Cardiac-related Hospitalization and/or Death Associated With Immune Dysregulation and Symptoms of Depression in Heart Failure Patients

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Objective: Congestive heart failure (CHF) patients with depressive symptoms have a greater risk of morbidity and mortality. Immune activity such as inflammation is increasingly implicated as underlying this relationship. However, it is unknown whether there is a broader spectrum of immune dysregulation beyond inflammatory activity. This study examined in CHF patients the relationship of depressive symptoms with cellular immune activity measured by Th1/Th2 ratios and cardiac rehospitalization and/or death. **Method:** Eighteen patients with CHF (mean age = 62, NYHA classes II–IV) were enrolled and depressive symptoms were measured with interviewer ratings using the Hamilton Rating Scale-Depression. For the determination of Th1/Th2 ratios, intracellular cytokine expression of interferon-gamma (IFN-gamma) and interleukin-10 (IL-10) CD4+ T cells were measured by flow cytometry. Plasma interleukin-6 levels were measured to ascertain circulating inflammatory cytokine activity. Patient records were examined for cardiac related rehospitalization or cardiac related death over a two-year period after baseline depression and immune measures were taken. **Results:** Higher depression scores were associated with a prospective increase in incidence of cardiac related hospitalizations and/or death ($p = .037$). Lesser IFN-gamma/IL-10 expressing CD4+ T cell ratios were related to higher depressive symptom scores at baseline ($p = .005$) and a prospective increased incidence of cardiac related hospitalization or death over a two-year period ($p = .05$). **Conclusions:** A shift in the Th1/Th2 ratio may play a role in the association between depressive symptoms and morbidity and mortality in CHF patients, suggesting broader immune dysregulation than previously considered. **Key words:** heart failure, B-type natriuretic peptide, ejection fraction, depressed mood, immune dysregulation, cardiac rehospitalization

CHF = congestive heart failure; **IFN** = interferon; **IL** = interleukin; **NYHA** = New York Heart Association; **BNP** = B-type natriuretic peptide; **HAM-D** = Hamilton Depression Scale; **EF** = ejection fraction; **BMI** = body mass index.

INTRODUCTION

Congestive heart failure (CHF) is often a final phase of cardiovascular diseases resulting from a variety of cardiovascular anomalies. Between five and six million North Americans are affected by CHF (1), which is one of the most common causes of chronic disability, reduction in tolerance for physical activity, and impaired quality-of-life in the elderly (2). It is not surprising that CHF patients are at greater risk for depression (3,4) particularly because the elderly are generally more prone to depressed mood than younger individuals (5). Depressive symptoms, in turn, can initiate a spiraling decline in physical and psychological well-being and affect the course of cardiovascular disease (6). Not only clinical depression but also sub-syndromal, or sub-clinical symptoms of depression can elevate an individual's risk for future cardiac events (7–9). Furthermore, depression symptoms are predictive of mortality in patients with CHF (10–13). Although the mechanisms associating depression and cardiac morbidity and mortality are not yet known, alterations in immunity may be one avenue.

Recently, inflammatory cytokines were implicated as a mediator of psychological symptoms of depression and cardiovascular disease etiology and progression (14–17). However, it is unclear if such inflammation reflects a broader immune dysregulation involving additional components of the immune system. Symptoms of depression are associated with reductions of cellular immune measures including natural killer (NK) cell cytotoxicity, lymphocyte proliferation (18,19), viral specific cell-mediated immunity (20), and reactivation of antibody titers to latent viruses (21), presumably by reducing cellular immunity. Yet, it is unknown if depressive symptom associated changes in cellular immunity are predictive of cardiac disease morbidity and mortality.

Cellular immunity is important for protection against infection. Th1 cells promote cellular immunity by rapidly producing a range of cytokines such as IFN-γ that activate other Th1 cells to fight infectious agents. Th1 cells also exert a negative regulatory role on Th2 cells that produce cytokines such as IL-4 and IL-10. Th2 cytokines attenuate immune defenses if they are locally over-expressed, by decreasing activities of major effectors such as Th1 cells (22,23). Maintaining Th1/Th2 homeostasis is important for preserving health. A Th2 shift may have a profound effect on the susceptibility of the organism to infection (24), increase inflammation and lead to dilated cardiomyopathy and heart failure (25). Examining Th1/Th2 ratios can provide information on the balance of cellular immune activation versus negative regulation of cellular immunity.

