Leukocyte count and the risk of adverse outcomes in patients with HFpEF

Zhaowei Zhu and Shenghua Zhou*

Abstract

**Background:** Inflammation is a key feature of heart failure including HFpEF. The leukocyte count is a marker of inflammation that is widely used in clinical practice. However, there is little available evidence for the relationship between leukocyte count and the outcomes of HFpEF.

**Methods:** We analyzed data from the TOPCAT (Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist) trial. The primary outcome was all-cause mortality, the secondary outcome was composite cardiovascular events and hospitalization for heart failure. Multivariable Cox proportional hazard models were used to compare the risk profiles of patients with leukocyte quartiles, subgroup study divided by sex was also analyzed.

**Results:** The present study included 2898 patients with HFpEF. 429 deaths, 671 composite cardiovascular events and 386 hospitalization for heart failure occurred during a mean 3.4 years follow-up. The association between leukocyte count and adverse outcomes followed a U-shaped curve. After multivariable adjustment, the patients with the lowest leukocyte count (Q1) and the highest leukocyte count (Q4) faced higher risk of all-cause death (Q1 vs. Q2, adjusted HR: 1.439; 95% CI: 1.060–1.953, \( p = 0.020 \); Q4 vs. Q2, adjusted HR, 1.901; 95%CI: 1.424–2.539, \( p < 0.001 \)). The subgroup analysis showed a consistent result in female but not male patients.

**Conclusions:** The association between leukocyte count and risk of adverse outcomes followed a U-shaped curve. Both higher and lower leukocyte count are associated with worse outcomes in patients with HFpEF, which may be attributed to the two sides of inflammation in cardiac remodeling.

**Keywords:** HFpEF, Leukocyte, Adverse outcomes

Background

Heart failure with preserved ejection fraction (HFpEF) has emerged as a pivotal problem with increasing prevalence and poor prognosis in recent years [1]. However, it is still not fully understood of the pathophysiology of HFpEF, which retards the improvement of its accurate diagnosis and efficient treatment. In fact, proven effective medical treatment has not yet appeared for this disease [2, 3].

Leukocyte, as an inflammation driver, plays an important role in cardiovascular disease. In further, it serves as an important predictor for various cardiovascular events [4–6]. Heart failure, which is an end stage of all kinds of cardiovascular disease, has been known to be involved in inflammation process and the concept of inflammation as a major component of HF is becoming more and more consolidated [7]. Recent studies confirmed that inflammatory processes could be part of the etiology of HF [8, 9]. Besides, it was shown that increased long-term incidence of HF hospitalizations were associated with high leukocyte counts [10]. Moreover, subclinical inflammation predicts adverse prognosis in patients with established HF [11–13]. Canakinumab (IL-1β inhibitor), as an inflammation inhibitor, has been found to be capable of reducing not only the incidence...
of hospitalization for heart failure but also heart failure-related mortality [13].

Although limit evidences indicate inflammation biomarkers are associated with adverse outcomes in patients with HFpEF [14, 15], the relationship between leukocyte count and HFpEF is still not fully clear. Therefore, this study aimed to examine the prognostic significance of leukocyte count on clinical outcomes in patients with HFpEF in the Treatment of Preserved Cardiac Function Heart Failure With an Aldosterone Antagonist Trial (TOPCAT).

**Methods**

**Study design and patients**

TOPCAT was a randomized, placebo-control, double-blind, multi-center clinical study. The study aimed to investigate the treatment efficacy of spironolactone in patients with HFpEF. The study information including background, design, inclusion and exclusion criteria, and baseline characteristics have been published previously [16, 17]. Briefly, this trial, beginning in August 2006 and ending in January 2012, enrolled 3445 patients with symptomatic HFpEF from 270 sites distributed in 6 countries. The primary goal of the trial was to clarify whether spironolactone could reduce the composite outcome of aborted cardiac arrest, cardiovascular mortality, or heart failure hospitalization in patients with HFpEF (e.g. documented ejection fraction $\geq 45\%$).

