Predictive Value of Low Relative Lymphocyte Count in Patients Hospitalized for Heart Failure With Reduced Ejection Fraction
Insights from the EVEREST Trial

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Background—Low lymphocyte count has been shown to be an independent prognostic marker in heart failure (HF) in the outpatient setting. Limited data exist regarding whether relative lymphocyte count correlates with postdischarge outcomes in patients hospitalized for HF.

Methods and Results—We performed a post hoc analysis of the Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study with Tolvaptan (EVEREST) trial, which randomized 4133 patients hospitalized for worsening HF with an ejection fraction ≤40% within 48 hours of admission to tolvaptan or placebo for a median follow-up of 9.9 months. The primary end points of all-cause mortality and cardiovascular mortality or HF hospitalization were analyzed in patients with available baseline complete blood counts (n=3717). Lymphocyte percentage was analyzed as a continuous variable. Times to events were compared using log-rank tests and multivariable Cox regression models. Patients with low lymphocyte percentage tended to be older and had higher rates of comorbid disease (diabetes mellitus, atrial fibrillation, and renal insufficiency). Low lymphocyte counts were associated with wide QRS duration, high natriuretic peptides, and low ejection fraction, blood pressure, and serum sodium. These patients were less likely to receive evidence-based HF medications. After adjusting for 22 known clinical risk factors, a 10% decrease in lymphocytes was associated with an increased hazard of all-cause mortality (adjusted hazard ratio 1.31 [95% CI: 1.14–1.150], \( P<0.001 \)) and cardiovascular mortality or HF hospitalization (adjusted hazard ratio 1.14 [95% CI: 1.04–1.25], \( P=0.007 \)) in the first 100 days postdischarge. Lymphopenia during hospitalization normalizes in majority of patients in the early postdischarge period.

Conclusions—Low relative lymphocyte count during hospitalization for HF is an independent predictor of poor outcomes in the early postdischarge period, beyond traditional prognostic indicators. (Circ Heart Fail. 2012;5:750–758.)

Key Words: heart failure ■ immune system ■ lymphocytes ■ prognosis

Heart failure (HF) accounts for >1 million primary admissions in the United States annually.1 Hospitalization represents one of the strongest prognostic indicators in patients with chronic HF.2 Patients hospitalized for heart failure (HHF) experience postdischarge mortality and rehospitalization that may be as high as 15% and 30%, respectively, within 60 to 90 days of hospitalization.3

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The identification of novel prognostic factors in this high-risk population may be necessary to guide future therapies and overall management. Few small studies have suggested that lymphopenia may confer increased risk for mortality in stable outpatient4–7 and patients with HHF.8–11 Although...
low lymphocyte count is currently recognized in certain risk stratification scores for chronic heart failure, contemporary HHF prognostication models lack inclusion of this potentially valuable parameter.

The Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study with Tolvaptan (EVEREST) trial allows for systematic and in-depth analysis of the role of lymphocytes in a large cohort of patients with HHF with reduced ejection fraction (EF), well treated with evidence-based HF therapies, with close follow-up and monitoring. Thus, we performed a post hoc analysis using the EVEREST trial database to determine the prevalence and prognostic use of low relative lymphocyte counts in HHF patients.

**Methods**

The study design and primary results of the EVEREST trial have been previously described. Briefly, the EVEREST program was a prospective, multicenter, international, randomized, double-blind, placebo-controlled trial evaluating the short- and long-term efficacy of tolvaptan, a vasopressin-2 receptor antagonist. Specific eligibility criteria for trial enrollment were the following: age >18 years, hospitalization for worsening HF, New York Heart Association class III or IV, EF ≤ 40%, and 2 or more signs and symptoms of fluid overload (eg, dyspnea, pitting edema, jugular venous distension). The only relevant exclusion criterion was comorbid conditions that limit survival to <6 months.

Patients were randomized within 48 hours of admission to receive oral tolvaptan (30 mg daily) or placebo for a minimum of 60 days. During this treatment period, all patients received standard HF therapies at the discretion of the treating physician. Explicit written informed consent was obtained from all enrolled patients and the study protocol was approved by the institutional review board or the ethics committee at each participating center. The present study includes patients in both study arms (tolvaptan and placebo) of the long-term outcomes study because tolvaptan has no known interaction with the immunological axis. Furthermore, patients randomized to tolvaptan in the EVEREST trial did not seem to experience increased rates of major or minor infections during the follow-up period.

