The association of total and differential leukocyte counts with cardiovascular disease, and mortality in UK Biobank.

Claire Welsh PhD*, Paul Welsh PhD*, Patrick B Mark MD, Carlos A Celis-Morales PhD, James Lewsey PhD, Stuart R Gray PhD, Donald M Lyall PhD, Stamatina Iliodromiti MD, Jason MR Gill PhD, Jill Pell MD, Pardeep S Jhund MD†, Naveed Sattar MD†
*Joint first authors
† Joint senior authors

From Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow (PW, CW, PBM, CAC-M, SRG, SI, JMRG, PSJ, NS); Institute of Health and Wellbeing, University of Glasgow, Glasgow (JL, DML, JP).

Running title: Leukocytes and CVD

Corresponding author:
Paul Welsh
BHF Glasgow Cardiovascular Research Centre,
Institute of Cardiovascular and Medical Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8TA, UK.
Tel: +44 (0)141 3302569
E-mail address: paul.welsh@glasgow.ac.uk

Keywords:
Inflammation, cardiovascular disease, white blood cell count, neutrophils, epidemiology

Subject codes:
Cardiovascular Disease; Epidemiology; Risk Factors

Word count:
6435

Figures and Tables:
3 tables 4 figures

TOC category:
Clinical and population studies

TOC subcategory:
Arteriosclerosis, Thrombosis, and Vascular Biology

Abstract

Objective

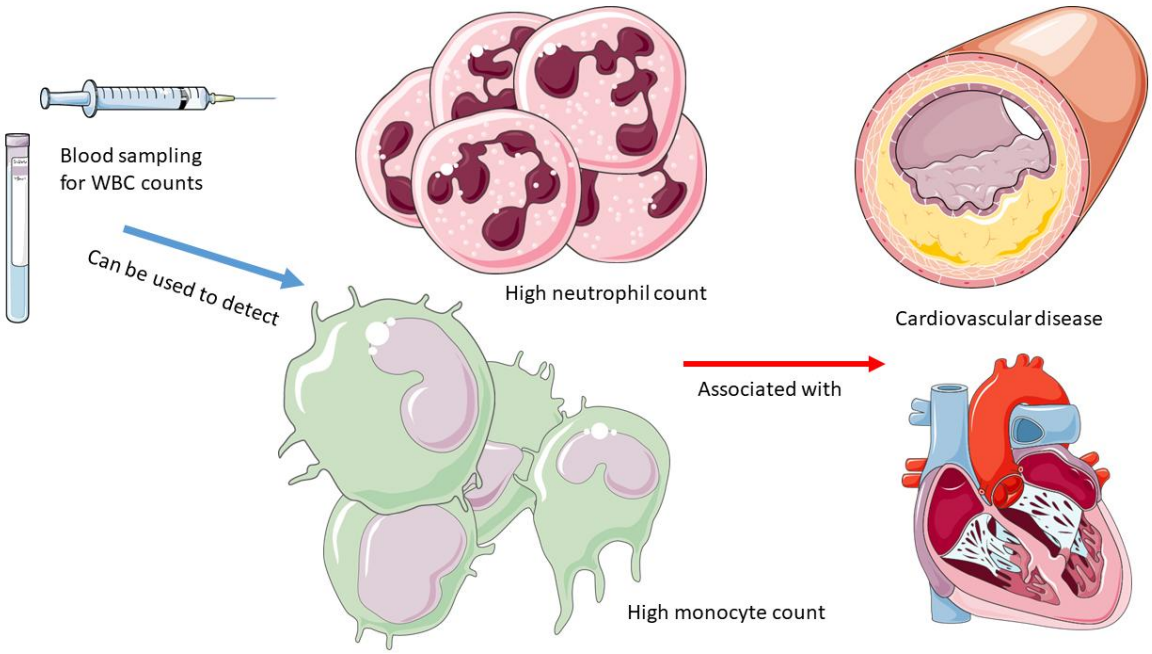
Elevated white blood cell count (WBC) is associated with a higher risk of cardiovascular disease (CVD). We aimed to investigate whether specific leukocyte subpopulations, which may more closely indicate a specific inflammatory pathway, are specifically associated with CVD.