According to the current guideline [18], this analysis in this investigation was limited to patients with ejection fraction $\geq 50\%$ ($n = 2930$). Patients with missed leukocyte count and outlier leukocyte count (over 20,000 cells/μL) ($n = 32$) were excluded. At last, total 2898 patients were enrolled in this study (Fig. 1). The association between leukocyte count on admission and the risk of all-cause death, the composite cardiovascular events and hospitalization for heart failure were analyzed.

**Baseline characteristics**

Basic information and medical histories were obtained in patients by a detailed baseline visit in TOPCAT study [17]. For example, age, sex, race, and current smokers were obtained by self-reported history. Medical history included: hypertension, diabetes, stroke, dyslipidemia, peripheral arterial disease, angina pectoris, myocardial infarction, percutaneous coronary revascularization, coronary artery bypass graft surgery, implanted cardioverter defibrillator, implanted pacemaker, thyroid disease, chronic obstructive pulmonary disease, New York Heart Association Class, and prior heart failure hospitalization. Systolic blood pressure, diastolic blood pressure and Body Mass Index (BMI) were obtained by trained staff. Laboratory data included serum creatinine, blood urea nitrogen (BUN), hematocrit, Brain Natriuretic Peptide (BNP), hemoglobin and platelet. Medication data included: aspirin, angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers, beta blockers, calcium channel blockers, and statins. The National Heart, Lung, and Blood Institute approved our use of TOPCAT data. Ethics approval and consent to participate were not applicable.

**Statistics**

Baseline characteristics were compared by quartiles of leukocyte counts. Data are presented as mean $\pm$ SD, non-normal variables were reported as median (interquartile range [IQR])—the distance between the 25th and 75th percentiles. Normally distributed continuous variables were analyzed with one-way ANOVA. Categorical variables were compared with Pearson $\chi^2$ test. Baseline plasma BNP levels were expressed as log-transformed data. Glomerular filtration rates were estimated by incorporating creatinine into the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [19]. Unadjusted Kaplan-Meier estimates of the time-to-event outcomes were generated according to baseline leukocyte count quartiles and compared via the log-rank test. Univariate and multivariable Cox regression analysis were used to test the risk of adverse outcomes associated with leukocyte count. Only variables with $p < 0.1$ on univariate analysis were incorporated into the multivariate Cox regression analysis. Subgroup analyses of multivariable models were done by sex. Two-sided P-values < 0.05 were considered statistically significant. All analyses were performed using Empower(R) (www.empowerstats.com, X&Y solutions, Inc Boston, MA) and SPSS version 25.0 (IBM, Armonk, New York).
Results
Study participants and baseline characteristics
A total of 2898 patients (mean age = 69 ± 9.6 years; 46% men; 89% white) were included in this analysis. Table 1 presented participants’ baseline characteristics based on leukocyte quartiles (Q): Q1: ≤ 5.5 × 10⁹/l; Q2: > 5.5 × 10⁹/l to ≤ 6.7 × 10⁹/l; Q3: > 6.7 × 10⁹/l to ≤ 8.0 × 10⁹/l; and Q4: > 8.0 × 10⁹/l. Leukocyte quartiles were not associated with any significant trends in age, race, prior heart failure hospitalization, hypertension, stroke, history of pacemaker or implantable cardioverter defibrillators (ICD) implanted, angina pectoris, systolic blood pressure, left ventricular ejection fraction (LVEF), heart rate, the use of β-blockers, calcium channel blockers, angiotensin-converting enzyme inhibitor/Angiotensin Receptor Blocker (ACEI/ARB) and spironolactone. However, male sex, smoker, dyslipidemia, previous myocardial infarction, percutaneous coronary intervention (PCI), Coronary artery bypass graft (CABG), diabetes mellitus, atrial fibrillation, chronic obstructive pulmonary disease (COPD), asthma, thyroid disease, peripheral arterial disease, use of statins and loop diuretics were more prevalent in participants with higher leukocyte quartiles. At the same time, higher leukocyte count was associated with higher heart rate, body mass index, BUN, hemoglobin and platelet. The higher leukocyte count was also associated with lower diastolic blood pressure, eGFR and prevalence of New York Heart Association class III-IV.