Laboratory testing was performed at 5 central facilities. Consistency of laboratory results was verified by cross-validation among testing sites. EVEREST protocol required a routine complete blood count with differential at the time of patient enrollment. Thus, baseline measurement occurred within the first 48 hours of hospital admission. Each complete blood count was performed using a commercially automated system. The relative lymphocyte count (%) was defined as the total number of lymphocytes/total number of leukocytes×100. Patients with missing data regarding baseline complete blood count or differential were excluded from analysis (n=416). Lymphocyte percentage as a continuous variable (per 10% decrease) was the primary predictor variable for all outcome analyses because of its normal distribution in this sample without evidence of nonlinearity and to avoid bias related to an arbitrary cut-off value. However, for the purposes of descriptive analyses, patients were stratified based on standard laboratory cutoffs for abnormal parameters. From the remaining 3717 patients with validated baseline lymphocyte data, 2 groups were identified: patients with low relative lymphocytes (<15.4%) and patients with normal relative lymphocytes (≥15.4%). There were only 20 patients with relative lymphocyte percentage >48.5 (the upper limit normal); these patients were retained in the normal group. Patient disposition and selection of the analytical cohort for the present study have been summarized in Figure 1. Follow-up lymphocyte percentage every 4 to 8 weeks were also obtained and documented up to 112 weeks postdischarge.

Demographic characteristics, clinical characteristics, vital signs, other laboratory and diagnostic testing, and medication use recorded.
at the time of enrollment were compared between low and normal lymphocyte count groups. Specific causes of death and reasons forrehospitalization were determined by an independent and blinded adjudication committee. The present study used the 2 prespecified primary EVEREST co-endpoints: all-cause mortality (ACM) and the composite of cardiovascular mortality or heart failure hospitalization (CVM+HFH). Secondary end points included CVM, noncardiovascular mortality, worsening HF (defined as death, rehospitalization, or unplanned outpatient visitation), HFH, and a composite of CVM and cardiovascular-related hospitalization. Median follow-up in the EVEREST trial and in the present study was 9.9 months (interquartile range 5.3–16.1 months).

All continuous variables were reported as mean±SD if normally distributed, or median (interquartile range) if non-normally distributed. Categorical variables were expressed as number (%). Baseline characteristics were compared using Kruskal-Wallis and \( \chi^2 \) tests, as appropriate. Time to first event was analyzed using the log-rank test and Cox proportional hazard models. The proportional hazards assumption was violated for both primary end points (by Kolmogorov-type supremum tests for nonproportionality, \( P<0.004 \)). Thus, the follow-up time period was divided into 2 phases at 100 days postrandomization (cut-off point established by visual inspection of Standardized Score Process plots). No residual nonproportionality was observed in the subsequent stratified analyses. For ACM, Kaplan-Meier curves were constructed for patients with normal and low relative lymphocyte percentage. For CVM+HFH, cumulative incidence curves (%CIF SAS Macro, SAS Global Forum 2012) were constructed and estimates of failure rates were adjusted for competing risks of noncardiovascular death. Multivariable regression models were adjusted for 22 preselected baseline covariates that are known to influence clinical end points: tolvaptan assignment, demographic characteristics (age, sex, region of origin), clinical characteristics (ischemic HF cause, atrial fibrillation on admission ECG, coronary artery disease, diabetes mellitus, hypertension, chronic obstructive pulmonary disease, New York Heart Association class class IV), vital signs (resting heart rate, systolic blood pressure), laboratory and diagnostic testing (QRS duration on admission ECG, EF, serum sodium, blood urea nitrogen, B-type natriuretic peptide, total white blood cell count), and baseline medication use (angiotensin converting enzyme-inhibitors/angiotensin II receptor blockers, \( \beta \)-blockers, mineralocorticoid receptor antagonists). No suspicion for collinearity was present for the estimates related to lymphocyte count in our final models (tolerance =0.81, variance inflation factor =1.23). Separate interaction analyses for both primary end points did not reveal significant interaction between continuous lymphocyte counts and tolvaptan trial assignment (\( P>0.17 \)).

Missing covariate data (\( \leq 5\% \) for QRS duration and B-type natriuretic peptide, \( \leq 2\% \) for ischemic HF cause, blood urea nitrogen and serum sodium and \( \leq 1\% \) for all other variables) were imputed using multiple imputation procedures (fully conditional specification method). Effect sizes are reported as hazard ratios and 95% CI. All statistical analyses were performed using SAS version 9.2 (Cary, NC) and \( P<0.05 \) was considered to be statistically significant.

