Leucocyte count predicts cardiovascular risk in heart failure with preserved ejection fraction: insights from TOPCAT Americas

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Abstract

Aims   Prior evidence has implicated leucocyte expansion in several cardiovascular disorders, including heart failure (HF) with reduced ejection fraction (rEF). However, the prognostic importance of leucocyte count in HF with preserved EF (HFpEF) remains largely unexplored.

Methods and results   The Americas cohort of the treatment of preserved cardiac function heart failure with an aldosterone antagonist (TOPCAT-Americas) was used to evaluate the association between total leucocyte count and clinical outcomes in HFpEF. The primary outcome was a composite of aborted cardiac arrest, cardiovascular mortality, or hospitalization for HF. Secondary outcomes were hospitalization for HF, aborted cardiac arrest, stroke, non-fatal myocardial infarction (MI), cardiovascular mortality, non-cardiovascular mortality, and all-cause mortality. Survival models were used to identify the risk of the primary and secondary outcomes in those with leucocyte count above the median (7100 cells/μL), as compared to those with leucocyte count below the median, during the follow-up period. A total of 1746 (out of 1767; 99%) patients from TOPCAT-Americas were available for the analyses with a median follow up of 2.4 (25th to 75th percentile 1.4–3.9) years. Patients with leucocyte count >7100 cells/μL were 36% more likely to experience the primary endpoint compared to those with ≤7100 cells/μL (hazard ratio: 1.36, 95% confidence interval: 1.14–1.61). This association remained significant after extensive adjustment for potential confounders (hazard ratio: 1.27, 95% confidence interval: 1.06–1.52). We also observed a greater incidence of HF hospitalization and non-fatal MI in patients with higher leucocyte count. These associations remained robust on sensitivity analyses, suggesting a low probability of confounding. Exploratory analyses suggested that both higher leucocyte count (integrating the combined influence of both myeloid and lymphoid immune cells) and augmented platelet count (as a surrogate for myeloid immune cell expansion) in the same model were associated with the primary outcome (both *P* < 0.05).

Conclusions   Leucocyte count >7100 cells/μL was independently associated with adverse clinical outcomes in HFpEF patients from TOPCAT-Americas. These results were primarily driven by the HF hospitalization outcome but were also accompanied by an excess of non-fatal MI. Further research is needed to define the mechanisms underlying our findings and their prognostic implications.

Introduction

Heart failure (HF) is a complex disease composed of a broad array of pathophysiological perturbations, including sustained and inappropriate inflammation.[1,2] In animal models of HF, chronic inflammation has been linked to the expansion of both myeloid (e.g. monocytes and macrophages) and lymphoid (e.g. T-cells) immune cells,[3–6] both systemically and in the failing myocardium. While most often studied in models of HF with reduced ejection fraction (HFrEF), a mouse model of HF with preserved EF (HFpEF) exhibited augmented macrophage density in the failing heart, accompanied by increased haematopoiesis, and similar haematopoietic activation (assessed by FDG-PET imaging) was also observed in a small cohort of humans with HFpEF.[7] Such data are consistent with the proposed concept that myocardial structural and functional abnormalities in HFpEF are driven by a systemic pro-inflammatory state that ultimately results in altered endothelial/myocyte function, myocyte survival, and myocardial fibrosis.[8,9]

Augmented haematopoiesis occurring with inflammatory activation is reflected in part by blood leucocyte count, particularly those of myeloid immune cells, which have short lifespans.[10] Several studies indicate that higher leucocyte and myeloid cell count in HF are negative prognostic markers. In middle-aged men, leucocyte count is associated with long-term incidence of HF hospitalizations,[11] and large population studies indicate that higher neutrophil count is associated with the development of incident HF.[12] In subjects with ischaemic left ventricular (LV) dysfunction, a leucocyte count of >7000 cells/μL is an independent predictor of all-cause mortality.[13] In a prospective observational study of a community-based cohort, elevated leucocyte count was associated with an increased risk of incident HFpEF.[14] Moreover, in acute HF, blood neutrophil count at hospital admissions was associated with long-term mortality,[15] whereas total blood monocytes were increased but with a shift of the monocyte subset profile towards that of healthy control after standard HF treatment.[16]

Despite the growing evidence base linking HFpEF with chronic inflammation, the importance of blood leucocyte count as an immune activity index and its relationship to cardiovascular and mortality outcomes in HFpEF are poorly defined. We sought to evaluate the association between leucocyte count and clinical events in HFpEF patients and test the hypothesis that higher leucocyte count, reflective of heightened immune cell expansion, is associated with higher rates of adverse cardiovascular outcomes. We investigated this hypothesis in a post hoc analysis of North and South American HFpEF patients enrolled in the treatment of preserved cardiac function heart failure with an aldosterone antagonist (TOPCAT) trial.

Methods

The analytical methods will be/have been made available to other researchers for purposes of reproducing the results or replicating the procedure. The study was conducted in accordance with the strengthening the reporting of observational studies in epidemiology (STROBE) guidelines (Supporting Information, *Table SS1*).

Study design and participants

The TOPCAT study was a multicentre, double-blind, placebo-controlled, randomized trial that evaluated the utility of spironolactone treatment for preventing adverse clinical outcomes in patients with HFpEF.[17,18] Patients were eligible for enrolment if they were age ≥50 years, had symptoms of HF, had an LV ejection fraction (LVEF) of ≥45% in the 6 months prior to randomization, had a systolic blood pressure ≤140 mmHg or <160 mmHg with ≥3 anti-hypertensive medications, a serum potassium ≤5.0 mmol/L, and had either been hospitalized in the 12 months prior to enrolment for management of HF or had elevated natriuretic peptides. Key exclusion criteria included severe systemic illness with a life expectancy of <3 years from randomization; significant chronic obstructive pulmonary disease (on home oxygen, oral steroid therapy, or were hospitalized for exacerbation within 12 months, or had significant chronic pulmonary disease in the opinion of the investigator); haemodynamically significant valvular disease; severe renal dysfunction with an estimated glomerular filtration rate (eGFR, based on modification of diet in renal disease equation) <30 mL/min/1.73 m^2; percutaneous coronary intervention in the 30 days preceding enrolment; and stroke, non-fatal myocardial infarction (MI), or coronary artery bypass grafting (CABG) in the 90 days prior to trial enrolment.[17,18] The primary outcome of the trial was a composite of cardiovascular mortality, aborted cardiac arrest, or hospitalization for the management of HF. The full details of the study population and outcomes have been published previously.[17]