Approach and results

478,259 participants from UK Biobank with data for WBC were included. Death due to CVD (n=1377), and non-CVD causes (n=8987) occurred during median follow up time of 7.0 years (IQR 6.3-7.6). In Cox models deciles of leukocyte counts (lymphocytes, monocytes, neutrophils, eosinophils and basophils) were examined using the 5th decile as the referent group. Models were stratified by sex and adjusted for a range of classical risk factors. A sensitivity analysis excluded participants with baseline comorbidites and the first 2 years of follow-up. Men (HR 1.59; 95%CI 1.22-2.08) and women (HR 2.15; 1.38-3.35) in the highest decile of neutrophil count were at higher risk of CVD mortality and non-fatal CVD (Men HR 1.28; 95% CI 1.16-1.42 and women HR 1.21; 95% CI 1.06-1.38). In the sensitivity analysis the power to investigate CVD mortality was limited, but for both sexes combined the linear HRs for a 1*10^{9}/L cell count increase in WBC and neutrophils respectively was 1.05 (1.03-1.07) and 1.07 (1.04-1.11).

Conclusions

Among circulating leukocyte subpopulations, neutrophil count in men was most consistently associated with fatal and non-fatal CVD. Further studies of interventions that lower circulating neutrophils, such as canakinumab, are required to investigate causality.



Abbreviations
CKD: Chronic kidney disease
CVD: Cardiovascular disease
NHS: National health service
SBP systolic blood pressure
WBC: White blood cell count

Introduction

Inflammation may be a key, potentially modifiable, process in the development of cardiovascular disease (CVD) [1,2]. Elevated levels of inflammatory mediators within healthy people may increase the risk of CVD. Many studies have reported that a range of different blood-based inflammatory biomarkers are associated with an increased risk of incident CVD events [3–7]. This initially led to an interest in reducing the risk of CVD by targeting patients with evidence of inflammation as illustrated by high sensitivity C-reactive protein [8]. More recently, with genetic evidence supporting interleukin-6 pathway as causal in the development of CVD [9], interest has emerged in directly inhibiting specific parts of the inflammatory pathway to prevent CVD [10,11].

One of the simplest, and most commonly measured markers of the immune response and inflammation, is the total white blood cell (WBC) count. A number of studies have reported that an elevated WBC is associated with higher rates of incident CVD [12–15] and non-CVD mortality [13,16]. The association between elevated WBC and elevated CVD risk may indicate that infection and inflammation are part of the pathway leading to the development of CVD. However, WBC may also reflect poor health and risk of death from any cause, and therefore be a non-specific association.

Different subpopulations of WBC (lymphocytes, neutrophils, monocytes, eosinophils and basophils) may also be associated with CVD and non-CVD mortality [14,17,18] . In particular, elevated neutrophil counts may be associated with a higher risk of CVD in routine population datasets [18], although the influence of clinical susceptibility bias is difficult to account for in such databases, and the influence of reverse causality requires consideration [19]. An additional consideration is that different associations in specific cell types may be suggestive of distinct pathways that lead to CVD. While neutrophils (granulocytes that comprise the majority of circulating WBCs) can cause tissue damage, including in the walls of blood vessels via the formation of neutrophil extracellular traps (NETs) [20] which in turn lead to macrophage-produced interleukin precursors [21], other white cell types such as monocytes (precursors of tissue macrophages) may also be important [22].

To explore these associations further we examined the association between WBC and the differential leukocyte counts with all-cause mortality, CVD mortality, and non-CVD mortality in the UK Biobank population. This is a large population-based cohort study with > 500,000 participants that have no clinical indication for WBC measurement. Simultaneous comparison of differential counts with both CVD and non-CVD mortality allows specificity of the associations to be investigated. To restrict the potential for reverse casualty - i.e. whereby pre-existing and subclinical illness might cause changes in leukocyte counts - we also examined these associations in a sensitivity analysis.

Materials and Methods

UK Biobank recruited 502,655 participants (aged 37 to 73) from 22 assessment centres across the UK between April 2007 and December 2010 [23]. Baseline biological measurements were recorded and touch-screen questionnaires were administered, as described elsewhere [1,2]. UK Biobank received ethical approval from the North West Multi-centre Research Ethics Committee (REC reference: 11/NW/03820). All participants gave written informed consent before enrolment in the study, which was conducted in accord with the principles of the Declaration of Helsinki.

