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

Vaduganathan et al: Lymphocytes in Heart Failure

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Abstract

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 post-discharge 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 (EF) ≤40% within 48 hours of admission to tolvaptan or placebo for a median follow-up of 9.9 months. The primary endpoints of all-cause mortality (ACM) and cardiovascular mortality and HF hospitalization (CVM+HFH) were analyzed in patients with available baseline complete blood counts (n=3717). Lymphocyte % was analyzed as a continuous variable. Times to events were compared using log-rank tests and multivariable Cox regression models. Patients with low lymphocyte % tended to be older and had higher rates of comorbid disease (diabetes, atrial fibrillation and renal insufficiency). Low lymphocyte counts were associated with wider QRS duration, higher natriuretic peptides and lower 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 ACM [adjusted HR 1.31 (95% CI 1.14-1.50), p<0.001] and CVM+HFH [adjusted HR 1.14 (95% CI 1.04-1.25), p=0.007] in the first 100 days post-discharge. Lymphopenia during hospitalization normalizes in the majority of patients in the early post-discharge period.

Conclusions—Low relative lymphocyte count during hospitalization for HF is an independent predictor of poor outcomes in the early post-discharge period, beyond traditional prognostic indicators.

Key Words: heart failure; immune system; lymphocytes; prognosis
Heart failure (HF) accounts for over 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 post-discharge mortality and
rehospitalization that may be as high as 15% and 30%, respectively, within 60-90 days of
hospitalization.\(^3\)

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 outpatients\(^4-7\) and HHF
patients.\(^8-11\) Although low lymphocyte count is currently recognized in certain risk stratification
scores for chronic heart failure,\(^12\) contemporary HHF prognostication models lack inclusion of
this potentially valuable parameter.\(^13, 14\)

The Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study with Tolvaptan
(EVEREST) trial\(^15-17\) allows for systematic and in-depth analysis of the role of lymphocytes in a
large cohort of HHF patients with reduced ejection fraction (EF), well-treated with evidence-
based HF therapies, with close follow-up and monitoring. Thus, we performed a \textit{post-hoc}
analysis using the EVEREST trial database to determine the prevalence and prognostic utility of
low relative lymphocyte counts in HHF patients.

\textbf{Methods}

The study design\(^16\) and primary results\(^15, 17\) of the EVEREST trial have been previously
described. Briefly, the EVEREST program was a prospective, multi-center, 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: age ≥ 18 years, hospitalization for worsening HF, New York Heart Association (NYHA) class III or IV, EF ≤ 40% and 2 or more signs and symptoms of fluid overload (e.g. dyspnea, pitting edema, jugular venous distension). The only relevant exclusion criterion was comorbid conditions that limit survival to less than 6 months.

Patients were randomized within 48 hours of admission to receive oral tolvaptan (30mg 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 17 since tolvaptan has no known interaction with the immunological axis. Furthermore, patients randomized to tolvaptan in the EVEREST trial did not appear to experience increased rates of major or minor infections during the follow-up period.15, 17

All 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 (CBC) with differential at the time of patient enrollment. Thus, “baseline” measurement occurred within the first 48 hours of hospital admission. Each CBC was performed using a commercially automated system. The relative lymphocyte count (%) was defined as the total number of lymphocytes / total number of leukocytes x 100. Patients with missing data regarding baseline CBC or differential were excluded from analysis (n=416). Lymphocyte % as a continuous variable (per 10% decrease) was the primary predictor variable for all outcome analyses due to its normal distribution in this sample without evidence of non-linearity 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 % >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 % every 4-8 weeks were also obtained and documented up to 112 weeks post-discharge.

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 for rehospitalization were determined by an independent and blinded adjudication committee. The present study utilized the two pre-specified primary EVEREST co-endpoints: all-cause mortality (ACM) and composite of cardiovascular mortality or heart failure hospitalization (CVM+HFH). Secondary endpoints included CVM, non-CV mortality, worsening HF (defined as death, rehospitalization or unplanned outpatient visitation), HFH and composite CVM and CV-related hospitalizations. Median follow-up in the EVEREST trial and in the present study was 9.9 months (interquartile range 5.3 to 16.1 months).

