Differential white blood cell count and incident heart failure in men and women in the EPIC-Norfolk study

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Aims
Markers of inflammation are associated with increased risk of heart failure, but data on differential white blood cell (WBC) count are lacking. We examined the prospective association between differential WBC count and incident heart failure events.

Methods and results
Hazard ratios (HRs) (per increase of 1000 cells/μL, 95% confidence interval) of total WBC count and individual components on heart failure were calculated in apparently healthy 7195 men and 8816 women aged 39–79 participating in the ‘European Prospective Investigation into Cancer and Nutrition’ (EPIC) study in Norfolk. During a mean follow-up of 12.4 years, 935 incident cases of heart failure occurred. In women, neither total WBC count (1.02, 0.96–1.09) nor individual components were associated with HR of heart failure after accounting for known risk factors. In men, HR of heart failure increased with increasing levels of total WBC count (1.09, 1.04–1.15) after accounting for established risk factors; analysis of WBC components showed increased hazard with increasing levels of granulocyte count (1.16, 1.09–1.24) and, independently of this, decreased hazard with increasing levels of monocyte count (0.71, 0.53–0.93); lymphocyte count was not significantly associated with heart failure (0.97, 0.83–1.13). Results did not change materially after excluding smokers, adjusting for intermediate myocardial infarction and coronary heart disease and C-reactive protein.

Conclusion
Inflammation as measured by WBC count was independently associated with incident heart failure in apparently healthy men but not women. The association observed in men was driven by granulocyte count, but there was an independent inverse association between monocyte count and incident heart failure.

Keywords
Heart failure • White blood cells • Differential leucocyte count • Inflammation

Introduction
There is a body of evidence suggesting that inflammatory processes are involved in the pathophysiology of heart failure, and inflammation is currently discussed as a therapeutic target in heart failure.1 Inflammatory markers such as C-reactive protein, tumour necrosis factor-α, and interleukin-6 are elevated in patients with left ventricular dysfunction and manifest heart failure and associated with adverse prognosis.2,3 In addition, experimental data indicate that inflammatory cytokines might substantially contribute to the development of myocardial dysfunction and heart failure by mediating, for example, myocyte apoptosis and ventricular remodelling processes.4,5 Indeed, inflammatory markers such as C-reactive protein, tumour necrosis factor-α, interleukin-6, and erythrocyte sedimentation rate were consistently shown to be associated with increased risk of future heart failure in population-based studies.6–9

White blood cell (WBC) count is a marker of systemic inflammation, but data on the association with the risk of heart failure are sparse.10,11 However, there is evidence to suggest an
association between WBC count and heart failure. Cytokines, which are associated with the development of heart failure, are mainly secreted by leucocytes and, conversely, were also shown to influence leucocyte levels. Additionally, WBC count is strongly associated with risk factors related to the development of heart failure such as coronary heart disease and smoking.

The aim of this study was to investigate the prospective association of WBC count and its individual components with the risk of developing heart failure in a population of apparently healthy middle-aged men and women.

**Methods**

**Participants**

The European Prospective Investigation into Cancer and Nutrition (EPIC) Norfolk is a prospective population study of 25 639 men and women aged between 39 and 79 years, resident in Norfolk, UK. Details of the recruitment process, study design, and population characteristics have been published earlier. The EPIC-Norfolk population is broadly similar to the UK population in terms of the distribution of anthropometric, smoking, and cardiovascular risk factors. The EPIC-Norfolk study was approved by the Norfolk Local Research Ethics Committee, and participants gave signed informed consent at each contact.

At the baseline survey between 1993 and 1997, participants completed a detailed health and lifestyle questionnaire including questions on history of diabetes, heart attack, stroke, cancer, smoking (current, former, never), steroid hormone use, hormone replacement therapy, menopausal status, and occupational social class (manual, non-manual). A four-level (inactive, moderately inactive, moderately active, and active) physical activity index was derived from the validated EPIC short physical activity questionnaire designed to assess combined work and leisure activity. Educational status was based on the highest qualification attained (O-level or less, A-level or higher).