**Results**

Of the total 4133 patients in the EVEREST trial, 416 had missing complete blood counts not available for analysis. No significant differences were observed in the primary and secondary end points between patients with missing data and those with available lymphocyte percentage data at baseline (Table in the online-only Data Supplement). Mean relative lymphocyte percentage in the remaining study cohort was 21.7±9.0% with a median of 21.0 (interquartile range 15.6–27.0) (Figure 2). At the time of enrollment, 924 (24.9%)...
Table 1. Baseline Characteristics and Clinical End Points

<table>
<thead>
<tr>
<th></th>
<th>Lymphocyte % &lt;15.4 (n=924)</th>
<th>Lymphocyte % ≥15.4 (n=2793)*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolvaptan, n (%)</td>
<td>453 (49)</td>
<td>1402 (50.2)</td>
<td>0.537</td>
</tr>
<tr>
<td><strong>Demographic characteristics</strong></td>
<td></td>
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<tr>
<td>Age, y, mean±SD</td>
<td>68.5±11.4</td>
<td>64.8±11.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>731 (79.1)</td>
<td>2040 (73)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Region of origin, n (%)</td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Eastern Europe</td>
<td>226 (24.5)</td>
<td>1285 (46.0)</td>
<td></td>
</tr>
<tr>
<td>Western Europe</td>
<td>398 (43.1)</td>
<td>681 (24.4)</td>
<td></td>
</tr>
<tr>
<td>North America</td>
<td>126 (13.6)</td>
<td>518 (18.6)</td>
<td></td>
</tr>
<tr>
<td>South America</td>
<td>174 (18.8)</td>
<td>309 (11.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Clinical characteristics</strong></td>
<td></td>
<td></td>
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<tr>
<td>Ischemic HF causes, n (%)</td>
<td>624 (68.4)</td>
<td>1798 (65.3)</td>
<td>0.081</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>422 (45.7)</td>
<td>1014 (36.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>660 (71.4)</td>
<td>1969 (70.5)</td>
<td>0.59</td>
</tr>
<tr>
<td>Chronic kidney disease, n (%)</td>
<td>385 (41.7)</td>
<td>588 (21.1)</td>
<td></td>
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<tr>
<td>Coronary artery disease, n (%)</td>
<td>682 (74)</td>
<td>1942 (69.6)</td>
<td>0.11</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease, n (%)</td>
<td>146 (15.8)</td>
<td>221 (7.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Prior coronary artery bypass graft, n (%)</td>
<td>267 (28.9)</td>
<td>501 (17.9)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Prior myocardial infarction, n (%)</td>
<td>499 (54.1)</td>
<td>1394 (49.9)</td>
<td>0.03</td>
</tr>
<tr>
<td>Prior percutaneous coronary intervention, n (%)</td>
<td>217 (23.5)</td>
<td>433 (15.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>New York Heart Association Class IV, n (%)</td>
<td>438 (47.6)</td>
<td>1031 (36.9)</td>
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<tr>
<td><strong>Vital signs</strong></td>
<td></td>
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<tr>
<td>Baseline systolic BP, mm Hg, Mean±SD</td>
<td>118.7±19.6</td>
<td>121.2±19.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Baseline diastolic BP, mm Hg, Mean±SD</td>
<td>70±12.9</td>
<td>73.7±12.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Baseline heart rate, per min, Mean±SD</td>
<td>81±16.1</td>
<td>79.4±15.4</td>
<td>0.011</td>
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<tr>
<td><strong>Laboratory and diagnostic testing</strong></td>
<td></td>
<td></td>
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<tr>
<td>QRS duration on electrocardiogram, Mean±SD</td>
<td>130.8±36</td>
<td>125.8±35.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ejection fraction, %, Mean±SD</td>
<td>26.7±7.8</td>
<td>27.9±8</td>
<td>&lt;0.0001</td>
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<tr>
<td>Serum sodium, mEq/L, Mean±SD</td>
<td>138.4±5.1</td>
<td>140.1±4.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Blood urea nitrogen, mg/dL, Mean±SD</td>
<td>37.4±20.1</td>
<td>27.6±13.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>B-type natriuretic peptide, pg/mL, Median (IQR)</td>
<td>1055 (510.8–1995)</td>
<td>590 (250–1331)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Amino terminal-pro-BNP, pg/mL, Median (IQR)</td>
<td>6712 (3303–15435)</td>
<td>3960 (1839–7954)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total white blood cell count, %, Mean±SD</td>
<td>8.8±3.2</td>
<td>7.1±2.3</td>
<td>&lt;0.0001</td>
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<tr>
<td><strong>Baseline medication use</strong></td>
<td></td>
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</tr>
<tr>
<td>ACE-inhibitors or ARBs, n (%)</td>
<td>730 (79)</td>
<td>2413 (86.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>β-blockers, n (%)</td>
<td>594 (64.3)</td>
<td>2045 (73.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mineralocorticoid receptor antagonists, n (%)</td>
<td>449 (48.6)</td>
<td>1624 (58.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Digoxin, n (%)</td>
<td>427 (46.2)</td>
<td>1217 (43.6)</td>
<td>0.162</td>
</tr>
<tr>
<td><strong>Primary end points</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All-cause mortality, n (%)</td>
<td>352 (38.1)</td>
<td>620 (22.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CV mortality+HF hospitalization, n (%)</td>
<td>473 (51.2)</td>
<td>1042 (37.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Secondary end points</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CV mortality, n (%)</td>
<td>261 (28.2)</td>
<td>484 (17.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-CV mortality, n (%)</td>
<td>64 (6.9)</td>
<td>69 (2.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Worsening HF, n (%)</td>
<td>426 (46.1)</td>
<td>913 (32.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HF hospitalization, n (%)</td>
<td>347 (37.6)</td>
<td>794 (28.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CV mortality+CV hospitalization, n (%)</td>
<td>523 (56.6)</td>
<td>1230 (44.0)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin converting enzyme; ARB, angiotensin II receptor blocker; BNP, B-type natriuretic peptide; BP, blood pressure; CV, cardiovascular; HF, heart failure; IQR, interquartile range.