All continuous variables were reported as mean ± standard deviation (SD) if normally distributed or median [interquartile range (IQR)] if non-normally distributed. Categorical variables were expressed as number (%). Baseline characteristics were compared using Kruskal-Wallis and Chi-square 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 endpoints (by Kolmogorov-type supremum tests for non-proportionality, p<0.004). Thus, the follow-up time period was divided into two phases at 100 days post-randomization (cut-off point established by visual inspection of Standardized Score Process plots). No residual non-proportionality was observed in the subsequent stratified analyses. For ACM, Kaplan-Meier curves were constructed for patients with normal and low relative lymphocyte %. 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 non-CV death. Multivariable regression models were adjusted for 22 pre-selected baseline covariates that are known to influence clinical endpoints: tolvaptan assignment, demographic characteristics (age, sex, region of origin), clinical characteristics (ischemic HF etiology, atrial fibrillation on admission electrocardiogram, coronary artery disease, diabetes, hypertension, chronic obstructive pulmonary disease, NYHA class IV), vital signs (resting heart rate, systolic blood pressure), laboratory and diagnostic testing (QRS duration on admission electrocardiogram, EF, serum sodium, blood urea nitrogen, B-type natriuretic peptide (BNP), total white blood cell count) and baseline medication use (ACE-inhibitors/ARBs, β-blockers and 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 endpoints did not reveal significant interaction between continuous lymphocyte counts and tolvaptan trial assignment (p>0.17).

Missing covariate data (≤5% for QRS duration and BNP, ≤2% for ischemic HF etiology, BUN and serum sodium and ≤1% for all other variables) were imputed using multiple imputation procedures (fully conditional specification method). Effect sizes are reported as hazard ratios (HR) and 95% confidence intervals (CI). All statistical analyses were performed
using SAS version 9.2 (Cary, North Carolina) 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 available for analysis. No significant differences were observed in the primary and secondary endpoints between patients with missing data and those with available lymphocyte % data at baseline (Supplemental Data Table). Mean relative lymphocyte percentage in the remaining study cohort was 21.7±9.0 with a median of 21.0 (IQR 15.6-27.0) (Figure 2). At the time of enrollment, 924 (24.9%) 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 vs.64.8±11.8 years, p<0.001), were more likely to be male (79.1% vs. 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, 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). Electrocardiogram 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 BUN and higher natriuretic peptides (all p<0.001). Patients
with lower relative lymphocyte count had higher mean total leukocyte counts (8.8±3.2 vs. 7.1±2.3, p<0.001). Despite this high-risk clinical profile, patients with low relatively lymphocyte % were less likely to receive evidence-based HF medications including ACE-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 % was strongly associated with increased hazard of both primary co-endpoints in both the early (≤100 days) and late (> 100 days) post-discharge period (p<0.001 for all analyses). Similarly, times to first event between patients with lymphocyte % <15.4 and ≥ 15.4 were significantly different by Kaplan-Meier method for ACM (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 endpoint were similar after controlling for the competing risk of non-CV death.

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

In both patients with low and normal baseline lymphocyte %, lymphocyte counts sharply increased as early as 1-week post-discharge. In patients with baseline lymphopenia, counts
continued to increase up to 112 weeks post-discharge and remained within normal limits by laboratory standards (Figure 5).

**Discussion**

Approximately a quarter of a large contemporary cohort of HHF patients has 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 baseline evidence-based HF medication use. Interestingly, relative lymphocyte count (as a continuous variable) was an independent predictor of both EVEREST primary endpoints, ACM and CVM+HFH, beyond these “traditional” prognostic indicators, in the early, but not late, post-discharge period. It appears 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 appear to predict incident HF, this measure may have prognostic significance in patients with an established diagnosis of HF. Several previous studies 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. These findings have been extended to special populations including patients with advanced chronic HF being evaluated for transplantation and patients with implantable cardioverter-defibrillators.