Smoking status was categorised into never, former or current smoking. Ethnicity was coded as white, South Asian, black, or mixed/other, with white as the referent group. Body mass index (BMI) was calculated as (weight (kg) /height^2 (m)). Area-based socioeconomic status was derived from postcode of residence, using the Townsend score [3]. White blood cell counts were measured on fresh samples as an absolute number per unit volume, and their

component leukocytes (lymphocytes, monocytes, neutrophils, eosinophils, and basophils) as absolute measures and proportions of the overall WBC; all using an automated, clinically validated, Coulter LH 750. Calibration and quality control were performed in line with the manufacturer's recommendations. Further details of these measurements can be found in the UK Biobank online showcase and protocol (http://www.ukbiobank.ac.uk).

Specific baseline comorbidities of interest were self-reported CVD (myocardial infraction, angina, stroke or transient ischaemic attack), diabetes, rheumatoid arthritis, chronic kidney disease (CKD), and atrial fibrillation or flutter. A category of "other baseline comorbidities" potentially associated with inflammation was defined as any of self-reported baseline diagnosis of; peripheral vascular disease, heart failure, any malignancy, dementia, Parkinson's disease, psoriasis or eczema, osteoporosis, polyarthropathies and systemic connective tissue disorders (other than rheumatoid arthritis), multiple sclerosis, chronic fatigue syndrome, chronic liver disease, endometriosis, polycystic ovarian syndrome, diverticular disease of the intestine, hypertension, depression, painful conditions, asthma, treated dyspepsia, thyroid disorders, chronic obstructive pulmonary disorder, inflammatory bowel disease or irritable bowel syndrome, alcohol problems, other psychoactive substance abuse, treated constipation, prostate disorders, glaucoma, epilepsy, migraines, chronic sinusitis, anorexia or bulimia, anxiety and other neurotic or stress related disorders, schizophrenia, viral hepatitis, bronchiectasis, Ménière's disease, and pernicious anaemia.

Date and cause of death were obtained from death certificates held by the National Health Service (NHS) Information Centre for participants from England and Wales and the NHS Central Register Scotland for participants from Scotland. There were three outcomes of interest in the current study; all-cause mortality, death related to CVD (primary cause of death including ICD-10 codes I20-I23, I24.1, I25.2 and I60-I64) and death unrelated to CVD (any ICD-10 codes excluding I00-I99). We also examined the association between WBC and non-fatal CVD outcomes. Non-fatal outcomes (codes I20-I23, I24.1, I25.2 and I60-I64) were ascertained from hospitalisation records using record linkage to national hospitalisation data. End of follow-up for the main study for each participant was recorded as the date of death or the date of end of follow-up for the assessment centre attended (30/11/2015 for Scottish centres, 31/01/2016 for English or Welsh centres), whichever came first. End of follow-up for the nonfatal events analysis was the date of admission of the first CVD hospitalisation, or the date of end of follow-up for the assessment centre attended, or the date of death, whichever came first. The period at risk per participant began on the date of their assessment. Participants who were hospitalised within 30 days of their assessment were excluded.

Statistical analyses

The distribution of classical risk factors (age, sex, systolic blood pressure (SBP), BMI, deprivation score, smoking, ethnicity, baseline CVD, baseline diabetes, family history of CVD (mother, father or sibling), rheumatoid arthritis, CKD (stage 4 or 5), atrial fibrillation, and other baseline illness), and leukocyte subpopulations, were investigated by decile of WBCs. We examined the relationship between WBC and its constituents and the outcomes using restricted cubic splines of each cell count restricted to values of their mean +/- 4 standard deviations, and found that the deciles adequately modelled the data (Figs sVII-sXXIV). We present the primary results on the basis of deciles. Classical risk factors were expressed as mean (standard deviation) if symmetrically distributed, median (interquartile range) if skewed, and number (%) if categorical. Tests for trends across WBC deciles were performed using regression, a Wilcoxon test for trend, or a chi-squared test, respectively. Associations between classical risk factors, WBC, and leukocyte subpopulations with mortality outcomes were also tabulated using these methods. Correlations between WBC and its components were tested by Spearman correlation coefficients.