Leukocyte count on admission and long-term clinical outcomes
Over a median follow-up of 3.4 years (25th–75th percentiles = 2.0–4.9 years), 429 deaths, 671 composite cardiovascular events and 386 hospitalization for heart failure occurred. Kaplan–Meier estimates of the cumulative incidence of all-cause death, the composite cardiovascular events and hospitalization for heart failure are depicted in Fig. 2. It seems both participants in the highest and lowest leukocyte count quartiles faced a greater risk for all-cause death (log-rank, P < 0.0001 for all; Q1 vs. Q2: P < 0.0001; Q3 vs. Q2: P < 0.0001; Q4 vs. Q2: P < 0.0001). The leukocyte count at Q2, and subgroup analysis reached a consistent result (Additional file 1: Table S3 and Table S4). Above results indicated that leukocyte count was not a prognostic factor for composite cardiovascular events and hospitalization for heart failure.

Discussion
This study found that the association between leukocyte count and the risk of adverse outcomes followed a U-shaped curve. Both lower and higher leukocyte count is related to a higher risk of adverse outcomes in the TOPCAT patients' cohort.

Several studies have reported that pro-inflammatory biomarkers including high sensitivity C-reactive protein, tumor necrosis factor-α, interleukin 6/8, monocyte chemotactant protein-1 and pentraxin 3 were significantly increased in patients with HFrEF [14, 20–22]. Consistent with previous studies, our results once again confirm that inflammatory responses may play an important role in the progression and development of HFrEF [20, 21, 23].
### Table 1: Baseline characteristics (n = 3421)