Less data are available evaluating the association between baseline lymphocyte counts and outcomes in HHF. All previous studies in HHF used relative lymphocyte % instead of absolute counts. Cutoffs for abnormally low values ranged from 13% to 25%. Patient
follow-up was variable in these studies with a few limited to the early post-discharge period.\textsuperscript{10} Despite this heterogeneity in study design, similar to our experience, low lymphocyte counts were highly related to known HHF predictors of adverse outcomes.\textsuperscript{8-11} Furthermore, all studies consistently demonstrated that relative lymphopenia was an independent predictor of post-discharge events.\textsuperscript{8-11} Earlier studies are limited since 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 endpoints; 4) use of lymphocytes expressed as a continuous variable to reduce bias from arbitrary cut-offs; 5) availability of multiple, longitudinal measurements of lymphocyte counts to assess the natural history and time-course of this immunological marker and 5) 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 post-discharge period.\textsuperscript{22} A number of potential, possibly inter-related 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.\textsuperscript{23} In HHF specifically, elevated biventricular filling pressures and subsequent splanchnic congestion may cause direct enteric losses of lymphocytes.\textsuperscript{24} 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 sub-populations), perhaps mediated by apoptotic mechanisms. In this regard, patients with baseline lymphopenia likely have more advanced disease with more 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 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 % in the setting of HHF. The snapshot of baseline immune status using lymphocyte % within 1-2 days of hospital admission may be a transient surrogate of severe abnormalities in multiple physiological axes. Upon resolution of these acute perturbations, lymphocyte % quickly normalizes in the early post-discharge period. This may help explain why the prognostic utility of relative lymphocyte counts was limited to the early post-discharge period. Reduced number of events occurring after 100 days may also reduce the predictive ability of baseline lymphocyte %.

Relative lymphocyte % 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 utilized 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 HHF patients.\(^3\) The development and testing of novel therapies in HHF has been challenging due to 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 anti-cytokine therapies in this population have not shown significant benefit.\(^32,\)\(^33\) Reduction in the sympathetic stress response via β-blockade may reduce leukocyte redistribution during hospitalization,\(^27\) but the relationship between this in-hospital phenomenon and post-discharge 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 (over 350) over 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 (e.g. cytokine levels) or specific lymphocyte fractions (e.g. 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 one 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 endpoints in HHF.34