*Includes 20 patients with lymphocyte counts >48.5% (upper limit normal based on central EVEREST laboratory).

†Defined as death, rehospitalization, or unscheduled outpatient HF-related visitation.
patients had low relative lymphocyte counts by EVEREST laboratory criterion (<15.4%), approximately correlating to the lower quartile of the study cohort. Table 1 summarizes the baseline characteristics of patients with low and normal baseline lymphocytes. Patients with low relative lymphocyte counts tended to be significantly older (68.5 ± 11.4 versus 64.8 ± 11.8 years, P < 0.001), were more likely to be male (79.1% versus 73.0%, P < 0.001), and be enrolled from Western Europe (P < 0.001). Low relative lymphocyte count was associated with an increased comorbidity profile including diabetes mellitus, chronic kidney disease, coronary artery disease, and chronic obstructive pulmonary disease (all P < 0.05). Furthermore, these patients were more likely to have a history of prior myocardial infarction and coronary revascularization. This subset of patients also had significantly lower presenting systolic and diastolic blood pressure and higher mean heart rates (all P < 0.001). ECG at the time of admission showed higher rates of atrial fibrillation/flutter and wider QRS durations in these patients (both P < 0.001). Other initial diagnostic testing revealed that patients with low relative lymphocyte counts had lower serum sodium levels, higher blood urea nitrogen, and higher natriuretic peptides (all P < 0.001). Patients with lower relative lymphocyte count had higher mean total leukocyte counts (8.8 ± 3.2 versus 7.1 ± 2.3, P < 0.001). Despite this high-risk clinical profile, patients with relatively low lymphocyte percentage were less likely to receive evidence-based HF medications, including angiotensin converting enzyme-inhibitors, β-blockers, and mineralocorticoid receptor antagonists (P < 0.001 for all comparisons).

A total of 1515 CVM+HFH and 972 ACM events occurred during a median follow-up of 9.9 months. Outcome analysis by 10% decreases in lymphocyte count (as a continuous variable) is displayed in Table 2. Univariate analysis revealed that reduced lymphocyte percentage was strongly associated with increased hazard of both primary co-endpoints in both the early (<100 days) and late (>100 days) postdischarge period (P < 0.001 for all analyses). Similarly, times to first event between patients with lymphocyte percentage <15.4 and ≥15.4 were significantly different by Kaplan-Meier analysis (Figure 3, log-rank P < 0.001) and cumulative incidence curves for CVM+HFH (Figure 4, P < 0.001). Estimates of failure rates for this composite end point were similar after controlling for the competing risk of non-CV death.

After accounting for known baseline risk factors, the impact of low lymphocyte percentage on CVM+HFH was reduced, but remained statistically significant (hazard ratios 1.14, 95% CI 1.04–1.25; P = 0.007) in the first 100 days after hospital discharge. Similarly, low baseline lymphocyte percentage remained an independent predictor of ACM after multivariable adjustment (hazard ratios 1.31, 95% CI 1.14–1.50; P < 0.001) in the early postdischarge period. The association between lymphocyte percentage and both primary end points was nonsignificant when analyzed after 100 days postdischarge. There was no difference in the effect of continuous lymphocyte count between patients with low and normal baseline lymphocyte values for CVM+HFH (P = 0.54) and ACM (P = 0.25).

In both patients with low and normal baseline lymphocyte percentage, lymphocyte counts sharply increased as early as 7 days postdischarge. In patients with baseline lymphopenia, counts continued to increase up to 112 weeks postdischarge and remained within normal limits by laboratory standards (Figure 5).