Trained nurses examined individuals at a clinic visit. Height and weight were measured and body mass index was estimated as weight (kg) divided by height (m²). Blood pressure was measured with an Accutorr non-invasive blood pressure monitor after the participant had been seated for 5 min. We used the mean of two measurements for analysis. Non-fasting blood samples were taken by EPIC-Norfolk technicians and transported to the EPIC-Norfolk laboratory in Attleborough, UK. An MD18 haematology analyser (Coulter Corporation, Miami, FL, USA) was used for the absolute blood cell enumeration (1000 cells/μL). Blood samples for assay were stored at 4°C and assayed at the department of clinical biochemistry, University of Cambridge, Cambridge, UK.

**Biochemical analysis**

We measured serum total cholesterol with the RA 1000 (Bayer Diagnostics, Basingstoke, UK). White blood cell count was measured in ~75% of the entire cohort, due to funding reasons. Blood samples for leucocyte count were stored overnight at room temperature and were collected each morning by EPIC-Norfolk technicians and transported to the EPIC-Norfolk laboratory in Attleborough, UK. An Olympus AU640 clinical chemistry analyser (Olympus UK Ltd). The coefficient of variation for the period of study was ≤3.0% (0.03). Respective standard deviations (SD) of granulocyte, monocyte, and lymphocyte count were 1.3, 0.3, and 0.6. Serum concentrations of C-reactive protein (mg/L) were measured in 2010 in all participants with baseline medical heart failure treatment. Participants with WBC and differential count values above mean ± 3 SD were excluded (n = 101) in order to reduce the effects of extreme blood cell count values arising from leucocyte-related disorders or from measurement errors or recording errors on the results, which left 7195 men and 8816 women for analysis. We performed secondary analyses excluding current smokers, people with the baseline use of steroids, and heart failure events occurring during the first 2 years of follow-up. To assess whether the association of leucocytes and heart failure was mediated by preceding myocardial infarction or ischaemic heart disease, we added hospitalization for myocardial infarction or ischaemic heart disease as a time-dependent variable to the Cox proportional hazard models in men and women, using the lowest quartile as a reference. The HR was also estimated per increase of 1000 cells/μL. Multivariable Cox’s regression was used to determine the independent contribution of leucocytes for incident heart failure. In multivariable analyses, we included established socio-demographic, cardiovascular, and lifestyle risk factors of heart failure which were also shown to be significantly associated with the risk of heart failure in the EPIC-Norfolk cohort in an earlier study. We performed secondary analyses excluding current smokers, people with the baseline use of steroids, and heart failure events occurring during the first 2 years of follow-up. To assess whether the association of leucocytes and heart failure was mediated by preceding myocardial infarction or coronary heart disease, we added hospitalization for myocardial infarction or ischaemic heart disease as a time-dependent variable to the model. To account for any possible effect of hormonal status of females on inflammatory response and subsequent impact on outcome, we also performed secondary analyses among females on inflammatory response and subsequent impact on outcome.
post-menopausal women not using hormone therapy. Potential collin-
earity between distinct WBC components was examined by pairwise
Pearson’s correlation.

All analyses were undertaken using Stata statistical software, version
11.1 (Stata Corporation, College Station, TX, USA).

Results

The mean (SD) age of the study population was 58.0 (9.4) years.
The mean (SD) WBC count was 6500/μL (± 1600/μL). Table 1
shows characteristics of the participants according to sex-specific
quartiles of WBC count. Increasing quartiles of WBC count
were associated with higher cholesterol levels, lower occupational
class, and lower educational level in men, and with higher age,
higher levels of BMI, systolic and diastolic blood pressure,
C-reactive protein, and higher rate of smoking and physical
inactivity in both men and women.

Among the 16011 men and women included in the analysis, 935
(90 fatal and 845 non-fatal) incident cases of heart failure were
identified in 527 men and 408 women during a mean follow-up of
12.4 years (incidence, 4.7 per 1000 person-years). Tables 2
and 3 show HRs for heart failure comparing each quartile of
total WBC count and individual WBC components with the
lowest quartile, and per every increase of 1000 cells/μL, for men
and women separately.