| Characteristic                                      | Leukocyte count |      |      |      |      | p-value |
|-----------------------------------------------------|-----------------|------|------|------|------|---------|
|                                                     | \(\leq 5.5\) n = 753 | 5.5–6.7 n = 707 | 6.7–8.0 n = 720 | > 8.0 n = 718 |      |         |
| Age, mean ± SD, years                               | 69 ± 9.2        | 69 ± 9.7 | 69 ± 10 | 69 ± 9 | 0.867 |         |
| Male (%)                                            | 289 (38)        | 304 (43) | 362 (50) | 372 (52) | 0.000 |         |
| Race                                                |                 |      |      |      |      | 0.620  |
| White (%)                                           | 671 (89)        | 629 (89) | 641 (89) | 629 (88) |      |         |
| Black (%)                                           | 69 (9)          | 58 (8)  | 63 (9)  | 66 (9)  |      |         |
| Other (%)                                           | 13 (2)          | 20 (2)  | 16 (2)  | 23 (3)  |      |         |
| Smoker (%)                                          | 237 (32)        | 241 (34) | 267 (37) | 306 (43) | 0.001 |         |
| Hypertension (%)                                    | 685 (91)        | 645 (91) | 673 (94) | 673 (94) | 0.077 |         |
| Dyslipidemia (%)                                    | 431 (57)        | 406 (57) | 423 (59) | 483 (67) | 0.000 |         |
| Previous myocardial infarction (%)                  | 143 (19)        | 154 (22) | 173 (24) | 192 (27) | 0.004 |         |
| Prior heart failure hospitalization (%)             | 562 (75)        | 511 (72) | 520 (72) | 504 (70) | 0.304 |         |
| Angina pectoris (%)                                 | 340 (45)        | 347 (49) | 345 (48) | 311 (43) | 0.112 |         |
| PCI (%)                                             | 89 (12)         | 87 (12)  | 89 (12)  | 132 (19) | 0.000 |         |
| CABG (%)                                            | 75 (10)         | 80 (11)  | 85 (12)  | 113 (16) | 0.006 |         |
| Hypertension (%)                                    | 198 (26)        | 198 (28) | 244 (34) | 318 (44) | 0.000 |         |
| Hypertension (%)                                    | 262 (35)        | 218 (31) | 239 (33) | 280 (39) | 0.011 |         |
| COPD (%)                                            | 58 (8)          | 67 (10)  | 89 (12)  | 124 (17) | 0.000 |         |
| Asthma (%)                                          | 36 (5)          | 56 (8)   | 43 (6)   | 61 (9)   | 0.016 |         |
| Stroke (%)                                          | 56 (7)          | 43 (6)   | 59 (8)   | 68 (10)  | 0.112 |         |
| Peripheral arterial disease (%)                     | 49 (7)          | 55 (8)   | 66 (9)   | 89 (12)  | 0.000 |         |
| Thyroid disease (%)                                 | 128 (17)        | 105 (15) | 104 (15) | 143 (20) | 0.021 |         |
| Pacemaker implanted (%)                            | 64 (9)          | 50 (7)   | 56 (8)   | 61 (9)   | 0.713 |         |
| ICD (%)                                             | 10 (1.3)        | 8 (1.1)  | 8 (1.1)  | 12 (1.7) | 0.773 |         |
| HR (b.p.m.)                                         | 69 ± 10.1       | 68 ± 9.9 | 68 ± 11.1 | 70 ± 11.3 | 0.078 |         |
| Systolic blood pressure, mean ± SD, mmHg            | 129 ± 12.6      | 130 ± 13.9 | 130 ± 14.6 | 129 ± 14.9 | 0.110 |         |
| Diastolic blood pressure                            | 76 ± 10.4       | 77 ± 10.6 | 76 ± 10.8 | 74 ± 11.1 | 0.000 |         |
| Body mass index, mean ± SD, kg/m²                    | 31 ± 6.6        | 32 ± 6.5 | 32 ± 7.1 | 34 ± 7.9 | 0.000 |         |
| eGFR (mL/min)                                       | 67 ± 18.2       | 69 ± 22.5 | 68 ± 19.8 | 65 ± 20.1 | 0.002 |         |
| BUN (mg/dL)                                         | 16.5 (6.8,22.1) | 16.2 (5.0,22.4) | 16.5 (5.6,23.0) | 17.6 (8.1,26.0) | 0.004 |         |
| Hematocrit (%)                                       | 39 ± 5.0        | 40 ± 4.8 | 40 ± 5.4 | 41 ± 5.7 | 0.000 |         |
| Hemoglobin (g/dL)                                   | 12.9 (12.0,14.0) | 13.2 (12.2,14.3) | 13.4 (12.3,14.5) | 13.5 (12.2,14.8) | 0.000 |         |
| Platelet (k/uL)                                     | 207 (1,73,243)  | 220 (188,254) | 223 (193,264) | 245 (208,294) | 0.000 |         |
| Albumin (g/dL)                                      | 3.9 ± 2.5       | 3.8 ± 2.7 | 3.7 ± 2.5 | 3.7 ± 2.8 | 0.000 |         |
| logBNP                                              | 2.6 ± 0.5       | 2.6 ± 0.5 | 2.6 ± 0.5 | 2.6 ± 0.5 | 0.627 |         |
| LVEF (%)                                            | 59 ± 6.5        | 59 ± 6.9 | 59 ± 6.0 | 59 ± 6.7 | 0.076 |         |
| New York Heart Association class III-IV (%)         | 514 (68)        | 509 (72) | 501 (70) | 428 (60) | 0.000 |         |
| Aspirin use (%)                                      | 453 (60)        | 458 (65) | 475 (66) | 458 (64) | 0.110 |         |
| b-blockers (%)                                       | 573 (76)        | 555 (79) | 565 (79) | 551 (78) | 0.599 |         |
| ACEI (%)                                            | 504 (66)        | 455 (64) | 455 (63) | 438 (61) | 0.120 |         |
| ARB (%)                                             | 107 (14)        | 113 (16) | 109 (15) | 132 (18) | 0.155 |         |
| Statins (%)                                         | 334 (44)        | 332 (47) | 362 (50) | 426 (59) | 0.000 |         |
| Calcium channel blockers (%)                        | 276 (37)        | 292 (41) | 272 (38) | 281 (39) | 0.300 |         |
| Spironolactone (%)                                  | 361 (48)        | 370 (52) | 346 (48) | 378 (53) | 0.118 |         |
| Loop diuretic (%)                                   | 326 (43)        | 329 (47) | 349 (49) | 458 (64) | 0.000 |         |
| Thiazide diuretic (%)                               | 322 (43)        | 278 (39) | 286 (40) | 216 (30) | 0.000 |         |

Values are presented as mean ± SD or median (25th-75th percentile) for continuous variables and number (%) for categorical variables. Statistical significance for continuous data was tested using the analysis of variance procedure and categorical data was tested using the \(\chi^2\) test.