The present study extends previous research by examining the relationship between stimulated intracellular Th1/Th2 ratios and depressive symptom severity in CHF patients. Systemic inflammatory activity was evaluated by measuring circulating levels of the proinflammatory cytokine IL-6. Finally, cardiac related hospital admissions and mortality were determined longitudinally over two years to explore the relationship among clinical outcomes, depression symptoms, and immune activity.

METHODS

Subjects

A sample of 18 males with CHF (age 50–78 years) New York Heart Association (NYHA) class II–IV were drawn from the Veteran Affairs Medical Center, La Jolla, CA, outpatient population and evaluated for depressive symp-

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toms, physical functioning (six-minute walk test), intracellular cytokine expression of interferon (IFN)-gamma and interleukin (IL)-10 and plasma IL-6. Patients were then followed for two years for cardiac related hospital readmissions or cardiac related death. Baseline data were collected during 2001 and follow-up data were examined through 2003. Inclusion in the study required symptoms of CHF for at least 3 months with optimal treatment with digitalis, diuretics, beta-blockers, and/or angiotensin-converting enzyme inhibitors. CHF patients had an ejection fraction less than 40% (systolic dysfunction), or symptoms of pulmonary congestion with normal systolic function and E to A reversal of flow on Doppler echocardiography, or excessive E dominance with short deceleration time of early rapid filling of the left ventricle (diastolic dysfunction). Participants were not eligible if they had a history of recent myocardial infarction (1 month), angina not adequately managed with nitrates, severe aortic or mitral stenosis, severe chronic obstructive pulmonary disease, a diagnosis of recent stroke or significant cerebral neurologic impairment, diagnosed psychopathology other than depression, or signs or symptoms of current infection, inflammatory disease, or were taking anti-inflammatory medication.

Patients were identified via cardiologists from the VA San Diego Healthcare System (VASDHS) cardiomyopathy clinics. They were screened by a trained psychologist and gave consent to participate in the study. Demographic and medical history data were collected from the patients' medical charts to verify inclusion and exclusion criteria. Seventy percent of CHF clinic patients who were screened were eligible to participate in the study. This was a pilot study to determine the feasibility of investigating immune activity, depression, CHF, and prognosis. The study was approved by the University of California, San Diego, Human Research Protections Program Institutional Review Board.

Psychosocial Assessments

Severity of depressive symptoms was evaluated with the Hamilton Rating Scale-Depression (HAM-D), a 21-item interview using a clinician-rated scale (26). The Structured Clinical Interview for DSM-IV (*Diagnostic and Statistical Manual for Mental Disorders*, fourth edition) (SCID) was administered for clinical diagnoses of major depression (27) for descriptive purposes.

CHF Severity Assessment

Echocardiography

A two dimensional echocardiogram was obtained in the standard fashion in parasternal long- and short-axis views and apical 4- and 2-chamber views. Pulsed Doppler spectral recordings were gathered from 4 × 4-mm sample volume placed at the tips of the mitral leaflets and in the pulmonary vein and that were adjusted to yield the maximal amplitude velocity signals. All data were copied to 0.5-inch VHS videotape for subsequent playback, analysis, and measurement. This method has been shown to be a reliable method of assessing left ventricular function and predicting mortality in patients with heart failure (e.g., (28)). Ejection fractions were derived from biplane apical (2- and 4-chamber) views with use of modified Simpson's rule algorithm (29).

Six-Minute Walk Test

This walk test is a reliable and reproducible method to assess the severity of heart failure in patients and physical functional capacity (30). Patients were instructed to walk as far as possible within six minutes in a straight corridor. The distance covered in that time period was recorded.