The study protocol was approved by the University of Alabama at Birmingham Institutional Review Board and National Heart, Lung, and Blood Institute Biologic Specimen and Data Repository Information Coordinating Center (NHLBI-BioLINCC) and does not necessarily reflect the opinions or views of TOPCAT or the NIH. No specific informed consent was needed for this current investigation. Due to prior concerns regarding the validation of the HFpEF diagnosis[19] and treatment adherence[20] for patients outside of North and South America, the analyses in this investigation were limited to patients that were enrolled from the Americas ('TOPCAT-Americas') who had leucocyte count measured at the time

Figure 1 Flow diagram for subject selection. *TOPCAT-Americas included patients from the United States, Canada, Argentina, and Brazil.
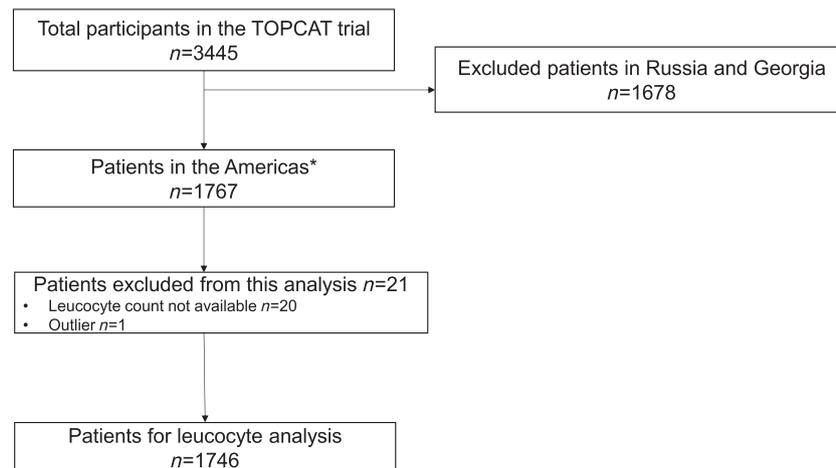
of enrolment into the trial. The TOPCAT-Americas population included patients that were enrolled from the United States, Canada, Argentina, and Brazil. *Figure 1* shows the inception of the study cohort. Twenty-one patients were excluded from our analysis due to non-availability (*n* = 20) of leucocyte count and one with a leucocyte count of 189200 cells/μL that was considered an outlier.

Study variables

The leucocyte and platelet counts that were used in this investigation were obtained at the time of the subject's enrolment into TOPCAT. The other demographic and clinical characteristics were also collected by the TOPCAT investigators into case report forms.[17] Because the leucocyte count was non-normally distributed, a cut-off of 7100 cells/μL was used to divide the cohort at the median leucocyte count in the TOPCAT-Americas cohort. Analyses were subsequently conducted to evaluate differences in the primary and secondary outcomes based on leucocyte count >7100 cells/μL and ≤7100 cells/μL. The associations between absolute leucocyte count, standard deviation rise in leucocyte count, and logarithmically transformed leucocyte count and the primary outcome were also examined.

Study outcomes

The primary outcome for this investigation was a composite of cardiovascular mortality, aborted cardiac arrest, or HF hospitalization during the follow-up. Secondary outcomes were individual components of the primary outcome, all-cause mortality, non-cardiovascular mortality, non-fatal MI, and stroke during follow-up. All clinical outcomes in TOPCAT were adjudicated by a blinded clinical events committee. The adjudication process has been described previously.[17,18]

Statistical analyses

Continuous variables were represented as medians with interquartile ranges, and categorical variables were represented as count with proportions. The Wilcoxon rank-sum test and chi-squared tests were used to identify the differences in baseline characteristics in continuous and categorical variables, respectively.

The time-to-event analyses were conducted with Cox proportional hazards models to identify the risk of the chosen primary and secondary outcomes in those with higher leucocyte count as compared to those with lower leucocyte count during the study follow-up period. The Cox proportional hazard assumption was checked using Schoenfeld residuals prior to reporting results of the model. The multivariable Cox proportional hazards model included the following clinically important covariates: age, gender, race, hypertension, diabetes, dyslipidaemia, coronary artery disease, estimated glomerular filtration rate, atrial fibrillation, body mass index (BMI), New York Heart Association (NYHA) class (III/IV vs. I/II), stroke, peripheral arterial disease, smoking, haematocrit, LVEF, and treatment with spironolactone. The baseline characteristics of prior angina, prior myocardial infarction, prior percutaneous coronary intervention, or prior CABG were combined to form the 'coronary artery disease' covariate, which was included in the aforementioned modelling. All of the covariates were included due to their possible association with cardiovascular prognosis in HFpEF patients. Hazard ratios (HRs) and 95% confidence intervals (CI) were reported for all primary and secondary outcomes in the adjusted and unadjusted analyses. Interactions between leucocyte count and treatment with

spironolactone, age, gender, race, hypertension, diabetes, coronary artery disease, BMI, NYHA class, and LVEF on the risk of the primary outcome were also evaluated. In addition, we estimated frailty index (FI) as previously described by Sanders et al.[21] and introduced leucocyte count and FI in a separate Cox model to determine association between leucocyte count and primary outcome. Notably, the FI index we used from Sanders et al.[21] incorporated ~40 variables and included most of the covariates in our multivariate Cox proportional hazards model described earlier.

Poisson regression analyses were also performed to estimate incidence rates (per 100 person-years) of primary and secondary outcomes. Finally, the associations between leucocyte count and incidence rates of primary and secondary outcomes that were statistically significant in Cox proportional hazard models were explored and plotted using restricted cubic spline Poisson models with the aforementioned clinically important variables. The number of knots was selected based on optimal values of the Akaike information criterion (AIC) to account for possible non-linearity in these associations.