White blood cell count

In men, we observed an increasing HR of heart failure with increas-
ing quartiles of WBC count in age-adjusted analysis, with border-
line significant results after multivariable adjustment (P for linear
trend 0.07). However, HR of heart failure significantly increased
by 9% with every 1000 cells/μL increase in WBC count. Among
women, there was also a trend for a positive association of
WBC count in age-adjusted analysis (P for linear trend 0.06), but
no significant association after multivariable adjustment, regardless
of whether WBC count was analysed as quartiles or per
1000 cells/μL increase. However, there was no evidence for a stat-
istically significant interaction between gender and WBC count on
HR of heart failure (P = 0.59).

Granulocytes

We observed a statistically significant interaction between gender
and granulocyte count (P = 0.03). The HR of heart failure
increased with increasing quartiles of granulocyte count in men.
In the multivariable-adjusted model, the HR for heart failure in
the highest quartile compared with the lowest quartile was 1.62
[95% confidence interval (CI) 1.25–2.11, P for linear trend
< 0.0001]. Among women, there was no significant association in
multivariable-adjusted analysis when comparing highest to lowest
quartile (HR of 1.04, 95% CI 0.78–1.38, P for linear trend 0.73)
or per increase of 1000 cells/μL.

Monocytes

We observed a statistically significant interaction between gender
and monocyte count (P = 0.01). The HR of heart failure decreased
with increasing quartiles of monocyte count in men. Those in the
highest quartile had an HR for heart failure of 0.70 (95% CI 0.53–
0.92, P for linear trend 0.009) compared with the lowest quartile.
There was no significant association between monocyte count and
HR of heart failure in women, with a multivariable-adjusted HR of
1.15 (95% CI 0.85–1.56, P for linear trend 0.46) in the highest
quartile compared with the lowest quartile.

Lymphocytes

There was no significant association between lymphocyte count
and risk of heart failure in men and women.

Additional analyses

The results were materially unchanged after further adjusting for
myocardial infarction or coronary heart disease occurring during
follow-up, and after excluding current smokers, participants with
steroid intake, and events within the first 2 years. The results
were not markedly different in post-menopausal women not
using hormone therapy from all women analysed together (data
not shown).

There was significant collinearity between individual compo-
nents of WBC count. Granulocyte count was inversely
correlated with monocyte count (Pearson’s correlation coeffi-
cient −0.08, P < 0.0001) and positively correlated with lympho-
cyte count (correlation coefficient 0.25, P < 0.0001), and
monocyte count was positively correlated with lymphocyte
count (correlation coefficient 0.26, P < 0.0001). We repeated
multivariable analyses for each WBC subpopulation adjusting
for the other components, which materially did not change the
results. In particular, after controlling for granulocyte count,
both monocyte count (HR 0.74 per increase of 1000 cells/μL,
95% CI 0.56–0.98, P = 0.04) and granulocyte count (HR 1.16
per increase of 1000 cells/μL, 95% CI 1.09–1.23, P < 0.0001)
remained significantly associated with the risk of heart failure in
men. Relative numbers (%) of granulocyte and monocyte count
were not significantly associated with heart failure risk when abso-
ute values were included in the multivariable analysis (data not
shown).

Adjustment for C-reactive protein

Measures of C-reactive protein were available in 5056 men (includ-
ing 355 heart failure cases) and 6515 women (including 277 cases).
HRs for heart failure differed slightly, particularly for monocyte
count, in the group with available C-reactive protein compared
with the total study population (Tables 2 – 4). Log-transformed
C-reactive protein levels were positively correlated with WBC
count (Pearson’s correlation coefficient 0.27, P < 0.0001), granulo-
cyte count (coefficient 0.26, P < 0.0001), and monocyte count
(coefficient 0.09, P < 0.0001).

