) vs 64.0(53.8-67.0); $P < 0.001$) between the two experimental groups, while IHF was more prevalent in male (62.0% vs 40.0%; $P < 0.05$). There was no difference in smoking state between the two groups (40.0% vs 26.0%; $P > 0.05$). The IHF group did not have any significant difference in the prevalence of hypertension than the control group (62.0% vs 74.0%; $P > 0.05$). Compared to the control group, the prevalence of diabetes was more frequently seen in IHF group (40.0% vs 10.0%; $P < 0.001$), while dyslipidemia was more prevalent in the control group (18.0% vs 40.0%; $P < 0.01$). Laboratory tests including serum creatinine (75.50(61.25-92.50) vs 68.0(61.0-76.0); $P < 0.05$), hs-CRP (5.32(2.35-13.67) vs 1.10(0.54-2.71); $P < 0.001$) and NT-proBNP (2302.0(1126.8-4503.0) vs 50.0(50.0-75.8); $P < 0.001$) were significantly higher in IHF group in comparison with the control group, while hemoglobin level was significantly lowered in IHF subjects (130.00(119.00-142.75) vs 137.00(129.50-145.25); $P < 0.01$). The value of LDL (2.80±0.98 vs 2.75±0.90; $P > 0.05$) and HDL (1.15(0.95-1.35) vs 1.20(1.06-1.34); $P > 0.05$) were similar in both the groups. In medical usage history, the frequency of angiotensin-converting enzyme inhibitor (ACEI) (29.0% vs 10.0%; $P < 0.01$), beta blocker (59.0% vs 34.7%; $P < 0.01$), diuretics (54.0% vs 2.0%; $P < 0.001$), statins (82.0% vs 59.2%; $P < 0.01$), and insulin (23.0% vs 8.0%; $P < 0.05$) usage was significantly higher in IHF group than in the control group, while calcium channel blocker (CCB) (18.0% vs 44.0%; $P < 0.01$) usage was more frequent in control patients. As expected, Echocardiography parameters of LVEF (47.0(40.0-52.3) vs 61.0(60.0-63.0); $P < 0.001$) and CI (2.7(2.2-3.1) vs 3.2(2.8-3.5); $P < 0.001$) was significantly lower in IHF than in the control group. Compared to the control group, IHF group had a significantly higher value of LAD (41.0(36.8-45.0) vs 35.0(31.0-37.0); $P < 0.001$), LVDd (52.0(45.3-57.0) vs 47.0(45.0-49.0); $P < 0.001$) and LVSd (37.5(29.0-44.3) vs 28.0(26.8-29.0); $P < 0.001$) suggesting left ventricle dilation in HF. IHF patients had a significantly lower levels of peripheral $CD4^+$ (468.0(297.0-657.0) vs 702.0(600.0-912.0); $P < 0.001$), $CD8^+$ (312.0(193.8-460.8) vs 624.0(479.0-842.0); $P < 0.001$), and $CD3^+$ (816.0(607.0-1161.0) vs 1395.0(1112.0-1820.0); $P < 0.001$), while significantly higher CD4/CD8 ratio (1.67±0.73 vs 1.15±0.30; $P < 0.001$) in comparison to the control group.