Sensitivity analysis

E-values were estimated for HRs for significant associations between leucocyte count >7100 cells/μL and outcomes to assess the potential effect of unmeasured confounding on these associations.[22] This method estimates the minimum strength of the association that would be required between an unmeasured confounder and both higher leucocyte count and risk of incident clinical outcome to overcome the statistically significant effect observed in a study. A large E-value implies that considerable unmeasured confounding would be needed to explain away an effect estimate. A small E-value implies little unmeasured confounding would be needed to explain away an effect estimate. The calculation was derived from the HR obtained from an adjusted analysis in our study. We also estimated sub-distributional hazard ratios for leucocyte categories and plotted cumulative incidence plots using competing risk regression in accordance with method described by Fine et al.[23] for primary outcome, CV mortality, non-fatal MI, and HF hospitalizations.

Exploratory analyses

Leucocyte differential count was not captured in the TOPCAT laboratory data set, and so we could not specifically assess the importance of myeloid (innate) vs. lymphoid (adaptive) immune populations. Instead, we performed an exploratory analysis of the association between augmented platelet count (also a marker of myeloid expansion[24]) and incidence rate of the primary outcome using restricted cubic spline Poisson

models with the aforementioned clinically important variables. The number of knots was selected based on optimal values of the AIC to account for possible non-linearity in these associations. Given the presence of a V-shaped relationship between platelet count and the incidence rate of primary outcome, we explored the relationship between platelet count and risk of clinical outcomes in the second and the third tertiles of platelet count. The lower tertile was excluded from this exploratory analysis given that reduced platelet counts are often related to multiple factors that do not represent true myeloid contraction (e.g. immune and non-immune platelet destruction, sequestration, haemodilution, drugs, infections, and toxins),[25] whereas platelet augmentation is usually associated with myeloid expansion and hence can be a useful surrogate for the same.[26,27]

To ascertain the nature of immune cell expansion as to whether myeloid or lymphoid cells were associated with risk of incident cardiovascular outcomes, we used Cox proportional hazards models to examine the association between covariates and primary efficacy events, sequentially adding demographic and clinical factors, followed by leucocyte count (as a marker of combined myeloid and lymphoid expansion) and then augmented platelet count (as a marker of myeloid expansion). We hypothesized that an isolated association with lymphoid expansion was possible if clinical outcomes were associated with leucocyte count >7100 cells/μL but not associated with the addition of platelet count. However, if clinical outcomes were associated with both the leucocyte and platelet count, this would suggest either a predominantly myeloid response or combined response to both myeloid and lymphoid expansion.

To test these hypotheses, four models were created for multivariate analyses to evaluate the relationship between leucocyte count, platelet count, and the primary efficacy outcome in TOPCAT-Americas enrolees. Model 1 included the demographic variables of age, gender, and race. Model 2 was adjusted for demographics and clinically important risk factors including diabetes mellitus, hypertension, dyslipidaemia, BMI, coronary artery disease, eGFR, atrial fibrillation, NYHA class (III and IV vs. I and II), stroke, peripheral arterial disease, and treatment with spironolactone. Model 3 included all of the demographic and clinical covariates in Model 2 along with leucocyte count (a marker of myeloid plus lymphoid expansion). Finally, Model 4 included all of the clinical covariates in Model 2 along with both leucocyte count and platelet count above median of the top two tertiles (>246 × 10^3 cells/μL, n = 1156) as marker of myeloid expansion. The HR and 95% CI for leucocyte and platelet count, model chi-square, AIC, and Bayesian information criterion were also calculated for each of the models. A P-value of less than 0.05 was used to define statistical significance. All statistical analyses were conducted in Stata Version 14.2 (StataCorp, College Station, TX, USA).

Role of the funding source

There was no funding source for the current investigation. The funders of TOPCAT had no role in study design, data collection, data analysis, data interpretation, or writing of the report. N. S. B., K. G., and S. D. P. had full access to all study data, and S. D. P. had final responsibility for the decision to submit for publication.

Results

Baseline characteristics

Of the 3445 patients with HFpEF enrolled in TOPCAT, 1767 (51%) were enrolled in the Americas. Within the

TOPCAT-Americas cohort, 1746 (99%) patients had leucocyte count available for the analyses (*Figure 1*). The characteristics of HFpEF patients in TOPCAT-Americas with available leucocyte count are presented in *Table 1*. The median age of the participants in this cohort was 72 years (interquartile range: 64–79) with equal representation of males and females. The majority of patients were non-blacks (1452, 83%). The median BMI in the cohort was 32.8 kg/m^2 (interquartile range: 27.9–38.4 kg/m^2), and the majority of the cohort had hypertension (1571, 90%), obesity (1128, 65%), and dyslipidaemia (1236, 71%). Coronary artery disease (46%), diabetes mellitus (45%), atrial fibrillation (42%), and peripheral artery disease (12%) were also prevalent in the overall cohort. The median leucocyte count for the entire cohort was 7100 cells/µL (*Table 1*).

The patients in the leucocyte count >7100 cells/µL stratum were younger with a lower prevalence of black patients,