Additional adjustment for C-reactive protein attenuated all esti-
mates except that of monocyte count in men, which was marginally
increased. Among men, there was no significant association
between WBC count and HR of heart failure after additional ad-
justment for C-reactive protein (HR 1.03 per increase of
1000 cells/μL, 95% CI 0.96–1.10). This might be due to reduced
sample size and loss of power as the association was also not sig-
nificant without adjustment for C-reactive protein in this subgroup,
in contrast to the total study population. The association of gran-
ulocyte (HR 1.10, 95% CI 1.02–1.19) and monocyte count

Differential WBC count and incident heart failure

525
<table>
<thead>
<tr>
<th>Quartiles of WBC count</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>P-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (n)</td>
<td>1690</td>
<td>1742</td>
<td>1862</td>
<td>1901</td>
<td></td>
</tr>
<tr>
<td>WBC count (cells/µL)</td>
<td>4700 (600)</td>
<td>5800 (300)</td>
<td>6800 (300)</td>
<td>8700 (1300)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Range</td>
<td>&lt;5400</td>
<td>5400–6200</td>
<td>6300–7300</td>
<td>&gt;7300</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.1 (9.3)</td>
<td>57.9 (9.4)</td>
<td>59.1 (9.3)</td>
<td>59.1 (9.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.0 (3.2)</td>
<td>26.4 (3.2)</td>
<td>26.6 (3.2)</td>
<td>26.7 (3.5)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>134 (17)</td>
<td>136 (17)</td>
<td>139 (17)</td>
<td>140 (18)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>83.0 (10.9)</td>
<td>84.0 (10.4)</td>
<td>85.4 (11.1)</td>
<td>86.1 (11.2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.91 (1.05)</td>
<td>6.02 (1.08)</td>
<td>6.08 (1.11)</td>
<td>6.14 (1.11)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.9 (4.2)</td>
<td>2.2 (4.1)</td>
<td>2.5 (3.5)</td>
<td>4.5 (8.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>2.8</td>
<td>3.4</td>
<td>3.0</td>
<td>4.3</td>
<td>0.07</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>3.7</td>
<td>7.6</td>
<td>11.9</td>
<td>24.7</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

| Occupational social class (%) | | | | | |
| Non-manual                  | 59.5 | 60.0 | 58.4 | 55.9 | 0.04 |
| Manual                      | 40.5 | 40.0 | 41.6 | 44.1 |     |

| Education                   | | | | | |
| A-level or higher           | 65.1 | 65.8 | 62.2 | 59.5 | 0.0002 |
| Less than A-level           | 35.0 | 34.2 | 37.8 | 40.5 |     |

| Physical activity           | | | | | |
| Inactive                    | 27.4 | 27.3 | 32.1 | 34.7 | <0.0001 |
| Mod. inactive               | 23.7 | 24.4 | 24.8 | 23.0 |     |
| Mod. active                 | 24.5 | 24.5 | 21.2 | 21.6 |     |
| Active                      | 24.4 | 23.9 | 22.0 | 20.7 |     |

| Women (n)                   | 2127  | 2090  | 2387  | 2212  |     |
| WBC count (cells/µL)        | 4500 (600) | 5700 (300) | 6700 (300) | 8600 (1100) | <0.0001 |
| Range                      | <5300 | 5300–6100 | 6200–7300 | >7300  |     |
| Age (years)                | 57.8 (9.1) | 58.3 (9.4) | 58.1 (9.4) | 57.0 (9.6) | 0.004 |
| BMI (kg/m²)                | 25.5 (3.8) | 26.1 (4.2) | 26.2 (4.2) | 26.7 (4.7) | <0.0001 |
| Systolic BP (mmHg)         | 131 (18) | 133 (19) | 134 (19) | 136 (19) | <0.0001 |
| Diastolic BP (mmHg)        | 79.4 (10.6) | 80.6 (11.2) | 81.4 (11.1) | 82.0 (11.1) | <0.0001 |
| Total cholesterol (mmol/L) | 6.26 (1.58) | 6.32 (1.15) | 6.37 (1.25) | 6.27 (1.21) | 0.10 |
| C-reactive protein (mg/L)<sup>b</sup> | 2.0 (3.1) | 2.6 (5.1) | 3.0 (6.0) | 4.5 (8.1) | <0.0001 |
| Diabetes (%)               | 1.8    | 2.7    | 2.2    | 2.8    | 0.16  |
| Smoking (%)                | 5.0    | 7.2    | 10.9   | 21.7   | <0.0001 |