### Table 2. Analysis of Primary Co-Endpoints

<table>
<thead>
<tr>
<th>10% Decrease in Lymphocytes (days)</th>
<th>Unadjusted Hazard Ratio (95% CI)</th>
<th>P</th>
<th>Adjusted Hazard Ratio (95% CI)*</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVM +HFH</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;100</td>
<td>1.48 (1.36–1.62)</td>
<td>&lt;0.001</td>
<td>1.14 (1.04–1.25)</td>
<td>0.007</td>
</tr>
<tr>
<td>&gt;100</td>
<td>1.28 (1.17–1.39)</td>
<td>&lt;0.001</td>
<td>1.02 (0.93–1.12)</td>
<td>0.649</td>
</tr>
<tr>
<td>≤100</td>
<td>1.86 (1.63–2.12)</td>
<td>&lt;0.001</td>
<td>1.31 (1.14–1.50)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ACM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;100</td>
<td>1.40 (1.27–1.55)</td>
<td>&lt;0.001</td>
<td>0.94 (0.85–1.05)</td>
<td>0.321</td>
</tr>
</tbody>
</table>

Hazard ratios were calculated using Cox proportional hazard models. The follow-up time was divided into 2 phases at 100 days postrandomization because the proportional hazards assumption did not hold. ACM indicates all-cause mortality; CVM, cardiovascular mortality; HFH, heart failure hospitalizations.

*Adjusted for Tolvaptan assignment, demographic characteristics (age, sex, region), clinical characteristics (ischemic HF cause, atrial fibrillation on admission EKG, coronary artery disease, diabetes mellitus, hypertension, chronic obstructive pulmonary disease, NYHA class IV), vital signs (resting heart rate, systolic blood pressure), laboratory and diagnostic testing (QRS duration on admission EKG, ejection fraction, serum sodium, blood urea nitrogen, B-type natriuretic peptide, total white blood cell count) and baseline medication use (ACE-inhibitors/ARBs, β-blockers, and mineralocorticoid receptor antagonists).

### Discussion

Approximately a quarter of a large contemporary cohort of patients with HHF had evidence of low relative lymphocyte counts within 48 hours of hospital admission. Lymphopenia was associated with a number of known HHF prognostic factors for increased morbidity and mortality including multiple comorbidities, hyponatremia, elevated natriuretic peptide levels, and reduced rates of baseline evidence-based HF medication use. Interestingly, relative lymphocyte count (as a continuous variable) was an independent predictor of both EVEREST primary end points, ACM and CVM+HFH, beyond these traditional prognostic indicators, in the early, but not late, postdischarge period. It seems that the lymphopenia during hospitalization is short lived and rapidly normalizes early after hospital discharge.

Although recent studies have shown that lymphocyte counts do not seem to predict incident HF,18,19 this measure may have prognostic significance in patients with an established diagnosis of HF. Several previous studies conducted in patients with chronic HF in stable outpatients have shown that lymphocyte counts (using different cutoffs and definitions) predict survival up to 1 year after measurement.4–7 These findings have been extended to special populations including patients with advanced chronic HF being evaluated for transplantation20 and patients with implantable cardioverter-defibrillators.21

Less data are available evaluating the association between baseline lymphocyte counts and outcomes in HHF. All previous studies in HHF used relative lymphocyte percentage instead of absolute counts.8–11 Cutoffs for abnormally low values ranged from 13%10,11 to 25%.8 Patient follow-up
was variable in these studies with a few limited to the early postdischarge period. 

Despite this heterogeneity in study design, similar to our experience, low lymphocyte counts were highly related to known HHF predictors of adverse outcomes. 

Furthermore, all studies consistently demonstrated that relative lymphopenia was an independent predictor of postdischarge events. Earlier studies are limited because findings were based on relatively small sample sizes and largely single-center experiences. The present study represents the largest experience to date to investigate the prognostic role of lymphopenia in the setting of HHF. Other major strengths are inclusion of: (1) data from multiple international centers; (2) independently adjudicated outcomes; (3) robust multivariable modeling including 22 clinical covariates known to influence clinical end points; (4) use of lymphocytes expressed as a continuous variable to reduce bias from arbitrary cutoffs; (5) availability of multiple, longitudinal measurements of lymphocyte counts to assess the natural history and time-course of this immunological marker; and (6) a well-treated patient population with close clinical follow-up, thus reducing the confounding influence of other systemic factors.