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; BNP, B-type natriuretic peptide; BUN, blood urea nitrogen; ICD, Implantable Cardioverter Defibrillator; COPD, chronic obstructive pulmonary disease; CABG, Coronary Artery Bypass Grafting; PCI, percutaneous coronary intervention; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HR, heart rate; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; SBP, systolic blood pressure

eGFR by the Chronic Kidney Disease Epidemiology Collaboration formula
However, although leukocyte count acts as an important marker for inflammation level in body, few previous studies have assessed the association between leukocyte count and cardiovascular events in patients with HFpEF. Previous studies only showed that the prognostic value of relative lymphocyte count in patients with chronic HFrEF [12, 24–26]. In further, high leukocyte count was found to be associated with increased long-term incidence of HF hospitalizations in middle-aged men [10]. Besides, Kim et al. found that neutrophil-to-lymphocyte ratio was prospectively associated with heart failure [5]. In line with above studies, present finding indicates that leukocyte count is associated with both all-cause death and composite cardiovascular events specifically in HFpEF patients, reaffirming this important link between leukocyte count and heart failure regardless of ejection fraction. Recently, Bajaj NS et al. [27] did a similar study and they found that leukocyte count > 7100 cells/μL was independently associated with adverse clinical outcomes especially HF hospitalization in HFpEF patients from TOPCAT-Americas. In our study, we focused on the whole population in TOPCAT study and patients with LVEF < 50% were excluded, which may be attributed to the different result from the study by Bajaj NS. In our study, we found a U-shaped relationship between the risk of clinical outcomes especially all-cause death and leukocyte count. Besides, the subgroup analysis showed that female may contribute more to such relationship of leukocyte count and all-cause death. However, the U-shaped relationship also showed an increased risk of clinical outcomes for patients with higher leukocyte count in our study, which was confirmed by the study by Bajaj NS. Besides, although similar trend was found, leukocyte count was not a prognostic factor for composite cardiovascular events and hospitalization for heart failure in this study. This may be caused by the heterogeneity of HFpEF, the shortage of the second analysis and the limit sample volume. Further
Table 2: Univariate and multivariable Cox regression analysis of all-cause mortality (n = 2898)