| Occupational social class (%) | | | | | |
| Non-manual                  | 62.0 | 62.6 | 62.8 | 62.5 | 0.97  |
| Manual                      | 38.0 | 37.4 | 37.2 | 37.5 |     |

| Education                   | | | | | |
| A-level or higher           | 48.1 | 48.2 | 48.3 | 46.6 | 0.60  |
| Less than A-level           | 51.9 | 51.8 | 51.7 | 53.4 |     |

| Physical activity           | | | | | |
| Inactive                    | 27.9 | 29.6 | 30.7 | 33.8 | <0.0001 |
| Mod. inactive               | 31.7 | 32.5 | 33.4 | 30.2 |     |
| Mod. active                 | 23.1 | 22.2 | 22.3 | 21.3 |     |
| Active                      | 17.4 | 15.7 | 13.6 | 14.7 |     |

Data are presented as mean (SD) unless indicated otherwise. WBC, white blood cell; BMI, body mass index; BP, blood pressure.

<sup>a</sup>Linear regression was used for continuous variables, and a χ² test was used for categorical variables.

<sup>b</sup>Available in 5245 men and 6809 women.
We demonstrate an increased risk of heart failure with increasing WBC count with HR of heart failure remained non-significant after additional adjustment for C-reactive protein. Among women, the associations of WBC, granulocyte, and monocyte count with HR of heart failure remained non-significant after additional adjustment for C-reactive protein.

**Discussion**

We demonstrate an increased risk of heart failure with increasing WBC count in a prospective population-based study of apparently healthy middle-aged men and women. In men, the association between WBC count and risk of heart failure was independent of established risk factors and was explained by granulocyte count. However, we also observed an independent association between monocyte count and risk of heart failure, with a decreased risk with increasing monocyte count. Women showed a strongly attenuated association between WBC count and risk of heart failure, which was not apparent after multivariable adjustment, and individual WBC components were not significantly associated with heart failure risk.

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**Table 2** Hazard ratios for incident heart failure by quartiles of white blood cell and differential blood cell count in men: European Prospective Investigation of Cancer-Norfolk Study 1993–2009

<table>
<thead>
<tr>
<th>Quartiles</th>
<th>WBC (events/n)</th>
<th>Age-adjusted</th>
<th>Multivariable</th>
<th>Granulocytes (events/n)</th>
<th>Age-adjusted</th>
<th>Multivariable</th>
<th>Monocytes (events/n)</th>
<th>Age-adjusted</th>
<th>Multivariable</th>
<th>Lymphocytes (events/n)</th>
<th>Age-adjusted</th>
<th>Multivariable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100/1690</td>
<td>1.00</td>
<td>1.00</td>
<td>89/1689</td>
<td>1.00</td>
<td>1.00</td>
<td>112/1602</td>
<td>1.00</td>
<td>1.00</td>
<td>112/1568</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>112/1742</td>
<td>1.01 (0.77–1.32)</td>
<td>0.93 (0.71–1.23)</td>
<td>111/1727</td>
<td>1.20 (0.91–1.59)</td>
<td>0.86 (0.64–1.16)</td>
<td>85/1237</td>
<td>1.12 (0.87–1.45)</td>
<td>1.07 (0.81–1.39)</td>
<td>121/1568</td>
<td>1.20 (0.86–1.52)</td>
<td>0.88 (0.65–1.19)</td>
</tr>
<tr>
<td></td>
<td>131/1862</td>
<td>1.02 (0.78–1.32)</td>
<td>0.94 (0.71–1.23)</td>
<td>134/1895</td>
<td>1.25 (0.96–1.63)</td>
<td>0.82 (0.65–1.05)</td>
<td>205/2807</td>
<td>1.01 (0.78–1.29)</td>
<td>0.97 (0.74–1.25)</td>
<td>136/1899</td>
<td>0.88 (0.65–1.19)</td>
<td>0.83 (0.64–1.07)</td>
</tr>
<tr>
<td></td>
<td>184/1901</td>
<td>1.44 (1.13–1.84)</td>
<td>1.24 (0.96–1.60)</td>
<td>193/1884</td>
<td>1.83 (1.42–2.35)</td>
<td>0.74 (0.57–0.96)</td>
<td>214/3221</td>
<td>1.13 (0.88–1.44)</td>
<td>0.96 (0.74–1.25)</td>
<td>158/2126</td>
<td>0.70 (0.53–0.92)</td>
<td>0.71 (0.53–0.93)</td>
</tr>
<tr>
<td></td>
<td>0.002</td>
<td>0.07</td>
<td>0.0001</td>
<td>&lt;0.0001</td>
<td>0.75 (0.57–0.98)</td>
<td>0.02</td>
<td>1.05 (0.91–1.21)</td>
<td>0.94</td>
<td>0.97 (0.83–1.13)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.12 (1.07–1.18)</td>
<td>1.09 (1.04–1.15)</td>
<td>1.19 (1.12–1.26)</td>
<td>0.75 (0.57–0.98)</td>
<td>0.71 (0.53–0.93)</td>
<td>1.05 (0.91–1.21)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Results are presented as hazard ratio (95% CI). Multivariable analysis were adjusted for age, body mass index, systolic blood pressure, known diabetes, cholesterol, social class, educational level, smoking, and physical activity.