Parameter	Ischemic Heart Failure (n=100)	Control (n=50)	*P* value
Age（years）	71.5(61.0-77.0)	64.0(53.8-67.0)	< 0.001
Gender (male) %	62.0	40.0	< 0.05
BMI (kg/m^2)	23.84±4.15	25.25±3.66	< 0.05
Medical history			
Smoking, %	40.0	26.0	> 0.05
Hypertension, %	62.0	74.0	> 0.05
Diabetes, %	40.0	10.0	< 0.001
Dyslipidemia, %	18.0	40.0	< 0.01
Blood investigation			
LDL (mmol/L)	2.80±0.98	2.75±0.90	> 0.05
HDL (mmol/L)	1.15(0.95-1.35)	1.20(1.06-1.34)	> 0.05
HGB (g/L)	130.00(119.00-142.75)	137.00(129.50-145.25)	< 0.01
Creatinine (μmol/L)	75.50(61.25-92.50)	68.0(61.0-76.0)	< 0.05
hs CRP (mg/L)	5.32(2.35-13.67)	1.10(0.54-2.71)	< 0.001
NT –proBNP (pg/ml)	2302.0(1126.8-4503.0)	50.0(50.0-75.8)	< 0.001
CD3 (cells/μL)	816.0(607.0-1161.0)	1395.0(1112.0-1820.0)	< 0.001
CD4 (cells/ μL)	468.0(297.0-657.0)	702.0(600.0-912.0)	< 0.001
CD8 (cells/ μL)	312.0(193.8-460.8)	624.0(479.0-842.0)	< 0.001
CD4/CD8	1.67±0.73	1.15±0.30	< 0.001
Echocardiography			
LAD (mm)	41.0(36.8-45.0)	35.0(31.0-37.0)	< 0.001
LVDd (mm)	52.0(45.3-57.0)	47.0(45.0-49.0)	< 0.001
LVSd (mm)	37.5(29.0-44.3)	28.0(26.8-29.0)	< 0.001
CI (L/min/m^2)	2.7(2.2-3.1)	3.2(2.8-3.5)	< 0.001
LVEF (%)	47.0(40.0-52.3)	61.0(60.0-63.0)	< 0.001
Medication history			
ACEI, %	29.0	10.0	< 0.01
ARB, %	43.0	32.0	> 0.05
b-blocker, %	59.0	34.7	< 0.01
CCB, %	18.0	44.0	< 0.01
Diuretics, %	54.0	2.0	< 0.001
Statins, %	82.0	59.2	< 0.01
Insulin, %	23.0	8.0	< 0.05

Table 1: Main characteristics and laboratory investigations of the IHF (n=100) and control patients (n=50)

Values are designated as median (interquartile), mean ± SD or number (%).

ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin type II receptor blocker; CCB = calcium channel blocker; BMI = body mass index; HGB = hemoglobin; LAD = left atrial diameter; LVSd = left ventricular end-systolic dimension; CI = cardiac index; LVDd = left ventricular end-diastolic dimension; LVEF = left ventricular ejection fraction; hs-CRP = high-sensitivity C-reactive protein; NT-proBNP = N-terminal-pro brain natriuretic peptide; HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol.

Comparison of the clinical parameters between NYHA functional classes:

In accordance with NYHA functional grade, IHF patients were further sub-grouped as NYHA II (n=17), III (n=31) and IV (n=52). Comparisons of clinical characteristics between these subgroups are shown in Table 2. Unlike age distribution in the previous group, there was no significant difference in age in these subgroups. There was no significant difference in the value of hsCRP, CD8, CD3, and CD4/CD8 ratio among the groups. Similarly, echocardiography parameters of LAD, LVDd, LVSd, and CI were also not significantly different between the groups. Peripheral $CD4^+$ T cells decreased significantly in NYHA III group in comparison to NYHA II group (416.0(252.0-628.0) vs 592.0(392.0-900.0); $P < 0.05$). As anticipated, NT-pro BNP value was significantly higher in NYHA III (2578.0(1296.0-4128.0) vs 998.0(550.0-1312.0); $P < 0.05$) and NYHA IV (3100.0(1249.0-4691.3) vs 998.0(550.0-1312.0); $P < 0.001$) groups as compared with the NYHA II group. In comparison to NYHA II group, LVEF was significantly reduced in NYHA III (48.0(44.0-55.0) vs 54.0(43.0-60.0); $P < 0.05$) and NYHA IV (43.0(38.0-50.0) vs 54.0(43.0-60.0); $P < 0.001$) groups.