B-Type Natriuretic Peptide (BNP)

Known to be a marker of cardiac disease severity (31) BNP is a cardiac neurohormone secreted from the cardiac ventricles in response to ventricular volume expansion and pressure overload (32). BNP levels are elevated in patients with symptomatic left ventricular dysfunction, correlate with New York Heart Association (NYHA) classification, and are predictive of hospital readmission and mortality (31). For the quantitative determination of BNP levels in plasma, 7 ml of whole blood were collected into tubes containing potassium ethylenediamenetetraacetic acid (EDTA; 1 mg/ml blood). Blood samples were collected and kept on ice until separated in a refrigerated centrifuge and plasma was stored at −80°C until assay. BNP was measured using the Triage B-Type Natriuretic Peptide test (Biosite Diagnostics Inc.), which is a fluorescence immunoassay that draws the plasma by capillary action into a reaction chamber to form a reaction mixture. After the incubation period, the reaction mixture flows through the device detection lane. Complexes of BNP and fluorescent antibody conjugates are captured on a discrete zone in the detection lane. Excess plasma from the blood sample being processed is then used to wash the unbound fluorescent antibody conjugates from the detection lane into a waste reservoir. The concentration of BNP in the specimen is proportional to the fluorescence bound in the detection lane and is quantified by the portable triage meter.

Immune Measures

Flow Cytometry Analyses of Intracellular Cytokines

Whole blood was stimulated with either 10 μg Staphylococcal Enterotoxin-B (SEB) and 2 μg alpha-CD28 or a comparable volume of sterile RPMI media (control sample) and incubated at 37°C at 5% CO_2 for 2 hours. SEB was chosen because it stimulates Th1 and Th2 cytokine generation from CD4+ T cells (33) and is thought to be more physiologic than phorbol esters plus calcium ionophore, which stimulate maximal levels of cytokines and does not involve T cell receptor signaling (34). Brefeldin A, 10 μg (Sigma, St. Louis, MO) was added and the samples were incubated for 4 hours at 37°C at 5% CO_2. Next, 100 μl of 4 C 20 mmol/L EDTA was added and samples were incubated for 10 minutes at room temperature. Ten ml of FACS lysing solution (BD Biosciences, San Jose, CA) was then added and samples were incubated for 10 minutes at room temperature. The samples were then centrifuged and supernatant was aspirated. The pellet was resuspended in 5 ml of wash buffer (phosphate buffered saline) followed by an overnight incubation at 4°C. Two ml of sample were transferred into polystyrene tubes, and 2 ml of wash buffer were added to each tube followed by centrifugation. The supernatant was aspirated and 500 μl of 1X FACS permiabilizing solution (BD Biosciences, San Jose, CA) was added. Samples were incubated for 10 minutes at room temperature and washed 2 times in 4 ml of wash buffer per tube. After centrifugation, the supernatant was aspirated to 100 ml, and cells were combined with antibody cocktail consisting of monoclonal antibodies conjugated with fluorochromes for either IL-10 or IFN-gamma/PE (BD Biosciences, San Jose, CA), alpha-CD69 and alpha-CD4/PerCP, gently vortexed, and incubated in the dark for 50 minutes at room temperature. Cells were washed twice in 4 ml wash buffer, centrifuged, and supernatant aspirated. The pellet was resuspended in 400 μl of 2% Formaldehyde for fixation. Samples were analyzed using a flow cytometer Beckman/Coulter Epics XL equipped with a 488 nm laser. Lymphocytes were identified on a linear forward and side scatter scatterplot, and then CD4+ T cells were identified using CD4+ gating. Activated CD4+ T cells positive for intracellular IFN gamma and IL-10 were then measured. Intracellular cytokines were expressed as a stimulation index (SI), the increase from nonstimulated to stimulated percentages of lymphocytes analyzed by flow cytometry. Spectral compensation was established daily. Quality control included the optimization for lymphocyte recovery, purity of the gate of analysis and replicate determinations.

Plasma IL-6 Levels

Plasma IL-6 levels were quantified using Quantikine High Sensitivity human IL-6 kits (R&D Systems, Inc., Minneapolis, MN) with an intra-assay coefficient of variation of 4% and an inter-assay coefficient of variation of 10%. The minimal detectable limit of IL-6 is 0.156 pg/ml, well below levels identified in our subjects.

Cardiac Related Admissions/Deaths

Patients' medical charts were examined over a two-year period after their baseline laboratory visit for cardiovascular related hospitalizations. Death certificates were obtained to verify cardiovascular related deaths. NonVA emergency care was noted in the follow-up visit physician notes inpatient VASDHS charts.

Statistics

HAM-D scores were maintained in continuous form for all primary analyses (survival analyses, and the relationships between depression and

Th1/Th2 ratios). In some secondary analyses, we categorized patients into "high" and "low" depressive symptoms based on a dichotomized breakdown of HAM-D scores (35,36). The use of dichotomized depression scores allowed us to assess whether relationships were better explained as a linear association or an effect present among those with "clinical" or "sub-clinical" depression.