| All-cause mortality                                      | Univariate analysis | Multivariate analysis |
|---------------------------------------------------------|---------------------|-----------------------|
|                                                         | HR  | 95% CI     | p-value | HR  | 95% CI     | p-value |
| Age                                                     | 1.054 | 1.043–1.065 | 0.000   | 1.046 | 1.033–1.059 | 0.000   |
| Sex                                                     | 0.67  | 0.554–0.810 | 0.000   | 1.698 | 1.368–2.106 | 0.000   |
| Race                                                    | 1.528 | 1.251–1.867 | 0.000   | –     | –           | –       |
| BMI                                                     | 1.007 | 0.993–1.021 | 0.330   | –     | –           | –       |
| Smoker                                                  | 1.170 | 1.037–1.320 | 0.011   | –     | –           | –       |
| LVEF                                                    | 0.998 | 0.984–1.013 | 0.820   | –     | –           | –       |
| Angina pectoris                                         | 0.613 | 0.504–0.745 | 0.000   | 0.815 | 0.653–1.017 | 0.071   |
| Prior heart failure hospitalization                     | 0.810 | 0.657–0.997 | 0.047   | 1.124 | 0.901–1.403 | 0.301   |
| Previous myocardial infarction                          | 1.266 | 1.026–1.563 | 0.028   | 0.777 | 0.603–1.002 | 0.052   |
| Stroke                                                  | 1.558 | 1.151–2.110 | 0.004   | 0.940 | 0.686–1.289 | 0.702   |
| CABG                                                    | 1.655 | 1.293–2.118 | 0.000   | 1.066 | 0.800–1.422 | 0.661   |
| PCI                                                     | 1.483 | 1.161–1.893 | 0.002   | 1.061 | 0.803–1.403 | 0.677   |
| COPD                                                    | 1.629 | 1.257–2.111 | 0.000   | 0.936 | 0.713–1.228 | 0.634   |
| Asthma                                                  | 1.601 | 1.152–2.226 | 0.005   | 0.812 | 0.574–1.148 | 0.239   |
| Hypertension                                            | 0.815 | 0.586–1.133 | 0.223   | –     | –           | –       |
| Peripheral arterial disease                             | 2.154 | 1.669–2.779 | 0.000   | 0.615 | 0.468–0.809 | 0.001   |
| Dyslipidemia                                            | 1.271 | 1.043–1.550 | 0.018   | 1.105 | 0.857–1.426 | 0.441   |
| ICD                                                     | 1.605 | 0.797–3.230 | 0.185   | –     | –           | –       |
| Pacemaker                                               | 1.983 | 1.500–2.621 | 0.000   | 0.978 | 0.724–1.320 | 0.884   |
| Atrial fibrillation                                     | 1.530 | 1.264–1.851 | 0.000   | 1.016 | 0.821–1.258 | 0.884   |
| Thyroid disease                                         | 1.219 | 0.957–1.553 | 0.108   | –     | –           | –       |
| Diabetes mellitus                                       | 0.595 | 0.491–0.721 | 0.000   | 0.857 | 0.685–1.071 | 0.175   |
| Heart rate                                              | 1.017 | 1.008–1.026 | 0.000   | 1.021 | 1.012–1.031 | 0.000   |
| Systolic blood pressure                                 | 0.981 | 0.974–0.988 | 0.000   | 0.992 | 0.984–1.000 | 0.050   |
| Diastolic blood pressure                                | 0.959 | 0.951–0.967 | 0.000   | 0.994 | 0.982–1.006 | 0.300   |
| Fasting glucose                                         | 1.002 | 0.998–1.005 | 0.343   | –     | –           | –       |
| New York Heart Association class III-IV                 | 1.723 | 1.423–2.086 | 0.000   | 0.806 | 0.658–0.988 | 0.038   |
| eGFR                                                    | 0.979 | 0.973–0.984 | 0.000   | 0.994 | 0.988–1.000 | 0.055   |
| Leukocyte group                                         | 1.249 | 1.146–1.361 | 0.000   | 1.439 | 1.060–1.953 | 0.020   |
| 1                                                       | –     | –           | –       | –     | –           | –       |
| 2 Reference                                             | –     | –           | –       | –     | –           | –       |
| 3                                                       | –     | –           | –       | –     | –           | –       |
| 4                                                       | –     | –           | –       | 1.510 | 1.113–2.050 | 0.008   |
| Hemoglobin                                              | 0.833 | 0.786–0.882 | 0.000   | 0.898 | 0.843–0.958 | 0.001   |
| BUN                                                     | 1.030 | 1.025–1.036 | 0.000   | 1.009 | 1.001–1.017 | 0.023   |
| Albumin                                                 | 0.983 | 0.945–1.023 | 0.411   | –     | –           | –       |
| Aspirin                                                 | 1.301 | 1.074–1.576 | 0.007   | 1.089 | 0.884–1.341 | 0.424   |
| b-blockers                                              | 1.16  | 0.915–1.471 | 0.220   | –     | –           | –       |
| ACEi                                                    | 1.355 | 1.116–1.643 | 0.002   | 0.945 | 0.770–1.160 | 0.591   |
| ARB                                                     | 0.862 | 0.670–1.109 | 0.248   | –     | –           | –       |
| Statin                                                  | 0.726 | 0.599–0.878 | 0.001   | 1.072 | 0.837–1.372 | 0.581   |
| Loop diuretic                                           | 0.304 | 0.245–0.377 | 0.000   | 0.553 | 0.423–0.724 | 0.000   |
| Thiazide Diuretic                                       | 0.494 | 0.398–0.612 | 0.000   | 1.080 | 0.840–1.388 | 0.548   |
| Spironolactone                                          | 1.029 | 0.851–1.243 | 0.769   | –     | –           | –       |

CI: confidence interval; HR: hazard ratio
well-designed study was warranted to investigate the actual role of leukocyte in patients with HFpEF. Although the association between leukocyte and heart failure is strongly supported by current clinical evidences [26]. It is not known whether leukocytes are involved directly in the pathogenesis of heart failure or are only accompany with the disease. Several systemic pro-inflammatory conditions including obesity, hypertension, diabetes or metabolic syndrome were usually combined in patients with HFpEF, which might be the fundamental mechanism that leads to inflammation and oxidative stress [28]. The increased pro-inflammatory state and oxidativeredress may in turn result in coronary microvascular endothelial dysfunction and myocardial fibrosis, consequently leading to adverse cardiovascular events finally. This may explain the increased risk of adverse outcomes of HFpEF patients with higher level of leukocyte count in this study.