Parameter	**NYHA II (n=17)**	**NYHA III (n=31)**	**NYHA IV (n=52)**
Age (years)	66.0(60.5-76.0)	66.0(61.0-78.0)	72.0(61.5-77.8)
NT–proBNP (pg/ml)	998.0(550.0-1312.0)	2578.0(1296.0-4128.0) *	3100.0(1249.0-4691.3) **
hsCRP (mg/L)	2.25(0.58-6.63)	4.89(2.66-15.74)	6.20(3.34-16.29)
CD4 (cells/ μL)	592.0(392.0-900.0)	416.0(252.0-628.0) *	474.0(299.0-620.0)
CD8 (cells/ μL)	404.0(298.0-584.0)	312.0(180.0-448.0)	282.0(188.3-417.0)
CD3 (cells/ μL)	1056.0(816.0-1684.0)	768.0(545.0-1020.0)	780.0(600.0-1124.0)
CD4/CD8	1.69(1.17-1.88)	1.29(1.00-2.00)	1.94(1.08-2.25)
LVEF (%)	54.0(43.0-60.0)	48.0(44.0-55.0) *	43.0(38.0-50.0) **
LAD (mm)	37.0(33.0-43.5)	41.0(33.0-44.0)	42.0(40.0-49.0)
LVDd (mm)	48.0(44.0-59.0)	53.0(46.0-57.0)	52.0(47.0-58.5)
LVSd (mm)	31.0(27.5-47.0)	36.0(28.0-44.0)	38.0(30.0-46.0)
CI ($L/min/m^2$)	2.8(2.2-3.2)	2.9(2.3-3.2)	2.5(2.2-2.9)

Table 2: Main characteristics and laboratory investigations between NYHA functional classes

Compared with NYHA II,*P<0.05, **P<0.001. Values are designated as median (interquartile). Abbreviations are as shown in table 1.

Independent predictors of IHF:

ROC curves were plotted to determine the optimum cutoff values of the different clinical indices of the IHF patients including age, CD4, CD8, CD3, CD4/CD8, hsCRP, NT-proBNP, LAD, LVDd, LVSd, LVEF, and CI (figure 1). The univariable binary logistic regression model was used to find the potential risk factors of IHF (table 3). According to the table 3 Age (≥69.5), gender, diabetes, dyslipidemia, hsCRP (≥3.01), LVEF (≤59.5), CD3 (≤1111.1), CD4 (≤588.2), CD8 (≤384.6), and CD4/CD8 (≥1.68) ratio were significantly associated with IHF. Furthermore, multivariable logistic regression analysis (table 4) done on these factors showed that age (≥69.5) (odds ratio [OR] 13.986; 95% confidence interval for OR [CI] 2.801-69.846; P < 0.01), diabetes (OR 19.606; 95% CI 3.142-122.337; P < 0.01), CD3 (≤1111.1) (OR 0.079; 95% CI 0.018-0.346; P < 0.01), CD4/CD8 ratio (≥1.68) (OR 49.920; 95% CI 4.425-497.510; P < 0.01), and LVEF (≤59.5) (OR 0.051; 95% CI 0.011-0.228; P < 0.001) were independently associated with IHF.

Variable	OR	95% CI for OR	*P*
Age (≥69.5)	10.560	3.871-28.839	<0.001
Gender	2.447	1.221-4.904	<0.05
Diabetes	6.000	2.192-16.422	<0.001
Dyslipidemia	0.329	0.154-0.705	<0.01
CD4 (≤588.2)	0.152	0.069-0.333	<0.001
CD8 (≤384.6)	0.152	0.069-0.333	<0.001
CD3 (≤1111.1)	0.097	0.043-0.221	<0.001
CD4/CD8 (≥1.68)	49.000	6.511-368.749	<0.001
hsCRP (≥3.01)	9.333	4.139-21.071	<0.001
LVEF (≤59.5)	0.048	0.017-0.132	<0.001

Table 3: Associations of various clinical parameters with IHF according to univariable logistic regression analysis

OR = odds ratio; CI = confidence interval. Abbreviations are as shown in table 1.

Variable	OR	95% CI for OR	*P*
Age (≥69.5)	13.986	2.801-69.846	<0.01
Diabetes	19.606	3.142-122.337	<0.01
CD3 (≤1111.1)	0.079	0.018-0.346	<0.01
CD4/CD8 (≥1.68)	49.920	4.425-497.510	<0.01
LVEF (≤59.5)	0.051	0.011-0.228	<0.001

Table 4: Independent predictors of IHF as according to the multivariable logistic regression analysis

Multivariable regression model included all variables at baseline with P < 0.05 by univariable regression analysis.