Table 1 Baseline characteristics stratified by leucocyte count group

		Leucocyte count >7100 cells/µL		
	TOPCAT-Americas (*n* = 1746)	No (*n* = 900)	Yes (*n* = 846)	*P*-value
Demographics				
Age at randomization (years)	72 (64, 79)	74 (65, 80)	71 (63, 78)	<0.001
Female	872 (49.9%)	471 (52.3%)	410 (47.4%)	0.039
Black race	294 (16.8%)	168 (18.7%)	126 (14.9%)	0.035
Anthropometric parameters				
Height (cm)	167.3 (157.5, 175.3)	167.3 (157.5, 175.3)	167.6 (157.5, 175.3)	0.900
Weight	90.3 (75.7, 108.9)	87.5 (75, 104.8)	94.5 (77, 112.5)	<0.001
Body mass index (kg/m^2)	32.8 (27.9, 38.4)	31.9 (27, 37.1)	33.9 (28.8, 39.7)	<0.001
Clinical parameters				
Atrial fibrillation	738 (42.3%)	404 (44.9%)	334 (39.5%)	0.023
Chronic obstructive pulmonary disease	288 (16.5%)	134 (14.9%)	154 (18.2%)	0.061
Coronary artery disease	807 (46.2%)	414 (46%)	393 (46.5%)	0.850
Angina pectoris	481 (27.5%)	247 (27.4%)	234 (27.7%)	0.910
Prior myocardial infarction	378 (21.6%)	167 (18.6%)	191 (22.6%)	0.036
Prior CABG	333 (19.1%)	164 (18.2%)	169 (20%)	0.340
Prior PCI	343 (19.7%)	170 (18.9%)	173 (20.5%)	0.410
Diabetes mellitus	779 (44.6%)	330 (36.7%)	449 (53.1%)	<0.001
Dyslipidaemia	1236 (70.8%)	621 (69%)	615 (72.8%)	0.082
Hypertension	1571 (90%)	794 (88.2%)	777 (92%)	0.009
Systolic blood pressure (mmHg)	129 (118, 138)	128 (118, 138)	129 (118, 139)	0.210
Diastolic blood pressure (mmHg)	70 (62, 80)	70 (62, 80)	70 (62, 80)	0.550
Obesity	1128 (64.6%)	545 (60.6%)	583 (68.9%)	<0.001
Peripheral arterial disease	205 (11.7%)	97 (10.8%)	108 (12.8%)	0.190
Smoking (current)	115 (6.6%)	46 (5.1%)	69 (8.2%)	0.010
Stroke	155 (8.9%)	80 (8.9%)	75 (8.9%)	0.990
Treatment with spironolactone	876 (50.2%)	451 (50.1%)	425 (50.2%)	0.960
Laboratory parameters				
Leucocyte count (*1000 cells/µL)	7.1 (5.9, 8.5)	5.9 (5.2, 6.5)	8.6 (7.8, 9.7)	<0.001
Haematocrit (%)	38.6 (35.5, 41.9)	38.1 (35.2, 41.4)	39 (35.7, 42.2)	0.002
Platelet count (*1000 cells/µL)	219 (181, 265)	202 (165, 241)	237 (201,287)	<0.001
Estimated GFR (mL/min/1.73 m^2)	61 (48.9, 76.5)	61.6 (50.7, 76.7)	59.71 (48.1, 76.3)	0.071
Left ventricular ejection fraction (%)	58% (52%, 64%)	60% (53%, 65%)	57 (51%, 63%)	0.028
Serum albumin (g/dL)	3.9 (3.6, 4.2)	3.9 (3.6, 4.2)	3.9 (3.6, 4.2)	0.300
Serum creatinine (mg/dL)	1.1 (0.9, 1.4)	1.1 (0.9, 1.3)	1.1 (0.9, 1.2)	0.015
Serum potassium (mEq/L)	4.2 (3.9, 4.5)	4.2 (3.9, 4.5)	4.2 (3.9, 4.5)	0.200

Glomerular filtration rate was estimated by the modification of diet in renal disease (MDRD) 4-component study equation. Coronary artery disease was defined as a composite of angina pectoris, previous MI, PCI, or CABG. Data are represented as median (25th to 75th percentile), number (percentage).
BMI, body mass index; CABG, coronary artery bypass grafting; GFR, glomerular filtration rate; MI, myocardial infarction; PCI, percutaneous coronary intervention; mEq = milliequivalents per litre.
*P-values are from chi-squared test for categorical comparisons and Wilcoxon rank rum test for continuous comparisons between patients with leucocyte count ≤7100 and >7100 cells/µL.

a greater prevalence of male patients, and a higher BMI. Patients with leucocyte count >7100 cells/μL had a higher prevalence of prior myocardial infarction, diabetes mellitus, hypertension, and obesity but a lower prevalence of atrial fibrillation. Patients with leucocyte count >7100 cells/μL had higher mean haematocrit and platelet count compared to patients with leucocyte count ≤7100 cells/μL. Patients with leucocyte count >7100 cells/μL had a marginally lower LVEF compared to patients with leucocyte count ≤7100 cells/μL (*Table 1*).

There were no differences in other haemodynamic parameters, baseline comorbidities, or laboratory parameters between the patients with leucocyte count >7100 cells/μL and patients with leucocyte count ≤7100 cells/μL. Importantly, there were no differences in the proportions of patients treated with spironolactone in the two arms (*Table 1*).

Associations between leucocyte count and clinical outcomes

The patients in the assembled cohort were followed for a median duration of 2.4 (25th–75th percentile 1.4–3.9) years for the occurrence of the primary outcome. During the follow-up period, 517 primary outcome events occurred at an annualized rate of 11.4% (95% CI: 10.5–12.4%) (*Table 2*).

As depicted in the Kaplan–Meier curves in *Figure 2*, in the unadjusted analyses for either the Cox proportional hazard model or competing risk model (competing events of non-HF hospitalization and non-CV mortality), patients with leucocyte count >7100 cells/μL were 35–36% more likely to experience the primary outcome than patients with leucocyte count ≤7100 cells/μL (HR: 1.36, 95% CI: 1.14–1.61). This association remained significant in fully adjusted analyses after accounting for clinically important confounders such that patients with leucocyte count >7100 cells/μL were 27% more likely to experience the primary outcome than the patients with leucocyte count ≤7100 cells/μL (HR 1.27, 95% CI: 1.06–1.52) (*Table 2*). There was no evidence of interaction between total leucocyte count and clinically important variables on risk of primary outcome (Supporting Information, *Table S2*). Given the non-normal distribution of total leucocyte count, we checked the association between every unit rise in log-transformed leucocyte count and risk of the primary outcome (HR: 1.36, adjusted *P* = 0.039). We also estimated HRs for each standard deviation rise in leucocyte count and primary outcome (HR: 1.09, adjusted *P* = 0.026) (Supporting Information, *Table S3*). Also, leucocyte count ≥7100 cells/μL remained significantly associated with risk of primary outcome after introducing frailty index in a separate Cox model (HR:1.21, *P* = 0.031). All analyses indicated robustness of the association. There were no differences in the incidence of cardiovascular mortality, aborted cardiac arrest, stroke, or non-cardiovascular/other mortality between the patients with leucocyte count >7100 cells/μL and with leucocyte count ≤7100 cells/μL in the unadjusted and adjusted analyses (*Table 2* and *Figures 3* and *4*).