Table 3. Subgroup analysis of Cox proportional-hazards model divided by sex for All-cause mortality

| All-cause mortality                           | Male                      | Female                   |
|-----------------------------------------------|---------------------------|--------------------------|
|                                               | HR | 95% CI | p-value     | HR | 95% CI | p-value     |
| Age                                           | 1.047 | 1.029–1.066 | 0.000      | 1.038 | 1.019–1.057 | 0.000     |
| Race                                          | 0.473 | 0.328–1.424 | 0.310      | 0.344 | 0.175–0.676 | 0.002     |
| Smoker                                        | 0.858 | 0.354–1.982 | 0.687      | 0.319 | 0.143–0.709 | 0.005     |
| Angina pectoris                               | 0.857 | 0.743–0.991 | 0.037      | 0.864 | 0.697–1.070 | 0.180     |
| Prior heart failure hospitalization           | 1.269 | 0.933–1.727 | 0.130      | 0.928 | 0.665–1.296 | 0.661     |
| Previous myocardial infarction                | 0.742 | 0.536–1.025 | 0.071      | 0.893 | 0.583–1.366 | 0.602     |
| Stroke                                        | 0.851 | 0.551–1.315 | 0.468      | 0.993 | 0.619–1.593 | 0.978     |
| Cabinetian                                     | 1.109 | 0.772–1.592 | 0.576      | 0.953 | 0.580–1.566 | 0.850     |
| PCI                                           | 1.168 | 0.805–1.693 | 0.414      | 0.927 | 0.594–1.447 | 0.739     |
| COPD                                          | 1.118 | 0.780–1.602 | 0.544      | 0.687 | 0.445–1.061 | 0.091     |
| Asthma                                        | 0.602 | 0.356–1.018 | 0.058      | 1.070 | 0.660–1.736 | 0.783     |
| Peripheral arterial disease                   | 0.562 | 0.394–0.801 | 0.001      | 0.657 | 0.420–1.029 | 0.067     |
| Dyslipidemia                                  | 1.158 | 0.816–1.643 | 0.412      | 1.121 | 0.770–1.634 | 0.550     |
| Pacemaker                                     | 0.863 | 0.574–1.298 | 0.479      | 1.116 | 0.697–1.787 | 0.647     |
| Atrial fibrillation                           | 1.139 | 0.852–1.521 | 0.380      | 0.860 | 0.622–1.189 | 0.360     |
| Diabetes mellitus                             | 0.986 | 0.729–1.334 | 0.926      | 0.737 | 0.527–1.032 | 0.075     |
| Heart rate                                    | 1.019 | 1.006–1.032 | 0.005      | 1.028 | 1.014–1.042 | 0.000     |
| Systolic blood pressure                       | 0.993 | 0.982–1.005 | 0.244      | 0.994 | 0.982–1.005 | 0.286     |
| Diastolic blood pressure                      | 0.990 | 0.974–1.007 | 0.251      | 0.992 | 0.975–1.010 | 0.332     |
| New York Heart Association class III-IV       | 0.805 | 0.604–1.072 | 0.138      | 0.756 | 0.557–1.026 | 0.072     |
| eGFR                                          | 0.995 | 0.986–1.003 | 0.211      | 0.993 | 0.984–1.002 | 0.143     |
| Leukocyte group                               | 0.088 | 0.002     |            | 0.088 | 0.002     |            |
| 1.134 | 0.745–1.726 | 0.557      | 1.907 | 1.188–3.059 | 0.007     |
| 2. reference                                  | 1.150 | 0.768–1.721 | 0.498      | 2.088 | 1.291–3.375 | 0.003     |
| 3.150 | 0.768–1.721 | 0.498      | 2.088 | 1.291–3.375 | 0.003     |
| 4.150 | 0.768–1.721 | 0.498      | 2.088 | 1.291–3.375 | 0.003     |
| Hemoglobin                                    | 0.899 | 0.816–0.968 | 0.007      | 0.910 | 0.822–1.006 | 0.066     |
| BUN                                           | 1.011 | 1.001–1.021 | 0.032      | 1.005 | 0.993–1.018 | 0.419     |
| Aspirin                                       | 1.335 | 1.021–1.795 | 0.035      | 0.838 | 0.609–1.153 | 0.277     |
| ACEI                                          | 1.007 | 0.759–1.335 | 0.963      | 0.884 | 0.650–1.202 | 0.432     |
| Statin                                        | 1.006 | 0.713–1.420 | 0.972      | 1.142 | 0.790–1.651 | 0.479     |
| Loop Diuretic                                 | 0.627 | 0.441–0.892 | 0.010      | 0.467 | 0.308–0.707 | 0.000     |
| Thiazide Diuretic                             | 0.925 | 0.666–1.285 | 0.642      | 1.303 | 0.880–1.930 | 0.186     |
level and cardiovascular outcomes in HFrEF patients. Leukocytes can not only facilitate the proteolysis of the collagen matrix but also promote interstitial myocardial fibrosis, which eventually contribute to the cardiac remodeling and heart failure [4]. Confirming this, recent study demonstrated that by activating fibroblasts and stimulating collagen deposition, IL-10 derived from T cells and macrophages can induce myocardial stiffness and impair myocardial relaxation [29, 30]. But on the other hand, through secretion of angiogenesis-promoting cytokines, leukocytes also protect the nonischemic remote myocardium in ischemic heart disease [4]. This indicates that too low leukocyte may be harmful for some heart disease.