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Disclosures

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References


Table 1. Baseline Characteristics and Clinical Endpoints

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<th>Lymphocyte% &lt;15.4 (n=924)</th>
<th>Lymphocyte% ≥15.4 (n=2793) *</th>
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<td><strong>Tolvaptan, n (%)</strong></td>
<td>453 (49)</td>
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<td>64.8±11.8</td>
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<td>Male, n (%)</td>
<td>731 (79.1)</td>
<td>2040 (73)</td>
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<td>Region of Origin, n (%)</td>
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<td>398 (43.1)</td>
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<td>South America</td>
<td>174 (18.8)</td>
<td>309 (11.1)</td>
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<td><strong>Clinical Characteristics</strong></td>
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<td>Ischemic HF Etiology, n (%)</td>
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<td>Diabetes, n (%)</td>
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<td>Hypertension, n (%)</td>
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<td>1969 (70.5)</td>
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<td>Chronic Kidney Disease, n (%)</td>
<td>385 (41.7)</td>
<td>588 (21.1)</td>
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<td>Coronary Artery Disease, n (%)</td>
<td>682 (74)</td>
<td>1942 (69.6)</td>
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<td>Chronic Obstructive Pulmonary Disease, n (%)</td>
<td>146 (15.8)</td>
<td>221 (7.9)</td>
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<td>Prior Coronary Artery Bypass Graft, n (%)</td>
<td>267 (28.9)</td>
<td>501 (17.9)</td>
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<td>Prior Myocardial Infarction, n (%)</td>
<td>499 (54.1)</td>
<td>1394 (49.9)</td>
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<td>Prior Percutaneous Coronary Intervention, n (%)</td>
<td>217 (23.5)</td>
<td>433 (15.5)</td>
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<td>New York Heart Association Class IV, n (%)</td>
<td>438 (47.6)</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>118.7±19.6</td>
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<td>Baseline Diastolic BP (mm Hg), Mean ± SD</td>
<td>70±12.9</td>
<td>73.7±12.5</td>
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<td>Baseline Heart Rate (per min), Mean ± SD</td>
<td>81±16.1</td>
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<td>QRS Duration on Electrocardiogram, Mean ± SD</td>
<td>130.8±36</td>
<td>125.8±35.2</td>
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<td>Ejection Fraction (%), Mean ± SD</td>
<td>26.7±7.8</td>
<td>27.9±8</td>
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<td>Serum Sodium (mEq/L), Mean ± SD</td>
<td>138.4±5.1</td>
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<td>Blood Urea Nitrogen (mg/dL), Mean ± SD</td>
<td>37.4±20.1</td>
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<td>B-type Natriuretic Peptide (pg/mL), Median (IQR)</td>
<td>1055 (510.8-1995)</td>
<td>590 (250-1331)</td>
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<td>Amino Terminal-pro-BNP (pg/mL), Median (IQR)</td>
<td>6712 (3303-15435)</td>
<td>3960 (1839-7954)</td>
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<td>Total White Blood Cell Count (%), Mean ± SD</td>
<td>8.8±3.2</td>
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<td><strong>Baseline Medication Use</strong></td>
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<td>ACE Inhibitors or ARBs, n (%)</td>
<td>730 (79)</td>
<td>2413 (86.4)</td>
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<td>594 (64.3)</td>
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<td>Mineralocorticoid Receptor Antagonists, n (%)</td>
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<td>Digoxin, n (%)</td>
<td>427 (46.2)</td>
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<td><strong>Primary Endpoints</strong></td>
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<td>All-Cause Mortality, n (%)</td>
<td>352 (38.1)</td>
<td>620 (22.2)</td>
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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.
Hazard ratios were calculated using Cox proportional hazard models. The follow-up time was divided into two phases at 100 days post-randomization because the proportional hazards assumption did not hold.

*Adjusted for Tolvaptan assignment, demographic characteristics (age, sex, region), clinical characteristics (ischemic HF etiology, atrial fibrillation on admission EKG, coronary artery disease, diabetes, 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).

Abbreviations: ACM = all-cause mortality; CI=confidence interval; CVM = cardiovascular mortality; HFH = heart failure hospitalizations.
Figure Legends

**Figure 1.** Patient disposition and selection of analytical cohort. Relative lymphocytes (total number of lymphocytes / total number of leukocytes x 100) were determined using enrollment complete blood counts. Cutoffs for abnormal lymphocyte parameters were pre-specified by the centralized laboratory analyzing EVEREST trial blood samples. Twenty patients with relative lymphocytes >48.5% (upper limit normal) were retained in the “normal group”. Abbreviations: EVEREST = The Efficacy of Vasopressin Antagonism in Heart Failure Outcome Study with Tolvaptan; HF = heart failure; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association.

**Figure 2.** Distribution and descriptive statistics of relative lymphocyte %. The primary predictor variable of relative lymphocyte count was normally distributed with a mean and median of approximately 21%. The distribution and descriptive statistics of lymphocyte % did not significantly differ based on tolvaptan assignment. The black curve represents the normal density distribution and the grey curve represents the kernel density distribution for this sample. Based on the standard cutoffs for abnormal parameters utilized in the EVEREST trial central core laboratory, approximately 25% (lower quartile, shaded) of the study sample were identified as having low relative lymphocyte counts (<15.4%). Abbreviations: IQR = interquartile range.

**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 endpoint of cardiovascular (CV) mortality and heart failure (HF) hospitalizations. Estimates of failure rates for this composite endpoint were similar across follow-up time-points using the traditional Kaplan-Meier (KM) method of survival analysis and cumulative incidence approach controlling for the competing risk of non-CV death.