Associations of leukocytes with all-cause mortality, CVD mortality, and non-CVD mortality were investigated using Cox-proportional hazard models. WBC and leukocyte counts at baseline were entered into the model as absolute counts, by sex-specific deciles. The fifth decile was used as the referent group. The adjusted model included continuous terms for age, deprivation index, systolic blood pressure and BMI and categorical variables for smoking, ethnicity, diabetes, family history of CVD, rheumatoid arthritis, atrial fibrillation, baseline CVD, and the binary composite variable for other baseline comorbidities (defined above). The results were reported as sex-specific hazard ratios for deciles of the leukocytes, together with 95% confidence intervals. We also investigated associations of leukocytes with outcomes using the exposure as a linear variable where this was an appropriate model fit. To examine the potential role of reverse causality, a sensitivity analysis was performed that excluded those with any baseline comorbidities (diabetes, rheumatoid arthritis, atrial fibrillation, baseline CVD, and other baseline illness) and, the first 2 years of follow-up. The results from this analysis had lower power; so only linear models combining sexes are presented. A further sensitivity analysis was conducted using a Fine and Gray model to adjust CVD mortality for the competing risk of non-CVD mortality (and vice-versa) [4]. These models did not meaningfully change hazard ratios, and so the more complex competing risk model was not utilised. We tested the interaction between smoking status and WBC and its components for CV mortality and found no significant interactions after adjusting for multiple testing.

All analyses were performed using STATA 14 (StataCorp LP). A p-value of <0.05 was considered statistically significant.

Results

Cross sectional associations

Of 502,634 people included in the study, WBC data was available in 478,279 (95.2%), lymphocytes, monocytes, neutrophils, eosinophils and basophils in 477,401 (95.0%). There were 258,966 women and 219,313 men with WBC measured.

Participants with higher WBC count were generally older, had higher systolic BP, higher BMI and were more likely to be a smoker, have a family history of CVD, and have baseline rheumatoid arthritis, chronic kidney disease, diabetes, CVD, atrial fibrillation, or other comorbidities (Table 1). A higher proportion of South Asians and a lower proportion of black ethnicities were observed in high WBC deciles. Deprivation scores were higher in the extreme deciles of WBC, particularly the lowest decile. The proportion of cells that are components of WBC varied by WBC count; proportions of neutrophils increased substantially as deciles of WBC increased, proportions of lymphocytes and monocytes fell, whereas proportions of eosinophils and basophils remained broadly similar.

In correlations of leukocyte counts with each other (Table 2), higher WBC counts were driven by higher absolute numbers of every differential leukocyte count, with the correlation being particularly high for neutrophils (r=0.90). Only neutrophil percentage was positively associated with WBC, whereas every other leukocyte percentages had an inverse association with WBCs. Percentage of neutrophils was strongly inversely associated with percentage of lymphocytes (r=-0.92).

Univariable association of leukocytes with mortality

Among participants with a WBC measurement, median follow up time for all cause mortality was 7.0 years (IQR 6.3-7.6). All cause mortality occurred in 5255 women (2.0%) and 8227 men (3.8%) in the full analysis, and in 753 women (0.9%) and 1146 (1.5%) men in the sensitivity analysis. CVD mortality occurred in 428 women (0.2%) and 949 men (0.4%) in the full analysis, and in 50 women (0.1%) and 107 (0.1%) men in the sensitivity analysis. Non-

CVD mortality occurred in 3982 women (1.5%) and 5005 men (2.3%) in the in the full analysis, and in 626 women (0.7%) and 814 (1.1%) men in the sensitivity analysis.

Death from any cause as well as CVD causes was generally associated with a more adverse clinical risk profile (Table 3), including older age, male sex, higher SBP, higher BMI, smoking, baseline CVD, diabetes, rheumatoid arthritis, CKD, atrial fibrillation and other baseline illness. The group who died from CVD or non-CVD causes during follow-up generally also had a lower lymphocyte count and lymphocyte proportion at baseline. In contrast, those who died from CVD or non-CVD causes during follow-up generally had slightly higher monocyte count and proportion of monocytes, and a substantially higher neutrophil count and proportion of neutrophils. The group who died from CVD or non-CVD causes also had a slightly higher eosinophil and basophil counts.