Primary Analyses

Two-tailed correlation analyses were performed to examine relationships between patient characteristics such as age, BMI, ejection fraction and log-transformed values for B-type natriuretic peptide levels (to reduce the effects of skewness in the distribution of BNP levels), and HAM-D scores. Two-tailed partial correlation analyses were performed to determine the linear relationship between Th1/Th2 ratios and HAM-D scores and the relationship between HAM-D scores and distance walked in a 6-minute walk test, controlling for disease severity using two measures, log BNP and ejection fraction (EF). To determine differences in HAM-D scores between patients with cardiac related rehospitalization(s) or death and those without, a Mann-Whitney *U* test was performed as a conservative initial test. Statistical assumptions were evaluated using the Levene's test for equality of variance and the Shapiro-Wilk test for normal distribution. Because the Mann-Whitney test produced a similar statistical conclusion, and the assumption tests were nonsignificant, an analysis was performed using Analysis of Covariance (ANCOVA), controlling for disease severity including log BNP and EF. A binary logistic regression approach was utilized to examine whether HAM-D scores predicted cardiac events over the following two years. Cytokine levels and Th1/Th2 ratios were entered in the equation as covariates to establish possible mediating factors. In these analyses, we used the method of Steyerberg et al. (37) in which prognostic modeling with logistic regression analysis was shown to be appropriate in small data sets. Power analyses were used to determine numbers of subjects needed to obtain statistical significance in analyses suggesting trends in predictions of outcome variables.

Secondary Analyses

CHF patients were stratified into two groups that differed in severity of depressive symptoms (<10 versus ≥ 10) (36). HAM-D scores below 10 are suggested to be indicative of a nondepressed clinically normal range, whereas scores of greater than 10 are suggested to encompass both clinical and sub-clinical depression (35,36). Values for B-type natriuretic peptide levels, EF, and cytokine levels and ratios were compared between groups who were classified as having "high" or "low" depressive symptom levels. This depressive symptom stratification method has been used previously beginning with Hamilton (1967) (35,36). In the present study, comparisons were made with Mann-Whitney *U* tests as a conservative initial test and then *t*-tests for independent samples and ANOVAs.

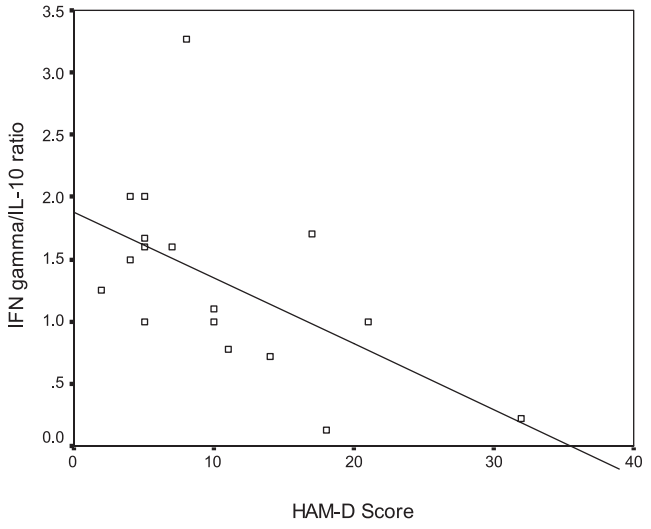

Figure 1. Relationship between IFN-gamma/IL-10 ratios and Hamilton Depression Scores in those with and without incidence of cardiac related rehospitalization and/or death.