Amongst the secondary outcomes, patients with leucocyte count >7100 cells/μL were more likely to be

Table 2 Hazard ratios for outcomes in the TOPCAT-Americas population stratified by median leucocyte count

Outcome	Events/total number event rate (person-years)	Median time to event	Analysis	Leucocyte count below median (≤7100 cells/μL)	Hazard ratio for leucocyte count above median (>7100 cells/μL)	P-value*	E-value
Primary outcome	517/1746	2.4	Unadjusted	Reference	1.36 (1.14–1.61)	0.001	—
	11.4 (10.5–12.4)		Adjusted		1.27 (1.06–1.52)	0.010	1.86
Secondary outcomes							
Heart failure admission	396/1746	2.4	Unadjusted	Reference	1.41 (1.15–1.72)	0.001	—
	8.7 (7.9–9.6)		Adjusted	Reference	1.30 (1.06–1.60)	0.011	1.92
Cardiovascular mortality	221/1746	2.9	Unadjusted	Reference	1.18 (0.90–1.53)	0.224	—
	4.2 (3.7–4.8)		Adjusted	Reference	1.16 (0.88–1.52)	0.291	—
Aborted cardiac arrest	6/1746	2.9	Unadjusted	Reference	1.12 (0.23–5.60)	0.886	—
	0.1 (0.1–0.3)		Adjusted	Reference	0.96 (0.12–7.63)	0.973	—
All-cause mortality	385/1746	2.9	Unadjusted	Reference	1.19 (0.97–1.45)	0.090	—
	7.4 (6.7–8.2)		Adjusted	Reference	1.16 (0.95–1.43)	0.152	—
Non-cardiovascular or other mortality	164/1746	2.9	Unadjusted	Reference	1.20 (0.89–1.64)	0.235	—
	3.1 (2.7–3.7)		Adjusted	Reference	1.17 (0.85–1.61)	0.940	—
Stroke	77/1746	2.8	Unadjusted	Reference	1.00 (0.64–1.56)	0.994	—
	1.5 (1.2, 1.9)		Adjusted	Reference	1.01 (0.64–1.61)	0.950	—
Non-fatal myocardial infarction	94/1746	2.6	Unadjusted	Reference	1.70 (1.13–2.58)	0.012	—
	1.9 (1.5–2.3)		Adjusted	Reference	1.71 (1.11–2.62)	0.014	2.81

The primary efficacy outcome was defined as the composite of death from cardiovascular causes, aborted cardiac arrest, or hospitalization for the management of heart failure. The covariates in the adjusted Cox proportional hazards model include age, sex, race, hypertension, diabetes, dyslipidaemia, coronary artery disease, smoking, body mass index, left ventricular ejection fraction, haematocrit, estimated glomerular filtration rate, atrial fibrillation, New York Heart Association class (III and IV vs. I and II), stroke, peripheral arterial disease, and treatment with spironolactone.
*P-values are from Cox proportional hazards modelling and E-values were calculated only for statistically significant adjusted associations.

Figure 2 Cumulative proportion using *Cox proportional hazard model* (A) and cumulative incidence using *competing risk regression* (B) for primary end point stratified by median leucocyte count. Red represents patients with leucocyte count >7100 cells/μL, and blue represents patients with leucocyte count ≤7100 cells/μL. The primary endpoint was a composite of death from cardiovascular causes, aborted cardiac arrest, or hospitalization for heart failure. Non-CV mortality and non-HF hospitalizations were considered competing events in the competing risk model. The adjusted models for both analyses included age, gender, race, hypertension, diabetes, dyslipidaemia, coronary artery disease, estimated glomerular filtration rate, atrial fibrillation, body mass index, New York Heart Association class (III/IV vs. I/II), stroke, peripheral arterial disease, smoking, haematocrit, LVEF, and treatment with spironolactone. HR, hazard ratio; sHR, sub-distributional hazard ratio.
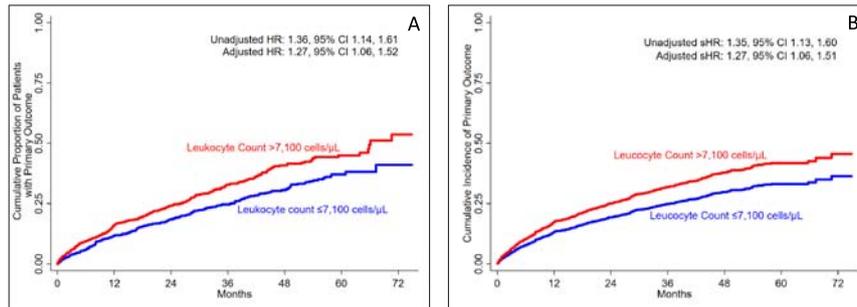
hospitalized for HF than patients with leucocyte count ≤7100 cells/μL over the study follow-up in the unadjusted and adjusted analyses (HR 1.41 and HR 1.30, respectively) (*Table 2*). Patients with leucocyte count >7100 cells/μL were also more likely to experience non-fatal myocardial infarction than patients with leucocyte count ≤7100 cells/μL in the unadjusted and adjusted analyses (HR 1.70 and HR 1.71, respectively) (*Table 2*). We also observed a significant linear relationship between increasing leucocyte count and higher incidence rates of the primary outcome, HF

hospitalization, and non-fatal MI (adjusted *P*-trends <0.05; *Figures 3* and *4*). Interestingly, there was a numerical increase in the risk of all-cause mortality when leucocyte count was dichotomized using 7100 cells/μL, which was not statistically significant (*Table 2*). In contrast, we observed a linear relationship between leucocyte count and all-cause mortality that was statistically significant in both unadjusted and adjusted analyses (*Figure 4*).