**Figure 5.** Changes in relative lymphocyte count over time. “Baseline” measurement of lymphocyte %, which has been used for all analyses in this study, was performed within 48 hours of hospital admission. The overall time course of mean lymphocyte % over the 112 week follow-up has been stratified by baseline lymphopenic status.
EVEREST Program Patients
- Age ≥18 Years
- Hospitalized for Worsening HF
- NYHA Class III or IV
- LVEF ≤40%
- Two or More Signs/Symptoms of Volume Overload
n=4133

Patients with Missing Data
n=416

Analytical Cohort
Patients with Validated Baseline Lymphocyte Data Available for Analysis
n=3717

Low
Patients with Relative Lymphocytes <15.4%
n=924 (24.9%)

Normal
Patients with Relative Lymphocytes ≥15.4%
n=2793 (75.1%)
**Descriptive Statistics (n=3,717)**

- **Mean**: 21.6% ± 8.9%
- **Median (IQR)**: 21.0% (15.6-27.0%)
All-Cause Mortality

Lymphocyte% $\geq 15.4$

Log rank $P<0.001$

Probability of Survival

No. at risk

| Lymph <15.4 | 924 | 572 | 321 | 124 | 12 | 0 |
| Lymph $\geq 15.4$ | 2793 | 1870 | 967 | 373 | 34 | 0 |
CV Mortality + HF Hospitalization

P < 0.001

Cumulative Incidence

Time (days)

Lymphocyte% < 15.4

Lymphocyte% ≥ 15.4

Estimates of Failure Rates

<table>
<thead>
<tr>
<th>Time</th>
<th>Competing Risk of non-CV Death</th>
<th>No Competing Risks (KM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lymph &lt;15.4%</td>
<td>Lymph ≥15.4%</td>
</tr>
<tr>
<td>6 months</td>
<td>0.400</td>
<td>0.270</td>
</tr>
<tr>
<td>1 year</td>
<td>0.510</td>
<td>0.396</td>
</tr>
<tr>
<td>2 years</td>
<td>0.631</td>
<td>0.492</td>
</tr>
</tbody>
</table>
The 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

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SUPPLEMENTAL MATERIAL
### Supplementary Table. Analysis of Missing Data