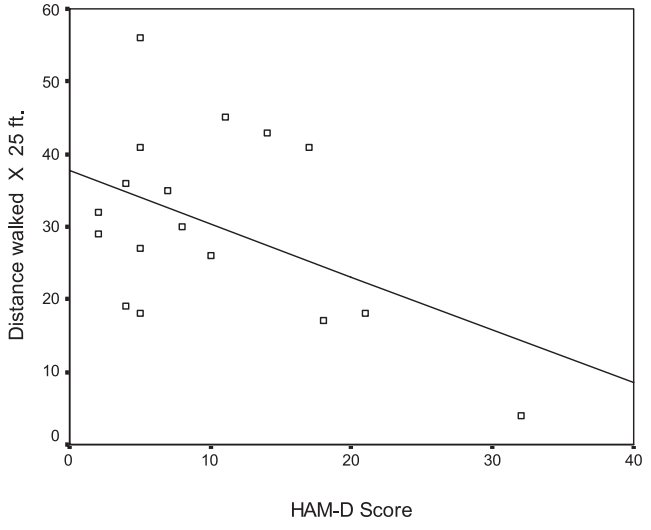

Figure 2. Relationship between Hamilton Depression Scores and distance covered in the six minute walk task.

RESULTS

Patient Characteristics

The sample of 18 men ranged in age from 50 to 78 years. Body mass index (BMI) was not associated with immune measures, HAM-D scores, or cardiac events. Over the two-year follow-up, 8 patients experienced at least one cardiac related hospital readmission or died from cardiac related complications (5 had one or more cardiac related admissions; 4 had cardiac related deaths). Eight of the 18 participants (44%) had HAM-D scores ≥ 10 of which four were diagnosed as having major depression (22.2%). The mean HAM-D scores for research participants was 10.0 (SD = 7.85) ranging from 2 to 32.

Primary Analyses

Clinical characteristics including age, BMI, level of BNP, and EF were not correlated with HAM-D scores in this cohort of CHF patients. There was a significant partial correlation between HAM-D and IFN-gamma/IL-10 ratios after controlling for disease severity markers, log BNP ($r = -0.62$, $p = .01$) or ejection fraction ($r = -0.56$, $p = .024$) (Figure 1). The distance covered in the six-minute walk task was significantly negatively correlated with HAM-D scores after controlling for disease severity with log BNP levels ($r = -0.52$, $p = .037$) and a trend when EF was used to control for disease severity ($r = -0.45$, $p = .08$) (Figure 2). Partial correlations were repeated with the removal of one IFN/IL-10 ratio outlier (>2 standard deviations of the mean) and the results remained statistically significant. Patients who later had a cardiac readmission or died had more severe depressive symptoms ($F(1, 17) = 5.2$, $p = .037$) (Figure 3), a trend for higher BNP levels ($F(1, 17) = 4.2$, $p = .058$) and significantly lower EFs ($F(1, 17) = 6.2$, $p = .024$) as compared with patients who did

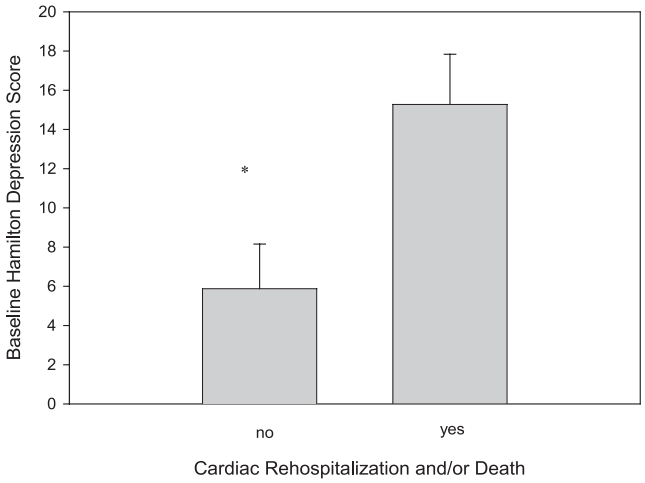

Figure 3. Significant baseline differences in Hamilton Depression Scores between those who later had an incidence of cardiac related rehospitalization and/or death and those who did not, * $p < .05$. Error bars represent standard error of the mean.