There were no differences in the incidence of cardiovascular mortality, aborted cardiac arrest, stroke, or non-

Figure 3 Relationship between leucocyte count and the primary outcome, cardiovascular disease (CVD)-related mortality, heart failure hospitalization, and aborted cardiac arrest in the TOPCAT-Americas population. The adjusted Poisson regression models were controlled for age, gender, race, hypertension, diabetes, dyslipidaemia, coronary artery disease, estimated glomerular filtration rate, atrial fibrillation, body mass index, New York Heart Association class (III/IV vs. I/II), stroke, peripheral arterial disease, smoking, haematocrit, LVEF, and treatment with spironolactone. Restricted cubic spline Poisson regression models estimates (red) are presented with 95% confidence intervals (blue). IRR, incidence rate ratio for every 1000 cells/μL rise in leucocyte count.
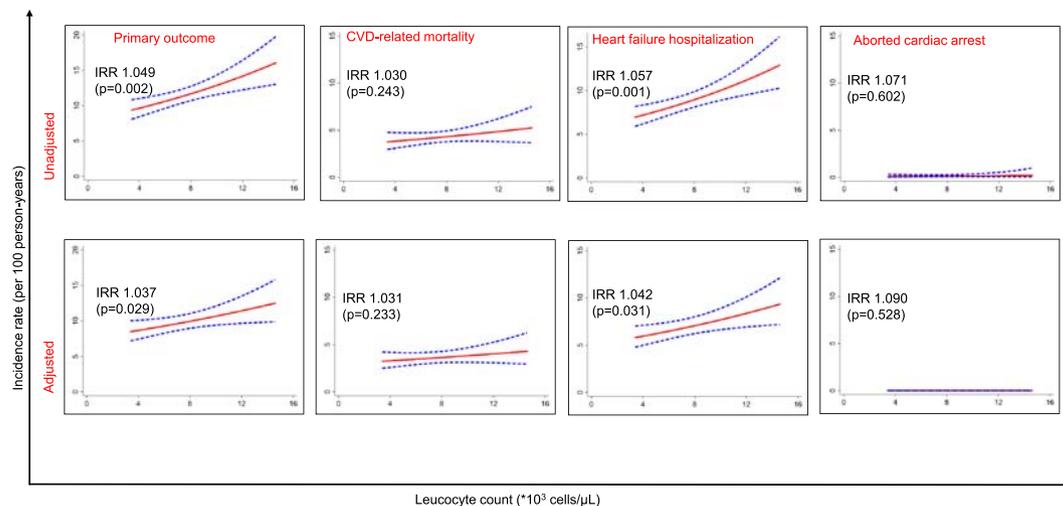
Figure 4 Relationship between leucocyte count and all-cause mortality, non-cardiovascular disease (CVD)-related mortality, non-fatal myocardial infarction and stroke in the TOPCAT-Americas population. The adjusted Poisson regression models were controlled for age, gender, race, hypertension, diabetes, dyslipidaemia, coronary artery disease, estimated glomerular filtration rate, atrial fibrillation, body mass index, New York Heart Association class (III/IV vs. I/II), stroke, peripheral arterial disease, smoking, haematocrit, LVEF, and treatment with spironolactone. Restricted cubic spline Poisson regression models are presented with 95% confidence intervals (blue). IRR, incidence rate ratio for every 1000 cells/μL rise in leucocyte count.
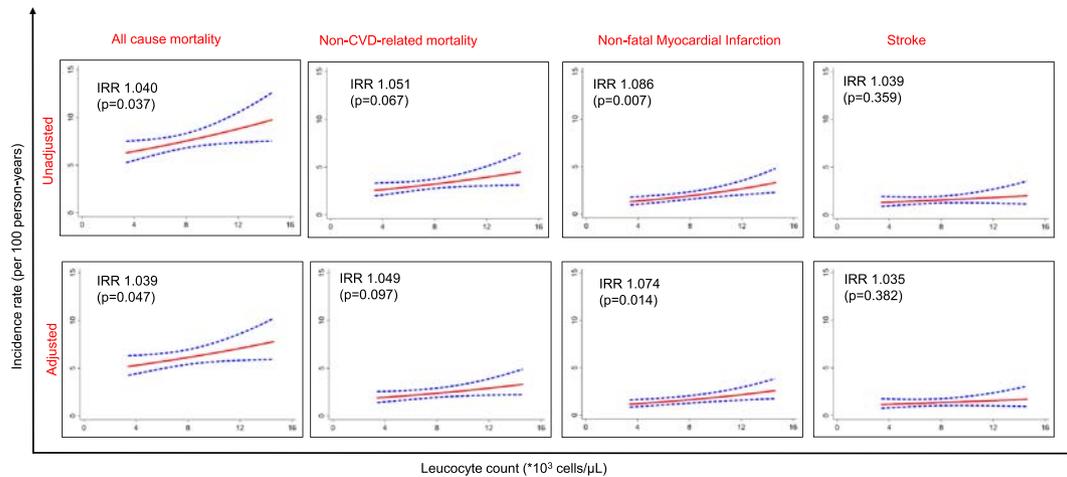
cardiovascular/other mortality between the patients with leucocyte count >7100 cells/μL and with leucocyte count ≤7100 cells/μL in the unadjusted and adjusted analyses (*Table 2* and *Figures 3* and *4*).

Sensitivity analysis

The *E*-values (HR) for the point estimate for the incident primary outcome, HF hospitalization, and myocardial infarction were 1.86, 1.92, and 2.81, respectively. The highest HR in the adjusted model for clinical comorbidities was 1.90 for smoking (primary outcome), 1.76 for NYHA class (HF hospitalization), and 2.17 for smoking (non-fatal myocardial infarction). It is unlikely that an unmeasured or unknown confounder would have a substantially greater effect on development of primary outcome, HF hospitalization, and myocardial infarction than the known clinically important comorbidities for which we adjusted in multivariate analysis by having an HR exceeding 1.86 for primary outcome, 1.92 for heart failure hospitalization, and 2.81 for myocardial infarction. The associations between leucocyte count and primary outcome, CV mortality, non-fatal MI, and HF hospitalization did not change in competing risk analysis indicating robustness of our results (Supporting Information, *Table S4* and *Figure S2*).