Multivariable association of leukocytes with all cause mortality
In adjusted Cox models, total WBC, neutrophils, basophils and monocytes showed generally J-shaped associations with all cause mortality in both sexes (Fig A-D). However, in the sensitivity analysis, these associations were generally attenuated to approximately more linear forms. For both sexes combined the linear HRs for a $1*10^9$/L cell count increase in WBC and neutrophils respectively was 1.06 (1.05-1.07) and 1.10 (1.07-1.12) respectively. Data were similar excluding smokers. For lymphocytes, those with low levels tended to be at far higher risk of all cause mortality than those with elevated levels (Fig D). The sensitivity analysis attenuated this towards the null (Table II).

Multivariable association of leukocytes with CVD mortality
Men in the highest decile of WBC were at greater risk of CVD mortality compared to those in the fifth decile (HR 1.64; 95% CI 1.24-2.16). Both men (HR 1.59; 1.22-2.08) and women (HR 2.15; 95% CI 1.38-3.35) in the highest decile of neutrophil count were at greater risk of CVD mortality. Monocyte count was also associated with CVD mortality in men (HR 1.57; 95% CI 1.26-1.97). For both sexes combined the linear HRs for a $1*10^9$/L cell count increase in WBC and neutrophils respectively was 1.04 (0.97-1.11) and 1.05 (0.94-1.17) respectively.

Multivariable association of leukocytes with non-CVD mortality
U-shaped associations were found between deciles of monocytes, neutrophils, WBC and basophils with non-CVD mortality. For WBCs and neutrophils, these associations were attenuated in the sensitivity analysis. For both sexes combined the linear HRs for a $1*10^9$/L cell count increase in WBC and neutrophils respectively was 1.06 (1.04-1.07) and 1.10 (1.071.13) respectively.

Multivariable association of leukocytes with nonfatal CVD
Associations between leukocyte deciles and non-fatal CVD were similar to, but generally less strong than, those with CVD mortality. Men (HR 1.28; 95% CI 1.16-1.42) and women (HR 1.21; 95% CI 1.06-1.38) in the highest decile of WBC were at greater risk of non-fatal CVD compared to those in the fifth decile (Table V). This trend was also true of neutrophils, and of monocytes among men. For both sexes combined the linear HRs for a $1*10^9$/L cell count increase in WBC and neutrophils respectively was 1.05 (1.03-1.07) and 1.07 (1.04-1.11) respectively. Most associations were removed in the sensitivity analysis, with the exception of high monocytes in women and low basophils in both sexes, which retained a higher risk of nonfatal CVD events.

Discussion
In this large, prospectively enrolled cohort of over 475,000 individuals from a general population, we found that a high WBC is associated with both CVD and non-CVD mortality, consistent with prior studies [12–15]. In addition we report that the leukocyte subpopulations are

differentially associated with CVD mortality and non-fatal CVD. Specifically we found that higher counts of neutrophils are associated with a higher risk of CVD mortality and non-fatal CVD, in line with previous data [14,17,18]. Our data emphasise the importance of reverse causality in influencing the association between differential blood counts and outcomes. Therefore, additionally, we have shown that the association of neutrophils with non-fatal CVD remains robust to a sensitivity analysis that attempts to limit the influence of reverse causality. We could not confirm this for fatal CVD, primarily due to lack of power. The distinct association of neutrophils with CVD is of particular interest given that inhibiting the IL-1β pathway with canakinumab substantially reduces circulating neutrophil counts [23,24] and has been shown to prevent CVD[25].

Our a priori hypothesis was that a specific leukocyte population might be more specifically associated with CVD mortality, in line with observations that neutrophils, macrophages, or lymphocytes might play a specific role in the immune response that causes, and results from, atherosclerosis [19,21,29-31]. In contrast to prior studies we simultaneously examined not only the association between WBC and mortality outcomes, but also subpopulations of leukocytes. Recent data from a large cohort study has shown that WBC was associated with CHD and cancer mortality among healthy women, even after excluding those with comorbidities (cardiovascular diseases, connective tissue disease, ulcerative colitis, liver disease, diabetes, or cancers) and early deaths during follow-up [13]. The association of WBC with CVD mortality among women in UK Biobank was fairly weak, but the association of neutrophils with CVD we report was strong in both sexes, and is also similar to a recent large cohort study [18]. Our sensitivity analysis attenuated associations of circulating leukocyte counts with mortality outcomes. This suggests an element of reverse causality [19]. Mechanisms that explain this observation likely include the association between neutrophilia and trauma, stress, bacterial infection, smoking, and indolent cancer [26]; any of which ultimately might increase the risk of death from any cause. Likewise, lymphopenia is likely to be caused by old age, viral infection, autoimmune disease, renal failure and immunosuppressive drugs [27]. By excluding those with a range of baseline diseases, and those who develop disease within 2 years of baseline, the present results provide models that limit the effect of baseline disease on counts themselves.