Marked abnormalities in clinical, neurohormonal, hemodynamic, and immunological parameters have been described in this patient population during hospitalization and into the postdischarge period. A number of potential, possibly interrelated mechanisms have been proposed regarding the interaction between lymphocytes and HHF. These mechanisms remain preliminary and require more rigorous testing in controlled settings. Lymphopenia has been described to occur in diverse clinical states and is reported to be fairly common in hospitalized patients. In HHF specifically, elevated biventricular filling pressures and subsequent splanchnic congestion may cause direct enteric losses of lymphocytes. Furthermore, this splanchnic congestive process may facilitate bacterial endotoxin translocation from the gut into the systemic circulation. Robust immune activation and release of cytokines, such as tumor-necrosis factor-1, may directly relate to reductions in lymphocyte counts (particularly T-helper cell and B-cell subpopulations), perhaps mediated by apoptotic mechanisms. In this regard, patients with baseline lymphopenia likely have more advanced disease with severe presenting congestion. Elevated natriuretic peptide levels and lower EF in patients with low relative lymphocyte counts in our study are supportive of this hypothesis. Another potential mechanism that has been described is activation of the hypothalamic-pituitary-adrenal axis as a physiological stress response in HHF. Exaggerated release of endogenous cortisol and catecholamines may contribute to reduced circulating lymphocyte subpopulations. This hypothesis is supported

Figure 3. Kaplan-Meier curve for all-cause mortality in patients with low (<15.4%) and normal (≥15.4%) relative lymphocytes.

Times to events were compared using log-rank tests.

Figure 4. Cumulative incidence curve for composite end point of CV mortality or HF hospitalization. Estimates of failure rates for this composite end point were similar across follow-up time-points using the traditional KM method of survival analysis and cumulative incidence approach controlling for the competing risk of non-CV death. CV indicates cardiovascular; HF, heart failure; KM, Kaplan-Meier.
indirectly by a study by von Haehling et al demonstrating that relative lymphopenia was more pronounced in patients who were β-blocker naïve. Furthermore, in our study, patients with low relative lymphocyte counts were less likely to be taking β-blockers at the time of enrollment.

This is the first study to describe the temporal course of lymphocyte percentage in the setting of HHF. The snapshot of baseline immune status using lymphocyte percentage within 1 to 2 days of hospital admission may be a transient surrogate of severe abnormalities in multiple physiological axes. Upon resolution of these acute perturbations, lymphocyte percentage quickly normalizes in the early postdischarge period. This may help explain why the prognostic use of relative lymphocyte counts was limited to the early postdischarge period. Reduced number of events occurring after 100 days may also reduce the predictive ability of baseline lymphocyte percentage.

Relative lymphocyte percentage represents a simple, inexpensive, and widely available immunological marker with potential prognostic significance. Contemporary risk stratification tools in HHF lack surrogates of immune status. Although our study needs to be confirmed by further prospective investigations, lymphocyte counts may help predict residual risk beyond currently used measures. Whether lymphocytes in HHF represent a marker of prognosis or a target for therapy remains to be determined. Despite advances in the overall care of patients with chronic HF with guideline-based therapies, novel therapies have lagged in patients with HHF. The development and testing of novel therapies in HHF has been challenging because of vast heterogeneity in patient population, incomplete understanding of the underlying pathophysiologic mechanisms, and lack of adequate characterization of patient profiles during and soon after hospitalization. Immunological parameters may help define clinically relevant subpopulations in which targeted, tailored therapies may be instituted.

However, results thus far from trials investigating the role of anticytokine therapies in this population have not shown significant benefit. Reduction in the sympathetic stress response via β-blockade may reduce leukocyte redistribution during hospitalization, but the relationship between this in-hospital phenomenon and postdischarge outcomes requires further clarification. There may be an important unmet need for novel immune-modulators in this area, perhaps targeting the underlying mechanisms contributing to lymphopenia including endotoxin translocation, lymphocyte apoptosis, and sympathetic activation.

The primary limitation of the present analyses is the post hoc nature of the study design and associated inherent biases. Furthermore, the patients included in this study were highly selected in the context of a randomized controlled trial, potentially limiting the external validity of these findings. Widespread variability in enrollment patterns due, in part, to the inclusion of a large number of clinical sites (>350) during a prolonged recruitment period (28 months), may further limit generalizability. Moreover, this study was conducted strictly in HF patients with reduced left ventricular EF. Despite covariate adjustment, other measured and unmeasured factors may have influenced these findings. Future prospective studies will need to externally validate these associations with longer follow-up durations. Our study did not measure hormone levels, indices of lymphocyte activity (eg, cytokine levels), or specific lymphocyte fractions (eg, T-cells, B-cells). The present analysis does not provide data elucidating the specific mechanisms underlying the prognostic significance of lymphopenia. Finally, lymphocytes represent only 1 component of the immunological axis, and recent studies suggest that more complete, global measures of immune function including the neutrophil-to-lymphocyte ratio may be more predictive of clinical end points in HHF.