Exploratory analyses

A total of 1738 patients had a platelet count (myeloid marker) measured and available for the platelet analyses (*Figure 1*).

We observed a V-shaped relationship between incidence rates of the primary outcome and platelet count (Supporting Information, *Figure S1*). After excluding the lower tertile of platelets, the final analysis was conducted in 1176 patients. In the adjusted analyses, patients with platelet count in the highest tertile were 27% more likely to experience the primary outcome than patients with platelet count in the middle tertile (HR 1.27, 95% CI: 1.01–1.60).

We also sought to distinguish whether lymphoid or myeloid cells were driving the clinical outcomes. To isolate the effect of the nature of immune cell expansion on the primary outcome, we sequentially added confounders to Cox proportional hazards models (*Table 3*). In Models 1 and 2, the model statistics improved with the addition of demographic and clinical factors. In Model 3, leucocyte count (aggregate myeloid and lymphoid expansion) improved model statistics as compared with Model 2 and was significantly associated with higher risk of primary outcome. In Model 4, the addition of platelet count (marker of myeloid expansion) in addition to leucocyte count improved model statistics further. Interestingly, both higher leucocyte and platelet count in the same model (Model 4) were associated with higher risk of primary outcome (*Table 3*), suggesting that leucocyte expansion in general and myeloid expansion in particular are associated with higher risk of the primary outcome.

Discussion

We demonstrated that higher leucocyte count, even after extensive multivariate modelling, was associated with the

composite endpoint of HF hospitalization, aborted cardiac arrest, and cardiovascular mortality in stable HFpEF patients enrolled in the America's cohort of the TOPCAT trial. This was primarily driven by increased hospitalizations for HF. We also observed a greater incidence of non-fatal myocardial infarction in patients with higher leucocyte count. These associations remained robust on sensitivity analyses that suggested a low probability of confounding. Our results support the hypothesis that immune cell expansion as evidenced by increased total blood leucocyte count (and potentially myeloid-specific cell expansion as reflected by increased platelet count) is an important pathogenetic component of HFpEF that impacts disease progression and outcomes.

There may be multiple mechanistic explanations for our findings. At present, the prevailing paradigm is that pathology and symptomatology in HFpEF are heavily driven by a subject's burden of comorbidities such as obesity, hypertension, diabetes mellitus, and coronary artery disease.[28,29] This comorbidity burden has been strongly linked to cardiac and systemic inflammation,[8] suggesting considerable interplay between the immune system and cardiovascular disease.[30–35] In our study, we found that most of these comorbidities were more prevalent in HFpEF patients with higher than median leucocyte count along with adverse CV outcomes. This suggests an interaction between immune cell expansion and the pathogenesis of HFpEF, and, subsequently, clinical outcomes.[8,36]

Prior data from animals and humans suggest multilevel involvement of the immune system in HFpEF. At the tissue level, reduced capillary density and augmented fibrosis are hallmarks of human HFpEF.[36] In animal models of aging-associated and hypertension-associated HFpEF, cardiac myeloid cell infiltration has been specifically linked to increased myocardial fibrosis.[7,37,38] There is also evidence of augmented bone marrow and splenic myelopoiesis and higher circulating monocytes and neutrophils in these models.[7] Myocardial biopsies from humans with HFpEF show augmented numbers of macrophages, CD3^{+} lymphocytes, and total CD45^{+} leucocytes as compared with nonfailing

control heart samples, with correlation between inflammatory cell infiltration and collagen expression.[7,39] Recent data, albeit from an ischaemic HFrEF model, have linked regulatory T-cell dysfunction to capillary rarefaction in the failing heart.[40] Taken together, these data indicate that myeloid and lymphoid expansion in HFpEF, presumably driven in part by co-morbidities, contribute importantly to adverse cardiac remodelling, lending mechanistic credence to our central observation that higher leucocyte count in human HFpEF is a powerful biomarker of detrimental clinical outcomes. The observation that the leucocyte count, while higher in HFpEF patients with adverse outcomes, was not significantly 'elevated' beyond the usual clinical ranges may imply qualitative immune cell dysfunction induced by comorbidities. This has been previously suggested for other types of cardiovascular disease.[12]

Our investigation adds important contributions to the existing literature base pertaining to immune cell expansion and HFpEF and is consistent with the aforementioned animal and human studies implicating myeloid and lymphoid expansion, and a general pro-inflammatory state, as disease drivers in HFpEF. To the best of our knowledge, there are no prior data linking the circulating leucocytes to clinical outcomes in a stable human HFpEF population. Prior observational data have, however, linked leucocyte (or neutrophil) count to later development of incident HF in broad population studies,[11,12] and to all-cause mortality in subjects with ischaemic LV systolic dysfunction[13] and acutely decompensated HF (HFrEF and HFpEF).[15] Shah *et al.*[12] posited that a chronic inflammatory substrate may combine with individual triggers to produce qualitative neutrophil dysfunction that leads to incident cardiovascular events in the general population. Importantly, they highlighted that neutrophil count may prognosticate within 'normal' ranges, with a neutrophil count of 6–7000 cells/μL associated with a hazard ratio of 2.04 for incident HF as compared to a neutrophil count of 2–3000 cells/μL. However, their analysis did not distinguish between types of HF or account for subject comorbidities. Engström *et al.*[11] evaluated the relationship between leucocyte count

Table 3 Multivariable adjusted association of leucocyte and platelet count with primary efficacy outcome (*N* = 1156)