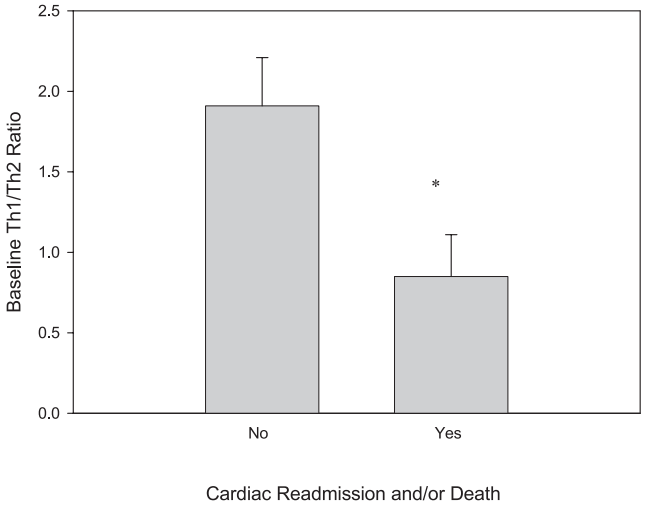

Figure 4. Significant baseline differences in IFN-gamma/IL-10 ratios between those who later had an incidence of cardiac related rehospitalization and/or death and those who did not, * $p = .05$. Error bars represent standard error of the mean.

not experience a cardiac event. Baseline Th1/Th2 cytokine ratios were lower in those who later had cardiac readmissions or died (F (1, 17) = 4.6, $p = .05$) (Figure 4) as compared with those with who did not experience such an event. IFN gamma, IL-10 and plasma IL-6 levels did not differ between those who did or did not have cardiac events Table 1. Using prognostic modeling with logistic regression analysis there was a trend for baseline HAM-D scores to predict cardiac related rehospitalization or death over a two-year period after controlling for disease severity with EF (Odds Ratio (O.R.) = 1.35, 95% CI = 0.99–1.84; $p = .059$) and with log BNP (O.R. = 1.46, 95% CI = 0.97–2.2; $p = .071$). Because IFN gamma/IL-10 ratio accounted for a greater amount of variance ($R^2 = 0.33$) for cardiac related hospitalization or death than IFN-gamma ($R^2 = 0.19$) or IL-10 ($R^2 = 0.23$) levels alone, IFN/IL-10 ratios were entered into the logistic regression equation to

TABLE 1. Cardiac Hospitalization and/or Death: Congestive Heart Failure Patient Characteristics Means and Standard Deviations

	Cardiac Rehospitalization and/or Death	SD	No Cardiac Rehospitalization and/or Death	SD
Age (yrs)	60.4	8.4	62.8	10.7
Body mass index	29.4	6.8	35.0	4.9
IFN gamma (SI)	4.5	3.3	14.6	20.7
IL-10 (SI)	9.4	10.6	4.6	3.9
IFN/IL-10 ratio*	0.93	0.70	1.66	0.73
Plasma IL-6 (ng/ml)	4.0	1.7	3.9	2.2
Ejection fraction* (%)	36.6	11.1	48.8	12.2
Log BNP	5.5	0.9	4.6	1.3
HAM-D*	15	9.8	7	4.5
Six-min walk (ft)	667.5	402.5	842.5	265

SI (stimulation index) = percentage of cells positive for cytokine expression after antigenic stimulation divided by percentage of cells expressing cytokines when unstimulated.

IFN = interferon; IL = interleukin; BNP = B-type natriuretic peptide; HAM-D = Hamilton Depression Scale.

* $p < .05$, significance between cardiac hospitalization and/or death or no cardiac hospitalization and/or death over a two year period.

determine IFN/IL-10 ratio as a possible mediating factor. The odds ratio for HAM-D scores for prediction of cardiac related rehospitalization or death when Th1/Th2 was also used as a covariate was reduced by 0.16 (O.R. = 1.22, 95% CI = 0.77–1.84; $p = .39$). Power analyses revealed that 22 patients ($n = 11$ with no cardiac events and $n = 11$ with cardiac events) would be necessary to obtain $p = .05$ at the existing level of power (50%) for HAM-D score prediction of cardiac events when controlling for EF. While, a total of 40 patients would be required to achieve 80% power. In controlling for BNP levels, a total of 40 subjects would be necessary to obtain $p = .05$ at the existing level of power, while 84 patients would be needed to achieve 80% power. These numbers are in contrast to the present pilot study consisting of a total of 18 patients (8 patients with cardiac events and 10 patients without) that were examined.

Secondary Analyses

As shown in Table 2, CHF patients with "high" HAM-D scores (≥ 10, $n = 8$) did not differ compared with patients with "low" HAM-D scores (<10, $n = 10$) in clinical characteristics including age, BMI, level of BNP, EF, and six-minute walk test. However, those patients with "high" HAM-D scores had lower Th1/Th2 ratios as compared with patients with "low" HAM-D scores (F (1, 17) = 10.86, $p = .005$) (Table 2). Plasma IL-6, INF, and IL-10 levels did not differ between those with "high" versus "low" HAM-D scores.