OR = odds ratio; CI = confidence interval. Abbreviations are as shown in table 1.

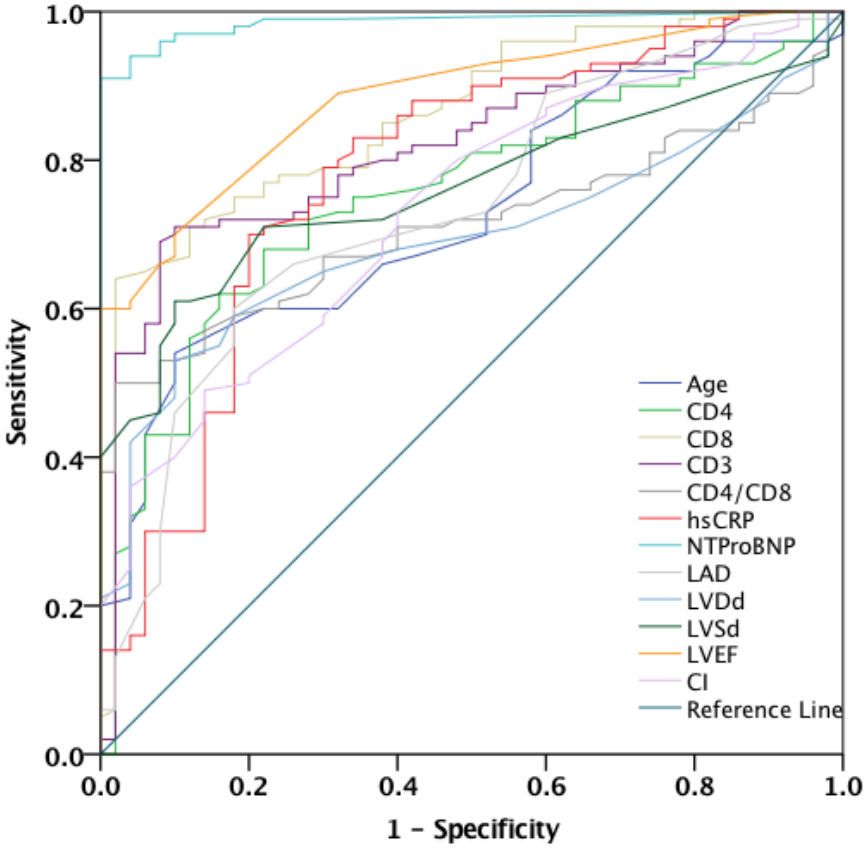

Figure 1: ROC curves to determine the optimal cutoff values in IHF.

Note: ROC curves for IHF were plotted to verify the optimum cutoff points for age, CD4, CD8, CD3, CD4/CD8, hsCRP, NT-proBNP, LAD, LVDd, LVSd, LVEF, and CI, which were 69.5years, 588.2, 384.6, 1111.1, 1.68, 3.01, 532.5, 38.5, 51.5, 33.5, 59.5, and 2.68, respectively. The AUC was 0.986 (95% CI: 0.970–1.000, *P*<0.001) for NT-proBNP, 0.860 (95% CI: 0.799–0.920, *P*<0.001) for CD8, 0.885 (95% CI: 0.843–0.936, *P*<0.001) for LVEF, 0.822 (95% CI: 0.754–0.891, *P*<0.001) for CD3, 0.782 (95% CI: 0.701–0.864, *P*<0.001) for hsCRP, 0.761 (95% CI: 0.687–0.836, *P*<0.001) for LVSd, 0.753 (95% CI: 0.674–0.833, *P*<0.001) for CD4, 0.735 (95% CI: 0.652–0.819, *P*<0.001) for LAD, 0.728 (95% CI: 0.648–0.809, *P*<0.001) for age, 0.712 (95% CI: 0.632–0.792, *P*<0.001) for CD4/CD8, 0.731 (95% CI: 0.650–0.812, *P*<0.001) for CI, and 0.693 (95% CI: 0.611–0.776, *P*<0.001) for LVDd.