**Table 3** Hazard ratios for incident heart failure by quartiles of white blood cell and differential blood cell count in women: European Prospective Investigation of Cancer-Norfolk Study 1993–2009

<table>
<thead>
<tr>
<th>Quartiles</th>
<th>WBC (events/n)</th>
<th>Age-adjusted</th>
<th>Multivariable</th>
<th>Granulocytes (events/n)</th>
<th>Age-adjusted</th>
<th>Multivariable</th>
<th>Monocytes (events/n)</th>
<th>Age-adjusted</th>
<th>Multivariable</th>
<th>Lymphocytes (events/n)</th>
<th>Age-adjusted</th>
<th>Multivariable</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>89/2127</td>
<td>1.00</td>
<td>1.00</td>
<td>106/2128</td>
<td>1.00</td>
<td>1.00</td>
<td>63/1775</td>
<td>1.00</td>
<td>1.00</td>
<td>93/1929</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>101/2090</td>
<td>1.10 (0.83–1.46)</td>
<td>0.95 (0.71–1.27)</td>
<td>94/2265</td>
<td>0.91 (0.69–1.20)</td>
<td>0.91 (0.69–1.22)</td>
<td>69/1617</td>
<td>1.19 (0.84–1.67)</td>
<td>1.08 (0.81–1.39)</td>
<td>72/1948</td>
<td>0.86 (0.64–1.15)</td>
<td>0.91 (0.69–1.22)</td>
</tr>
<tr>
<td></td>
<td>103/2387</td>
<td>0.99 (0.74–1.31)</td>
<td>1.09 (0.81–1.47)</td>
<td>99/2207</td>
<td>1.01 (0.77–1.33)</td>
<td>1.04 (0.78–1.38)</td>
<td>115/2514</td>
<td>1.23 (0.94–1.60)</td>
<td>0.73</td>
<td>117/2369</td>
<td>1.36 (1.03–1.80)</td>
<td>1.09 (0.81–1.47)</td>
</tr>
<tr>
<td></td>
<td>115/2212</td>
<td>1.36 (1.03–1.80)</td>
<td>0.75</td>
<td>109/2216</td>
<td>1.23 (0.94–1.60)</td>
<td>0.73</td>
<td>161/2910</td>
<td>1.09 (1.02–1.18)</td>
<td>1.05 (0.97–1.13)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>0.75</td>
<td>0.11</td>
<td>0.73</td>
<td>1.05 (0.97–1.13)</td>
<td>1.05 (0.97–1.13)</td>
<td>1.00 (0.82–1.48)</td>
<td>1.10 (0.82–1.48)</td>
<td></td>
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<tr>
<td></td>
<td>1.08 (1.01–1.14)</td>
<td>1.02 (0.96–1.09)</td>
<td>1.09 (1.02–1.18)</td>
<td>1.10 (0.97–1.13)</td>
<td>1.05 (0.97–1.13)</td>
<td>1.05 (0.97–1.13)</td>
<td>1.19 (0.90–1.57)</td>
<td>1.10 (0.82–1.48)</td>
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Results are presented as hazard ratio (95% CI). Multivariable analysis were adjusted for age, body mass index, systolic blood pressure, known diabetes, cholesterol, social class, educational level, smoking, and physical activity.
Our findings on the association of WBC count with the risk of heart failure are in line with analyses of a population-based study in men and the ‘Atherosclerosis Risk in Communities’ study in middle-aged adults.\(^{10,11}\) We extend previous findings by sex-stratified analyses demonstrating substantial differences in the association of total WBC count and individual WBC components with heart failure by sex. Moreover, our stratified analyses revealed a significant association of monocyte count in men only which might be diluted in earlier sex combined analyses.