Sources of Funding
Otsuka Inc. (Rockville, MD) provided financial and material support for the Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study with Tolvaptan (EVEREST) trial. Database management was performed by the sponsor.
Disclosures
Haris P. Subacius conducted all final analyses for this article with funding from the Center for Cardiovascular Innovation (Northwestern University Feinberg School of Medicine, Chicago, IL). The authors had full access to the data, take responsibility for its integrity, and had complete control and authority over article preparation and the decision to publish. Otherwise, the authors have no potential conflicts of interest to disclose.

References


**CLINICAL PERSPECTIVE**

The immunological axis is becoming increasingly recognized in the pathophysiology of heart failure (HF). Low lymphocyte counts are known to be predictive of cardiovascular outcomes in chronic HF, but its prognostic significance in patients hospitalized for HF is presently unclear. In this post hoc analysis of the Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study with Tolvaptan (EVEREST) trial, we evaluated 3717 patients hospitalized for HF and reduced ejection fraction with available relative lymphocyte counts at the time of enrollment. Lymphopenia was associated with an overall adverse clinical profile including older age, higher rates of medical comorbidities, lower use of evidence-based HF therapies, higher natriuretic peptides and lower ejection fraction, blood pressure, and serum sodium. However, even after accounting for 22 known clinical risk factors, lower lymphocyte counts were highly predictive of all-cause mortality and composite cardiovascular mortality and HF hospitalization in the first 100 days postdischarge. Lymphocytes represent a simple, inexpensive and widely available measure that may assist clinicians in identifying high-risk patients early during HF hospitalization. This study adds to the growing body of evidence supporting an interconnection between the immune system and HF. Future prospective investigations are required to determine whether lymphocytes represent a marker of disease severity or a potential target for therapy.
Predictive Value of Low Relative Lymphocyte Count in Patients Hospitalized for Heart Failure With Reduced Ejection Fraction: Insights from the EVEREST Trial
Muthiah Vaduganathan, Andrew P. Ambrosy, Stephen J. Greene, Robert J. Mentz, Haris P. Subacius, Aldo P. Maggioni, Karl Swedberg, Savina Nodari, Faiez Zannad, Marvin A. Konstam, Javed Butler and Mihai Gheorghiade

Circ Heart Fail. 2012;5:750-758; originally published online October 9, 2012;
doi: 10.1161/CIRCHEARTFAILURE.112.970525
Circulation: Heart Failure is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1941-3289. Online ISSN: 1941-3297

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circheartfailure.ahajournals.org/content/5/6/750

Data Supplement (unedited) at:
http://circheartfailure.ahajournals.org/content/suppl/2012/10/09/CIRCHEARTFAILURE.112.970525.DC1
Supplementary Table. Analysis of Missing Data