DISCUSSION

During the past decade, HF has been linked with chronic inflammatory and immune disease involving adaptive immune cells. Nevers et al demonstrated that in mice and human, pressure overload condition

activated intra-myocardial endothelium and promoted up-regulation of endothelial cell adhesion molecules (ICAM-1, VCAM-1) that lead to $CD4^+$ and $CD8^+$ T cells recruitment to the left ventricle resulting in cardiac fibrosis, hypertrophy and HF [4]. Similarly, chronic IHF mice were found to have local and systemic activation of $CD4^+$, and $CD8^+$ T cells and $CD4^+$ T cell subsets [5].$CD4^+$ T cell subsets can stimulate endothelial cells, fibroblasts, epithelial cells to release TNF-a, MMP-1, G-CSF, IL-1b, IL-6 potentially causing cardiac structural and functional dysfunction [17]. Post MI clonal proliferation of $CD8^+$ T cells into senescent $CD8^+CD28^{null}$ T cells destroyed healthy cardiomyocytes [13]. Indeed, $CD8^+CD28^{null}$ T cells carried a very high cytotoxic effect towards healthy cardiac tissues by activation of proinflammatory macrophages and the various signal induced death complexes. It can be concluded that IHF is characterized by the immune T cell activation as supported by the following evidence: (a) cytokine increase [5], (b) alteration of the T lymphocyte pattern [2], (c) attenuation of adverse cardiac remodeling after depletion of T cells [5].

Myocardial and systemic expansion of $CD3^+$, $CD4^+$, and $CD8^+$ T cells is seen in IHF [5]. However, T lymphocyte activation in heart draining lymph nodes and peripheral blood are not mirrored, with increased $CD4^+$ and B lymphocytes are seen in heart draining lymph nodes [18]. Our findings demonstrate that peripheral blood $CD3^+$, $CD4^+$, and $CD8^+$ T cells were significantly reduced in IHF patients in comparison with the control patients. Decreased number of $CD3^+$, $CD4^+$ and $CD8^+$ T cells in patients with chronic IHF, may be a phenomenon related to the duration of HF. This reduction in T-lymphocyte subsets is difficult to interpret, as $CD4^+$ T cells are the main pathological modifiers of IHF. We supposed the possible mechanisms for T-lymphocyte subset reduction could be:

a) A transitory change due to negative feedback modulation to the development of cardiac fibrosis and ventricular remodeling,
b) Migration of T-cell from peripheral blood to persistently inflamed heart tissue, or
c) Increased T cell death rate due to apoptosis.

In chronic IHF, prolonged inflammatory phase gives rise to progressive terminal differentiation of primed T cells into $CD4^+CD28^-$ and $CD8^+CD28^-$ phenotype which is short-lived and is highly vulnerable to spontaneous and unrestrained apoptosis. The increased T cell death rate is due to down-modulation of Bcl-2 gene and high expression of FAS surface molecule [19,20]. Chronic immune activation in IHF is found to be responsible for spontaneous lymphocyte apoptosis by down-regulation of Bcl-2 gene secondary to the deprivation of growth factors. Indeed, reshuffling of T lymphocytes from peripheral blood to heart tissue and heart draining lymph nodes can be due to the secretion of intra-myocardial chemokines and adhesion molecules which can cause peripheral T cell migration and home to the heart. These mechanisms together in association with long duration of IHF can be held responsible for the T cell subsets decline seen in our study.

Accumulating studies demonstrated that the $CD4^+$ T cell subsets dysfunction had a crucial role in the disease process of chronic HF, with an imbalance in Th1/Th2 [21,22] or Th17/Treg [5,21] ratio as the determining factor. It is well established that Th1 and Th17 cells activation promote inflammation in HF, while Treg cells reduce inflammation depending on the generation of certain cytokines. Taking atorvastatin in HF could

modulate Th1/Th2 response by inhibiting Th1 cytokine generation [22]. Indeed, studies have shown the application of Th1/Th2 and Th17/Treg ratio in determining the prevalence of IHF. These $CD4^+$ T cell subsets ratios could be evaluated by detecting their transcription factors and quantifying cytokine production, but applying this knowledge to general clinical practice is difficult since it's expensive for patients. However, by simply evaluating peripheral T lymphocyte subsets and CD4/CD8 ratio, it would be relatively economical to predict the prevalence of IHF.