What mechanisms might explain the association of leukocytes with CVD mortality? The inflammatory hypothesis of CVD is well established [28]. Underpinning this, cellular atherosclerotic plaques appear more prone to rupture [32]. It is hypothesised that leukocytes may play a direct role in destabilising the plaque itself [34, 33] through fibrous cap thinning, although it may be that circulating levels of leukocytes do not necessarily reflect resident cells within atherosclerotic plaques. Investigating specific differential counts, our data suggest that high circulating neutrophil levels are linearly associated with CVD mortality that occurs beyond a 2 year time horizon in ostensibly healthy people. The same does not appear to be true of other leukocytes. It is interesting that, until recently, neutrophils were a neglected leukocyte in atherosclerosis research. There are clear mechanisms that might explain why neutrophils might play an important role in causing CVD death [35]. Neutrophils interact with cholesterol crystals to produce pro- IL-1β [20]. They can form structures that bind bacteria and platelets, the end result of which is that neutrophils release nuclear material leading to cell death and thrombosis [37]. Neutrophils are, therefore, intimately linked to the inflammatory cascade, which can be promoted through a pro-atherogenic environment. Other environmental factors, such as smoking [36], might also promote neutrophilia and CVD death, resulting in confounding. However, our sensitivity analysis suggests there may be an independent association too. Inhibiting IL-1β in patients with established CVD reduces cardiovascular events [10,25], and strongly reduces circulating neutrophil counts [29,30]. Our findings suggest that further work should be done in patients without pre-existing CVD to

determine if IL-1β blockers, or other modulators of the immune response to inflammation, may have a role to play in the prevention of CVD, and the role of neutrophils in this context.

Higher neutrophil counts were also associated with a higher risk of non-CVD death. Here the association may be even more complex. Specific non-CVD conditions may be linked to higher neutrophil counts through different pathways. For instance, there is evidence that people with chronic inflammatory conditions such as RA have increased risk of death beyond that cause by CVD (http://onlinelibrary.wiley.com/doi/10.1002/acr.22752/abstract). There has also been speculation that tumor-infiltrating neutrophils may be important in the prognosis of several types of cancer (https://www.sciencedirect.com/science/article/pii/S1044579X13000138). As noted above, mechanisms that may underlie the associations we observe include the influence of indolent malignancy (both solid organ and haematological) and other confounders such as inflammatory diseases and smoking. While we corrected for as many of these as possible through multivariable analysis and sensitivity analysis there remains a significant element of confounding and reverse causality. More work is required to examine the association between neutrophils and specific non-CVD causes of death to determine potential mechanisms underlying our findings.

Our study has a number of strengths. The UK Biobank includes a wide sample of the UK general population in terms of age, sex, ethnicity and socioeconomic status, and as such is not derived from health records of patients with a clinical indication for leucocyte measurement, in contrast to prior work [18]. Further strengths of the study include its large sample size, comprehensive phenotyping, and simultaneous consideration of differential leukocyte counts derived from a central laboratory measurement, also in contrast to prior work [18]. The side-by-side comparison of CVD and non-CVD mortality gives an indication of the specificity of associations, although we did not further subdivide non-CVD morality due to lack of adjudicated outcomes. It is notable that inflammatory markers appear to have a stronger association with CVD mortality than with non-fatal events [38,39]. Weaknesses include the lack of data to adjust for lipids, although the extensive adjustment model will capture much of this confounding by proxy. Lack of adjustment for medication is a further limitation, although again adjustment for co-morbidity accounts for much of the association. Reverse causality is possible in any observational study, and whilst our results include a sensitivity analysis and an analysis excluding those with diagnosed co-morbidities, we cannot exclude the potential of further reverse causality explaining some of the associations that we report.