In addition, the U-shaped relationship between leukocyte count and the risk of adverse cardiovascular outcomes persisted even after controlling for baseline covariates. The U-shaped relationship may also be a potential reason for the unsuccessful clinical trials attempting to combat HFrEF by blocking inflammation [11]. Although canakinumab related to a dose-dependent reduction in heart failure related hospitalization and the composite of heart failure-related mortality and hospitalization, it is not efficient in all population but patients with elevated hsCRP [31]. Besides, interaction between inflammation and body weight, blood pressure, and blood glucose might jointly affect the outcomes of HFrEF patients and the sum of the complex interaction may be also responsible for the observed U-shaped relationship in this study [32–35].

Conclusions
In this study, we found a U-shaped relationship between leukocyte count and risk of clinical outcomes, and subgroup analysis showed that female contributed more to such relationship for all-cause death. Both higher and lower leukocyte count are associated with worse outcomes in patients with HFrEF, which may be attributed to the two sides of inflammation in cardiac remodeling.

Limitations
The findings of this study must be interpreted in the context of limitations inherent to the TOPCAT study design. First, there is heterogeneity in HFrEF, so these findings may not represent all HFrEF classifications. Secondly, we cannot exclude bias introduced by leukocyte levels measured at laboratories and there is lack of CRP value and serial measurements about leukocyte count in the database, which limit the strength of the conclusion. Thirdly, leukocyte count is elevated or decreased commonly in patient with acute infection or blood system diseases, no information is applied about the exclusion of such patients in the TOPCAT trial, the impact of acute infection or blood system diseases thus remain unknown and served as a limitation of present analysis. At last, although the subtype of leukocyte may play pivotal role in cardiovascular disease, we did not assess the specific role due to the unavailability of the related information in the present database.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12872-021-02142-y.

Additional file 1. Supplemental Table 1. Univariate and multivariable Cox regression analysis of Composite cardiovascular events (n=2898).
Supplemental Table 2. Subgroup analysis of Cox proportional-hazards model divided by gender for Composite cardiovascular events (n=2898).
Supplemental Table 3. Univariate and multivariable Cox regression analysis of hospitalization for heart failure (n=2898).
Supplemental Table 4. Subgroup analysis of Cox proportional-hazards model divided by gender for Hospitalization for heart failure (n=2898).

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Authors’ contributions
ZZ and SZ analyzed the data and wrote the main manuscript text. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations
Ethics approval and consent to participate
The study was approved by the ethics committee of The Second Xiangya Hospital and obeyed the Declaration of Helsinki (No. 2017YFC0908802). All patients have provided written consent to participate in this study.

Consent to publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.
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