<table>
<thead>
<tr>
<th>Missing Data (n=416)</th>
<th>Available Lymphocyte Data (n=3717)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tolvaptan, n (%)</strong></td>
<td>217 (52.2)</td>
<td>1855 (49.9)</td>
</tr>
<tr>
<td><strong>Demographic Characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years), Mean ± SD</td>
<td>66.5±12.4</td>
<td>65.7±11.8</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>304 (73.1)</td>
<td>2771 (74.5)</td>
</tr>
<tr>
<td>Region of Origin, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>East Europe</td>
<td>108 (26)</td>
<td>1511 (40.7)</td>
</tr>
<tr>
<td>West Europe</td>
<td>172 (41.4)</td>
<td>1079 (29)</td>
</tr>
<tr>
<td>North America</td>
<td>55 (13.2)</td>
<td>644 (17.3)</td>
</tr>
<tr>
<td>South America</td>
<td>81 (19.5)</td>
<td>483 (13)</td>
</tr>
<tr>
<td><strong>Clinical Characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic HF Etiology, n (%)</td>
<td>250 (60.7)</td>
<td>2422 (66.1)</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>162 (39.1)</td>
<td>1436 (38.6)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>303 (73.2)</td>
<td>2629 (70.7)</td>
</tr>
<tr>
<td>Chronic Kidney Disease, n (%)</td>
<td>134 (32.4)</td>
<td>973 (26.2)</td>
</tr>
<tr>
<td>Coronary Artery Disease, n (%)</td>
<td>287 (69.3)</td>
<td>2624 (70.7)</td>
</tr>
<tr>
<td>Chronic Obstructive Pulmonary Disease, n (%)</td>
<td>49 (11.8)</td>
<td>367 (9.9)</td>
</tr>
<tr>
<td>Prior Coronary Artery Bypass Graft, n (%)</td>
<td>94 (22.7)</td>
<td>768 (20.7)</td>
</tr>
<tr>
<td>Prior Myocardial Infarction, n (%)</td>
<td>194 (46.9)</td>
<td>1893 (51)</td>
</tr>
<tr>
<td>Prior Percutaneous Coronary Intervention, n (%)</td>
<td>88 (21.3)</td>
<td>650 (17.5)</td>
</tr>
<tr>
<td>New York Heart Association Class IV, n (%)</td>
<td>153 (37.0)</td>
<td>1469 (39.6)</td>
</tr>
<tr>
<td><strong>Vital Signs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline Systolic BP (mm Hg), Mean ± SD</td>
<td>119.3±18.6</td>
<td>120.6±19.8</td>
</tr>
<tr>
<td>Baseline Diastolic BP (mm Hg), Mean ± SD</td>
<td>71.8±12.6</td>
<td>72.8±12.7</td>
</tr>
<tr>
<td>Baseline Heart Rate (per min), Mean ± SD</td>
<td>80.1±16.3</td>
<td>79.8±15.6</td>
</tr>
<tr>
<td><strong>Laboratory and Diagnostic Testing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>QRS Duration on Electrocardiogram, Mean ± SD</td>
<td>125.7±34.2</td>
<td>127±35.5</td>
</tr>
<tr>
<td>Ejection Fraction (%), Mean ± SD</td>
<td>26.8±8.5</td>
<td>27.6±8</td>
</tr>
<tr>
<td>Serum Sodium (mEq/L), Mean ± SD</td>
<td>139.2±4.6</td>
<td>139.7±4.6</td>
</tr>
<tr>
<td>Blood Urea Nitrogen (mg/dL), Mean ± SD</td>
<td>32.6±18.3</td>
<td>30±16.2</td>
</tr>
<tr>
<td>B-type Natriuretic Peptide (pg/mL), Median (IQR)</td>
<td>735 (312-1621)</td>
<td>696 (291-1494)</td>
</tr>
<tr>
<td>Amino Terminal-pro-BNP (pg/mL), Median (IQR)</td>
<td>4448 (2252-9917)</td>
<td>4671 (2118-9676)</td>
</tr>
<tr>
<td>Total White Blood Cell Count (%), Mean ± SD</td>
<td>7.4±3.6</td>
<td>7.5±2.7</td>
</tr>
</tbody>
</table>

### Baseline Medication Use

| ACE Inhibitors or ARBs, n (%) | 335 (81.1) | 3144 (84.6) | 0.066 |
| β-Blockers, n (%) | 268 (64.9) | 2635 (70.9) | 0.011 |
| Mineralocorticoid Receptor Antagonists, n (%) | 202 (48.9) | 2036 (54.8) | 0.023 |
| * 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.

**Primary Endpoints**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 392)</th>
<th>Group 2 (n = 640)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-Cause Mortality, n (%)</td>
<td>108 (26.0)</td>
<td>972 (26.2)</td>
<td>0.934</td>
</tr>
<tr>
<td>CV Mortality + HF Hospitalization, n (%)</td>
<td>185 (44.5)</td>
<td>1515 (40.8)</td>
<td>0.144</td>
</tr>
</tbody>
</table>

**Secondary Endpoints, n (%)**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 392)</th>
<th>Group 2 (n = 640)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV Mortality, n (%)</td>
<td>84 (20.2)</td>
<td>745 (20.0)</td>
<td>0.943</td>
</tr>
<tr>
<td>Non-CV Mortality, n (%)</td>
<td>10 (2.4)</td>
<td>133 (3.6)</td>
<td>0.214</td>
</tr>
<tr>
<td>Worsening HF, n (%) †</td>
<td>157 (37.7)</td>
<td>1339 (36.0)</td>
<td>0.490</td>
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<tr>
<td>HF Hospitalization, n (%)</td>
<td>145 (34.9)</td>
<td>1141 (30.7)</td>
<td>0.082</td>
</tr>
<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>