Sequential models for primary efficacy outcome	Leucocyte hazard ratio (95% CI)	*P*-value	Platelet hazard ratio (95% CI)	*P*-value	Model statistics			
					Likelihood ratio chi-square	*P*-value	AIC	BIC
Multivariable Model 1: demographics	—	—	—	—	9.72	0.021	4434.87	4450.04
Multivariable Model 2: Model 1+ clinical factors	—	—	—	—	122.41	<0.001	4305.29	4391.16
Multivariable Model 3: Model 2 + Leucocyte	1.36 (1.08–1.71)	0.009	—	—	129.32	<0.001	4300.39	4391.30
Multivariable Model 4: Model 3 + Platelets	1.28 (1.01–1.62)	0.042	1.27 (1.01–1.60)	0.039	176.35	<0.001	4280.58	4376.42

The primary efficacy outcome was defined as the composite of death from cardiovascular causes, aborted cardiac arrest or hospitalization for the management of heart failure. Model 1 includes demographic factors age, gender, and race. Model 2: Model + clinical factors include hypertension, diabetes, dyslipidaemia, coronary artery disease, smoking, body mass index, left ventricular ejection fraction, haematocrit, estimated glomerular filtration rate, atrial fibrillation, New York Heart Association class (III and IV vs. I and II), stroke, peripheral arterial disease, and treatment with spironolactone. Model 3: leucocyte hazard ratio with reference as leucocyte count ≤7100 cells/μL. Model 4: platelets hazard ratio of with reference as platelet count ≤246 000 cells/μL.
AIC, Akaike information criterion; BIC, Bayesian information criterion; CI, confidence interval.

and incident HF hospitalization in a Swedish community cohort. A leucocyte count in the highest quartile (710019 200 cells/μL) was associated with incident HF hospitalization; the analysis was limited by lack of data on the HF phenotype (HFrEF or HFpEF) whereas HF hospitalizations were identified using ICD-9 codes.

In comparison, our data focus exclusively on well-characterized HFpEF patients with all clinical outcomes rigorously adjudicated in the setting of a prospective, double-blind, placebo-controlled randomized trial. We were able to link the prognostic effect of leucocyte count to other several clinical outcomes in HFpEF—HF hospitalization and non-fatal myocardial infarction—and adjust for a large number of potential confounders. Interestingly, we note that the cut-off for leucocyte count that Engström *et al.* linked to heart failure hospitalization ($>$7100 cells/μL) was identical to that seen in our investigation. A similar cut-off of $>$7000 leucocytes/ μL was predictive of all-cause mortality in patients with ischaemic left ventricular (LV) dysfunction enrolled in the studies of left ventricular dysfunction (SOLVD) trial.[13] Hence, our findings taken together with prior studies suggest that total leucocyte count may be broadly applied as a risk marker in HF.

Our investigation has important public health implications. We anticipate that invoking immune cell expansion in the pathophysiology of HFpEF will yield further investigation into specific immune pathways that may contribute to disease development and progression. At present, few quantitative tools exist for predicting long-term risk in HFpEF patients. If our findings are validated, our data support using immune cell function to stratify long-term clinical risk and pursuing further investigation into identifying potential therapeutic immunomodulatory targets for HFpEF. The promising results of immunomodulation for the treatment of coronary artery disease[34] and the emergence of immune checkpoint inhibitors for oncological disease[41] underscore the possibility that similar approaches may be feasible for HFpEF.

We acknowledge that our work has important limitations. We were limited in our ability to determine the causal relationships between leucocyte count and HFpEF outcomes. Also, the leucocyte and platelet count employed in our analyses were those that were measured only at the time of the subjects' study enrolment. However, our one-time measurement of leucocyte count at baseline would likely underestimate its association with poor outcomes, and the fact that an association is present suggests at least some temporal relationship between leucocyte count, platelet count, and clinical outcomes. We were also unable to characterize many important comorbidities and clinical characteristics that may influence leucocyte and platelet count as they were not captured in the TOPCAT trial. However, our sensitivity analyses suggested a low probability of an unmeasured confounder. We were also limited in our ability to evaluate the association of the clinical outcomes with direct measurement of the myeloid and lymphoid components of the leucocyte population, and instead used platelet count as a surrogate marker of myeloid cells. This was due to the lack of availability of leucocyte differential count amongst TOPCAT patients. This may be of particular importance in view of prior research demonstrating highly specialized roles of specific lymphoid and myeloid cells in key pathologic processes, such as adverse cardiac remodelling.[40,42] Due to the aforementioned limitations, the therapeutic window of opportunity and accurate timeframe for prognostication also remain unclear. Further research will be needed to delineate specific immunologic effect measure modifiers in the causal pathway between HFpEF and clinical outcomes.

Conclusion

In conclusion, leucocyte count was independently associated with the composite primary outcome of cardiovascular mortality, aborted cardiac arrest, or HF hospitalization during the follow-up in the HFpEF patients from TOPCAT-Americas. These results were primarily driven by the HF hospitalization outcome and were also accompanied by an excess of non-fatal MI amongst patients with leucocyte count $>$7100 cells/μL. Patients with platelet count (surrogate myeloid marker) in the highest tertile also had a higher incidence rate for the primary outcome. Further research is needed to define the mechanisms underlying our findings and validate their prognostic implications.

Acknowledgement

Conflict of interest

Funding

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. STROBE Statement

Table S2. Hazard ratios (HR) with 95% confidence interval for interaction between leucocyte count with clinically important variables used in the multivariable Cox proportional hazard model for the primary outcome.

Table S3. Hazard ratios (HR) with 95% confidence interval (CI) for leucocyte count and primary outcome using a Cox proportional hazard model.

Table S4. Association between Leucocyte counts and Outcomes in Cox proportional hazard Model vs Competing Risk models

Figure S1. Unadjusted relationship between platelet count and the primary outcome in the TOPCAT-Americas population

Figure S2. Cumulative subhazard plots stratified by median leucocyte count for **primary outcome** with non-primary outcome (non-CV mortality or non-HF hospitalization) as competing risk (Panel A), **CV mortality** with non-CV mortality as competing risk (Panel B), **non-CV mortality** with CV mortality as competing risk (Panel C), **HF hospitalization** with non-HF hospitalization as competing risk (Panel D) and **Non-Fatal Myocardial Infarction** with all-cause mortality as competing risk (Panel E).

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