<table>
<thead>
<tr>
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<th>Missing Data (n=416)</th>
<th>Available Lymphocyte Data (n=3717) *</th>
<th>p-value</th>
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<tr>
<td><strong>Tolvaptan, n (%)</strong></td>
<td>217 (52.2)</td>
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<td><strong>Demographic Characteristics</strong></td>
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<td>Age (years), Mean ± SD</td>
<td>66.5±12.4</td>
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<td>Male, n (%)</td>
<td>304 (73.1)</td>
<td>2771 (74.5)</td>
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<td>Region of Origin, n (%)</td>
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<td>East Europe</td>
<td>108 (26)</td>
<td>1511 (40.7)</td>
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<td>172 (41.4)</td>
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<td>North America</td>
<td>55 (13.2)</td>
<td>644 (17.3)</td>
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<td>South America</td>
<td>81 (19.5)</td>
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<tr>
<td><strong>Clinical Characteristics</strong></td>
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<tr>
<td>Ischemic HF Etiology, n (%)</td>
<td>250 (60.7)</td>
<td>2422 (66.1)</td>
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<td>Diabetes, n (%)</td>
<td>162 (39.1)</td>
<td>1436 (38.6)</td>
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<td>Hypertension, n (%)</td>
<td>303 (73.2)</td>
<td>2629 (70.7)</td>
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<td>Chronic Kidney Disease, n (%)</td>
<td>134 (32.4)</td>
<td>973 (26.2)</td>
<td>0.007</td>
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<tr>
<td>Coronary Artery Disease, n (%)</td>
<td>287 (69.3)</td>
<td>2624 (70.7)</td>
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<td>Chronic Obstructive Pulmonary Disease, n (%)</td>
<td>49 (11.8)</td>
<td>367 (9.9)</td>
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<tr>
<td>Prior Coronary Artery Bypass Graft, n (%)</td>
<td>94 (22.7)</td>
<td>768 (20.7)</td>
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<tr>
<td>Prior Myocardial Infarction, n (%)</td>
<td>194 (46.9)</td>
<td>1893 (51)</td>
<td>0.113</td>
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<tr>
<td>Prior Percutaneous Coronary Intervention, n (%)</td>
<td>88 (21.3)</td>
<td>650 (17.5)</td>
<td>0.055</td>
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<tr>
<td>New York Heart Association Class IV, n (%)</td>
<td>153 (37.0)</td>
<td>1469 (39.6)</td>
<td>0.320</td>
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<td><strong>Vital Signs</strong></td>
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<td>Baseline Systolic BP (mm Hg), Mean ± SD</td>
<td>119.3±18.6</td>
<td>120.6±19.8</td>
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<td>Baseline Diastolic BP (mm Hg), Mean ± SD</td>
<td>71.8±12.6</td>
<td>72.8±12.7</td>
<td>0.158</td>
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<td>Baseline Heart Rate (per min), Mean ± SD</td>
<td>80.1±16.3</td>
<td>79.8±15.6</td>
<td>0.758</td>
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<tr>
<td><strong>Laboratory and Diagnostic Testing</strong></td>
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<td>QRS Duration on Electrocardiogram, Mean ± SD</td>
<td>125.7±34.2</td>
<td>127±35.5</td>
<td>0.476</td>
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<tr>
<td>Ejection Fraction (%), Mean ± SD</td>
<td>26.8±8.5</td>
<td>27.6±8</td>
<td>0.086</td>
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<td>Serum Sodium (mEq/L), Mean ± SD</td>
<td>139.2±4.6</td>
<td>139.7±4.6</td>
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<td>Blood Urea Nitrogen (mg/dL), Mean ± SD</td>
<td>32.6±18.3</td>
<td>30±16.2</td>
<td>0.018</td>
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<td>B-type Natriuretic Peptide (pg/mL), Median (IQR)</td>
<td>735 (312-1621)</td>
<td>696 (291-1494)</td>
<td>0.509</td>
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<td>Amino Terminal-pro-BNP (pg/mL), Median (IQR)</td>
<td>4448 (2252-9917)</td>
<td>4671 (2118-9676)</td>
<td>0.998</td>
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<td>Total White Blood Cell Count (%), Mean ± SD</td>
<td>7.4±3.6</td>
<td>7.5±2.7</td>
<td>0.911</td>
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<td><strong>Baseline Medication Use</strong></td>
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<td>ACE Inhibitors or ARBs, n (%)</td>
<td>335 (81.1)</td>
<td>3144 (84.6)</td>
<td>0.066</td>
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<td>β-Blockers, n (%)</td>
<td>268 (64.9)</td>
<td>2635 (70.9)</td>
<td>0.011</td>
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<td>Mineralocorticoid Receptor Antagonists, n (%)</td>
<td>202 (48.9)</td>
<td>2036 (54.8)</td>
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<td></td>
<td>Study Group A</td>
<td>Study Group B</td>
<td>p-Value</td>
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<tr>
<td>--------------------</td>
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<td>--------------</td>
<td>---------</td>
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<tr>
<td>Digoxin, n (%)</td>
<td>191 (46.2)</td>
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<td><strong>Primary Endpoints</strong></td>
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<td>All-Cause Mortality, n (%)</td>
<td>108 (26.0)</td>
<td>972 (26.2)</td>
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<td>CV Mortality + HF Hospitalization, n (%)</td>
<td>185 (44.5)</td>
<td>1515 (40.8)</td>
<td>0.144</td>
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<tr>
<td><strong>Secondary Endpoints, n (%)</strong></td>
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<td></td>
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<td>CV Mortality, n (%)</td>
<td>84 (20.2)</td>
<td>745 (20.0)</td>
<td>0.943</td>
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<td>Non-CV Mortality, n (%)</td>
<td>10 (2.4)</td>
<td>133 (3.6)</td>
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<td>Worsening HF, n (%) †</td>
<td>157 (37.7)</td>
<td>1339 (36.0)</td>
<td>0.490</td>
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<td>HF Hospitalization, n (%)</td>
<td>145 (34.9)</td>
<td>1141 (30.7)</td>
<td>0.082</td>
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<tr>
<td>CV Mortality + CV Hospitalization, n (%)</td>
<td>211 (50.7)</td>
<td>1753 (47.2)</td>
<td>0.168</td>
</tr>
</tbody>
</table>

* Includes 20 patients with lymphocyte counts >48.5% (upper limit normal based on central EVEREST laboratory)

† Defined as death, rehospitalization or unscheduled outpatient HF-related visitation

Abbreviations: ACE = angiotensin converting enzyme; ARB = angiotensin II receptor blocker; BNP = B-type natriuretic peptide; BP = blood pressure; CV = cardiovascular; HF = heart failure; IQR = interquartile range; SD = standard deviation.