TABLE 2. Low Versus High Hamilton Depression (HAM-D) Scores: Congestive Heart Failure Patient Characteristics Means and Standard Deviations

	Low HAM-D (<10) $n = 10$	SD	High HAM-D (≥10) $n = 8$	SD
Age (yrs)	62.11	10.47	61.50	9.23
Body mass index	33.6	5.10	31.76	7.59
IFN gamma (SI)	12.71	21.81	6.20	5.78
IL-10 (SI)	3.0	3.0	11.26	9.60
IFN/IL-10 ratio*	3.28	2.18	0.82	0.73
Plasma IL-6 (ng/ml)	5.56	4.34	3.22	1.63
Ejection fraction (%)	41.0	14.3	44.6	12.9
Log BNP	5.19	1.40	4.85	1.01
HAM-D*	4.7	1.89	16.6	17.4
Six-min walk (ft)	807.5	274.25	692.75	393

SI (stimulation index) = percentage of cells positive for cytokine expression after antigenic stimulation divided by percentage of cells expressing cytokines when unstimulated.

* $p < .05$, significant difference between low and high HAM-D scores.

DISCUSSION

In spite of growing evidence that depression raises the risk of heart disease morbidity and mortality, the mechanisms involved are not well understood. Elevated levels of inflammatory markers including circulating cytokine levels are associated with psychological depression and worsening cardiovascular disease (14,38). However, little is known about the relationships among other parameters of immune function, depression, and heart disease. The present study examined whether cellular immune dysregulation is related to depressive symptoms and CHF morbidity and mortality. Our findings indicate that there is a negative linear relationship between the number of depressive symptoms and Th1/Th2 ratios in the CHF patients in our cohort. In addition, CHF patients with "high" depression scores (>10) had lower cellular immunity, implicated by a Th2 shift in IFN gamma/IL-10 ratios. Significant linear as well as group (higher versus lower depression) relationships between depression and cellular immunity suggest that even moderate changes in depression scores may have important effects on patients. This may be consistent with suggestions that even sub-clinical or sub-syndromal depression is related to future cardiac events (7–9). Indeed, patients who were rehospitalized and/or died due to cardiovascular events over a two-year period had higher depression scores as well as reduced Th1/Th2 ratios at baseline in our study. These preliminary findings suggest that cellular immunity, as indicated by IFN gamma/IL-10 ratios may partially explain the relationship between cardiac morbidity and mortality and depressive symptoms in CHF patients.

These results are in agreement with prior observations indicating depression is associated with reductions in cellular immune responses including NK cytotoxicity and lymphocyte blastogenesis (39) and reactivation of latent viruses (21) in medically healthy populations. Our findings also correspond with previous research that suggests reduced cellular immunity is related to cardiovascular disease progression. Th1 reductions and a shift toward Th2 have been associated with cardiac morbidity, such as cardiac dysfunction and eventual dilated cardiomyopathy and heart failure (24,25,40).

Although our results concur with prior studies, the explanations for co-occurrence of reduced cellular immunity with elevated inflammatory cytokine levels in circulation (e.g., TNF-α) within the context of depressive symptoms and heart disease morbidity and mortality are unclear. One possibility is that reduced cellular immunity associated with symptoms of depression compromises the immune system's ability to resist infection. This may allow chronic and latent infections to be activated and replicate within the body (e.g., periodontal disease, Chlamydia pneumoniae, and herpes virus infection) leading to inflammatory host responses to pathogens (41–43). Unexpectedly, the present study did not find a corresponding increase in the inflammatory marker IL-6 in association with Hamilton Depression Scores, cardiac morbidity, or a reduction of Th1/Th2 ratios. Then again, IL-6 may not be an ideal marker to investigate inflammation. Anti-inflammatory properties of IL-6 have been shown, where IL-6 stimulates the production of anti-inflammatory cytokines such as IL-10 (44), which inhibits the production of the proinflammatory cytokine TNF-α (45). Also, adiposity may interfere with IL-6-depression associations. Approximately 25% to 30% of systemic IL-6 is from adipose tissue (46–48), although the present study did not find an association between BMI and plasma IL-6 levels or depression scores. Other recent studies have also failed to find an association between symptoms of depression and plasma IL-6 levels (14,49,50). However, elevated circulating levels of inflammatory cytokine TNF-α have been related with depression (14,51).