Since early 1990, there have been studies conducted on finding out the relationship between $CD4^+$ T cell to $CD8^+$ T cell ratio in HF but with varying results. Accumulative studies have shown that dilated cardiomyopathy induced HF is associated with elevated peripheral CD4/CD8 ratio [23-25]. Indeed, alteration in T lymphocyte subset and CD4/CD8 ratio is associated with the severity of the HF and independent of any etiology [2,26]. Our study demonstrated that there was a significant elevation in CD4/CD8 ratio in patients with IHF in comparison to control patients. This finding was similar to data by Satoh et al [27] and Agnoletti et al [2], where peripheral CD4/CD8 ratio was elevated. In contrast to our result, a prospective study done by Cao et al showed a reduced CD4/CD8 ratio in a population of 96 hospitalized elderly patients with primary chronic IHF [26].

Hs-CRP is one of the established markers of inflammation. Elevated hs-CRP levels have already been acknowledged as a risk factor for atherosclerosis and coronary artery disease. Our studies demonstrated that patients with IHF had elevated blood hs-CRP levels compared to those of normal control patients. Additionally, hs-CRP levels increased with the increase in severity of heart failure functional grouping. We observed a negative correlation between hs-CRP levels and peripheral T-lymphocyte subsets which further demonstrates that the decreased levels of T lymphocyte subsets are linked with local cardiac inflammation and ventricular dysfunction in IHF.

Furthermore, on the comparison of different parameters among the IHF NYHA functional class groups, we found the level of plasma NT-proBNP in NYHA III and IV group was significantly higher than the NYHA II group, which indicates the severity of the heart failure. We did not find any statistically significant changes in the levels of hs-CRP, CD3, CD8, and CD4/CD8 ratio in between the three groups. Echocardiography parameters including LAD, LVDd, LVSd, and CI also did not have any significant changes between the NYHA class groups. LVEF was on decreasing trend with the increase in NYHA class and had a statistical significance. On multivariable regression analysis, we found that age, diabetes, LVEF, peripheral blood $CD3^+$ T cell, and CD4/CD8 ratio were independently associated with IHF.

In summary, subjects with IHF are characterized by a decreased T lymphocyte subsets and an elevated CD4/CD8 ratio. Our findings suggest that the evaluation and monitoring of the change in peripheral CD4/CD8 ratio could provide a useful noninvasive method for the diagnosis of IHF. However further research and large-scale longitudinal clinical trials should be organized focusing on evaluating the exact functional role of altered T lymphocyte subsets and CD4/CD8 ratio in the mechanism of ventricular remodeling in IHF.

Limitations of this study are the fact that it was a small-sized cohort study with a risk of selection bias. We

only evaluated the peripheral blood values of T-lymphocyte subsets which only comprise a minority of T cell populations as compared to the spleen, cardiac tissue and heart draining lymph nodes. Furthermore, detection of the changes in blood T lymphocytes value may not mirror the actual changes in the cardiac tissue and the coronary arteries. We only observed the changes in T lymphocyte subsets and CD4/CD8 ratio immediately after admission, while for deep correlation with IHF it is necessary to monitor the changes during discharge and after certain time intervals. In our study, over 60% of the control subjects had either hypertension or diabetes which may not be counted as a healthy control group. Finally, we didn't evaluate the levels of anti-inflammatory regulatory Treg cells, which are one of the main mediators of inflammation in IHF.

CONCLUSION

1. The peripheral levels of $CD3^+$ T cell, $CD4^+$ T cell, and $CD8^+$ T cell are decreased in patients with IHF, implying the chronic activation, altered expression and spontaneous apoptosis of T lymphocyte subsets could have a fundamental role in the pathogenesis of IHF.
2. Peripheral CD4/CD8 ratio is elevated in patients with IHF. Furthermore, the CD4/CD8 ratio (≥1.68) is independently associated with the prevalence of IHF implying its potential to be a clinically relevant biomarker tool in diagnosing IHF.
3. Peripheral CD4/CD8 ratio is not associated with the severity of IHF.

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