Significant sex differences in the inflammatory response have been long noted\(^{21}\) and there is a body of evidence showing a modulation of immune response by female sex hormones.\(^{22–24}\) Accordingly, albeit counts of blood leucocytes are similar in men and women, functional capacity of cells might differ due to sex hormone effects. Our results were similar in post-menopausal women not using hormone replacement compared with all women of the study; however, we could not account for effects of androgens on inflammatory cells.\(^{25}\)

Another explanation for the sex different findings is that leucocytes or inflammation in general does not contribute to the development of heart failure in women as much as in men. In the Rotterdam study, C-reactive protein was less strongly associated with the risk of heart failure in women compared with men and the association was attenuated more strongly after multivariable adjustment, which is in line with our findings on WBC count.\(^{26}\) The aetiology of heart failure differs between men and women, with women, for example, being more likely to have obesity or hypertension as underlying cause.\(^{27}\) Hence, in women, the role of traditional or yet unknown risk factors might be more relevant for the development of heart failure than inflammatory processes.

Leucocytes might contribute to the development of heart failure in distinct ways. Granulocytes were shown to be associated with risk of cardiovascular disease, and inflammatory processes are assumed to actively contribute to vascular injury and atherosclerosis.\(^{14,28}\) However, adjusting for interim coronary heart disease did not attenuate the association between WBC count and heart failure in our study. Smoking, which is also associated with heart failure risk,\(^{29}\) strongly affects granulocyte count.\(^{30}\) The association between WBC or granulocyte count and risk of heart failure was attenuated, but remained significant after adjusting for smoking or excluding smokers. Higher levels of granulocyte count might reflect a general systemic inflammatory response associated with early stages of myocardial dysfunction. The association of granulocyte count with the risk of heart failure remained significant after adjustment for C-reactive protein though, which is in line with findings of the ‘Atherosclerosis Risk in Communities’ study.\(^{11}\) Notably, in our study, C-reactive protein and WBC count were measured from blood samples taken during the same visit, whereas in the latter study, samples for C-reactive protein measurement were drawn about 10 years after the samples for WBC, which might have introduced bias to the adjustment. Nonetheless, the association between granulocyte count and heart failure seems to be independent of a general inflammatory response and granulocytes indeed might causally contribute to increased oxidative stress and adverse myocardial remodelling by secreting enzymes such as, for example, myeloperoxidase.\(^{30}\)

The inverse association between monocyte count and risk of heart failure was remarkably enhanced after adjustment for C-reactive protein, suggesting an underlying mechanism independent of inflammation. Monocytes as the main source of pro-inflammatory cytokines have long been regarded as detrimental, since associated with ischaemia induced myocardial damage, subsequent adverse myocardial remodelling, and development of heart failure.\(^{31}\) More recent data, though, indicate a potential role of monocytes in angiogenesis which is crucial for healing processes also in the myocardium.\(^{32}\) Furthermore, endothelial progenitor cells are a functional subpopulation of blood monocytes, which have been shown to improve vascular repair and left ventricular function in animal models.\(^{33}\) Importantly, selective depletion of monocytes was shown to lead to a rapid development of left ventricular dysfunction in a model of hypertensive animals prone to heart failure.\(^{34}\) To our knowledge, our study is the first to show an association between monocyte count and heart failure in humans. Together with the experimental data, our findings contribute evidence for a beneficial role of monocytes in the development of heart failure.