In light of implications that immune measures other than circulating inflammatory cytokines may be associated with depression and heart failure, future studies should examine immunity as an integrated system. Mechanisms that underlie the link among depression, immune changes, and heart failure progression may be better understood via studying the "system" and its regulation rather than primarily measuring biomarkers in circulation. Research shows that cellular and inflammatory systems interact with one another whereby Th1 cells are important in the regulation of inflammation (24) and disruption of cellular immunity with a shift in the Th1/Th2 balance is associated with exaggerated inflammatory responses (25). Thus, this phenomenon should also be examined in depression and heart failure. These observations lead to one of the limitations of the present study, a failure to measure additional inflammatory cytokines such as TNF-α to look at the interactions among cellular immunity and inflammatory systems, cardiac events, and depressive symptoms. Also, future investigations should determine not only whether there are increases in inflammatory cytokines but also the levels at which circulating inflammatory cytokines become associated with cardiac events. Although studies suggest that increases in TNF-α are deleterious in CHF, levels that are too low may have a negative impact as well. Two clinical trials testing an

anti-TNF cytokine medication in CHF were halted because of increased mortality rates in patients given the highest dose of anti-TNF treatments (52). Moderate physiologic levels of TNF are suggested to have a cytoprotective response in the heart during acute ischemic injury and likely to play an important role in tissue remodeling and repair (53,54). Further studies should examine cytokine-moderating interventions such as exercise, which may reduce inflammation (or restore the balance of the inflammatory system) but not eliminate inflammatory cytokines to deleterious levels.

Our findings are based on a small sample size, precluding more definitive conclusions pertaining to the predictive power of depression levels on CHF-associated morbidity and mortality and the role of Th1/Th2 ratios as a mediating factor. The prognostic modeling analyses were only marginally significant for the prediction of CHF-associated morbidity and mortality by depression scores when EF ($p = .059$) and log BNP ($p = .071$) were used to control for disease severity. Moreover, the addition of Th1/Th2 ratios into the equation further reduced the power needed to evaluate the mediating role of cellular immunity. Future studies with larger sample sizes will help draw more definitive conclusions.

Although variations in results were small when BNP versus EF were used to control for disease severity, such variation exemplifies the difficulty in controlling for heart failure severity. CHF can result from a number of etiologies and therefore finding a single measure that reflects CHF disease severity is challenging. Using EF may be problematic in cases where patients have more severe diastolic than systolic dysfunction, because EF is generally a reflection of systolic proficiency. BNP may also be imperfect as the sole marker for CHF severity. Heart ventricles produce BNP in response to increased mechanical load and wall stretch resulting from insufficient blood pumped from the left ventricle to the systemic circulation leading to backward congestion and renin-angiotensin-aldosterone system activation to compensate for reduced blood flow in the periphery. This leads to increased blood volume, hypertrophy of heart tissue, structural dilation, and even more BNP secretion. However, these factors can differ depending on left ventricle systolic versus diastolic dysfunction (55) and BMI (56). Using a functional measure, such as a six-minute walk task is not practical as a lone marker because it may overlap with symptoms of psychological depression (Figure 2). Finally, using a classification system such as NYHA stages I–IV may be variable over short periods of time due to changes in medication and level of disease compensation. Patients can move back and forth between stage II and III for years before moving on to stage IV. Therefore, there are at least three possibilities for controlling for disease severity, a) inclusion of multiple measures of disease severity in a study (this was opted for in the present study by measuring both EF and BNP), b) another measure of CHF disease severity needs to be determined as a novel marker, or c) creation of a new variable from a combination of CHF severity factors.

In conclusion both depressive symptoms and Th1/Th2 ratios were associated with morbidity and mortality outcomes in CHF patients. Future studies are needed to further the understanding of the mechanisms underlying morbidity and mortality among CHF patients with increased symptoms of depression. Studies with larger sample sizes and increased numbers of cellular and cytokine immune parameters are needed to answer these research questions. Additionally, determining an appropriate and more comprehensive indicator for heart failure severity is needed.

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