What implications might our findings have? Differential WBC count is a new risk factor for the development of heart failure.

### Table 4 Hazard ratios (95% confidence interval) for incident heart failure by white blood cell, granulocyte, and monocyte count and by sex with additional adjustment for C-reactive protein: European Prospective Investigation of Cancer-Norfolk Study 1993–2009

<table>
<thead>
<tr>
<th></th>
<th>WBC</th>
<th>Granulocytes</th>
<th>Monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P-value</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td><strong>Men (355 events/5056 total)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivariable-adjusted(^a)</td>
<td>1.06 (0.99–1.13)</td>
<td>0.07</td>
<td>1.15 (1.06–1.24)</td>
</tr>
<tr>
<td>Plus C-reactive protein</td>
<td>1.03 (0.96–1.10)</td>
<td>0.47</td>
<td>1.10 (1.02–1.19)</td>
</tr>
<tr>
<td><strong>Women (277 events/6515 total)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multivariable-adjusted(^a)</td>
<td>1.02 (0.95–1.10)</td>
<td>0.59</td>
<td>1.03 (0.94–1.12)</td>
</tr>
<tr>
<td>Plus C-reactive protein</td>
<td>1.01 (0.93–1.09)</td>
<td>0.83</td>
<td>1.01 (0.92–1.11)</td>
</tr>
</tbody>
</table>

\(^a\)Hazard ratios are per increase of 1000 cells/μL.
\(^b\)Adjustments as in the multivariable model (Table 2).
Importantly, in contrast to interleukin-6, tumour necrosis factor-α, and high-sensitive C-reactive protein, WBC count is established as a routine laboratory parameter in the clinical setting. Additionally, our findings provide pathophysiological insight into the development of heart failure. First, we add evidence suggesting a sex difference role of inflammation for the development of heart failure which might have implications for future trials on preventive interventions targeting inflammatory processes. Secondly, our results further support inflammation as a strong independent risk factor for heart failure, which warrants evaluation as a target for intervention not only in manifest heart failure, but also in prevention. Finally, we suggest low monocyte count as a novel risk factor of heart failure independent of the inflammatory pathway which warrants further study.

There are some limitations to our study. We defined prevalent heart failure by drug treatment only, which might be less sensitive and undetected cases might influence estimates if associated with WBC count. However, excluding events of the first 2 years as potential pre-existing heart failure cases did not change results. Moreover, our findings in apparently healthy individuals oppose data in patients with manifest heart failure which showed a prognostic impact of lymphocyte count but not of total WBC and monocyte count. The latter might be due to the pro-apoptotic effects of the renin–angiotensin system and the adrenergic system on lymphocytes and suggests that our findings are not due to undetected prevalent cases of heart failure. The approach of ascertaining cases through hospital records will tend to result in the detection of more severe cases and thus is a specific approach to finding heart failure but is likely to be relatively insensitive. This limits the generalizability of our conclusions to less severe heart failure. We have no information on the aetiology of the incident heart failure cases. Previous findings suggest that inflammatory markers are stronger associated with diastolic than systolic heart failure, however, even if the WBC count association is only with a subset, this would only attenuate the overall association with heart failure as a general group. Furthermore, we have no information on intercurrent diseases during drawing of blood samples which might affect WBC count. However, participants were recruited for the health examination from the general community as a research study and they would not have attended if they were acutely ill. If they had mild transient intercurrent illnesses such as a cold which might cause a transient increase in WBC count, such random occurrences would be likely only to attenuate any underlying associations. Finally, our observational study cannot provide insight into the underlying mechanism.

In conclusion, inflammation as measured by WBC count was independently associated with the risk of heart failure in apparently healthy middle-aged men but not women. The association observed in men was driven by granulocyte count, but there was an independent inverse association between monocyte count and heart failure risk which warrants further exploration of the underlying mechanism.

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References