In conclusion, we report that among leukocytes higher neutrophil count is particularly associated with higher CVD risk in a general population. This association appears to be robust in those without comorbidity at baseline or in those who may have unrecognised disease at baseline. Further work is required to determine if modulation of the immune responses that involve neutrophils, via canakinumab or other interventions, can lead to a reduction in CVD mortality.

Acknowledgments
This research was conducted using the UK Biobank resource. We thank the participants of the UK Biobank. The work was performed under UK biobank project number 9310. The graphical abstract was designed adapting images from Servier Medical Art by Servier (https://smart.servier.com/) under Creative Commons Attribution 3.0 Unported License

Sources of funding
The work in this study was supported by a grant from Chest, Heart, and Stroke Association Scotland [Res16/A165].

Disclosures

None

References

1. Welsh P, Grassia G, Botha S, Sattar N, Maffia P. Targeting inflammation to reduce cardiovascular disease risk: a realistic clinical prospect? *Br J Pharmacol* 2017;174:3898-3913
2. Bäck M, Hansson GK. Anti-inflammatory therapies for atherosclerosis. *Nat Rev Cardiol*. 2015;12:199–211.
3. Kaptoge S, Di Angelantonio E, Pennells L, Wood AM, White IR, Gao P, et al. C-reactive protein, fibrinogen, and cardiovascular disease prediction. *N Engl J Med*. 2012;367:1310–1320.
4. Welsh P, Woodward M, Rumley A, Lowe G. Associations of plasma pro-inflammatory cytokines, fibrinogen, viscosity and C-reactive protein with cardiovascular risk factors and social deprivation: the fourth Glasgow MONICA study. *Br J Haematol*. 2008;141:852–61.
5. Welsh P, Murray HM, Ford I, Trompet S, de Craen AJM, Jukema JW, et al. Circulating interleukin-10 and risk of cardiovascular events: a prospective study in the elderly at risk. *Arterioscler Thromb Vasc Biol*. 2011;31:2338–44.
6. Woodward M, Rumley A, Welsh P, Macmahon S, Lowe G. A comparison of the associations between seven hemostatic or inflammatory variables and coronary heart disease. *J Thromb Haemost*. 2007;5:1795–800.
7. Danesh J, Kaptoge S, Mann AG, Sarwar N, Wood A, Angleman SB, et al. Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review. *PLoS Med*. 2008;5:e78.
8. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto Jr. AM, Kastelein JJ, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*. 2008;359:2195–2207.
9. Sarwar N, Butterworth AS, Freitag DF, Gregson J, Willeit P, Gorman DN, et al. Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. *Lancet*. 2012;379:1205–13.
10. Ridker PM, Thuren T, Zalewski A, Libby P. Interleukin-1β inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). *Am Heart J*. 2011;162:597–605.
11. Ridker PM, Howard CP, Walter V, Everett B, Libby P, Hensen J, Thuren T. Effects of Interleukin-1 Inhibition With Canakinumab on Hemoglobin A1c, Lipids, C-Reactive Protein, Interleukin-6, and Fibrinogen: A Phase IIb Randomized, Placebo-Controlled Trial. *Circulation*. 2012;126:2739–2748.
12. ó Hartaigh B, Bosch JA, Thomas GN, Lord JM, Pilz S, Loerbroks A, et al. Which leukocyte subsets predict cardiovascular mortality? From the LUdwigshafen RIsk and Cardiovascular Health (LURIC) Study. *Atherosclerosis*. 2012;224:161–169.
13. Kabat GC, Kim MY, Manson JE, Lessin L, Lin J, Wassertheil-Smoller S, Rohan TE. White Blood Cell Count and Total and Cause-Specific Mortality in the Women's Health Initiative. *Am J Epidemiol*. 2017;186:63–72.
14. Madjid M, Fatemi O. Components of the complete blood count as risk predictors for coronary heart disease: in-depth review and update. *Texas Heart Inst J*. 2013;40:17–29.
15. Horne BD, Anderson JL, John JM, Weaver A, Bair TL, Jensen KR, et al. Which white blood cell subtypes predict increased cardiovascular risk? *J Am Coll Cardiol*. 2005;45:1638–1643.
16. Patterson CC, Blankenberg S, Ben-Shlomo Y, Heslop L, Bayer A, Lowe G, et al. Which biomarkers are predictive specifically for cardiovascular or for non-cardiovascular mortality in men? Evidence from the Caerphilly Prospective Study (CaPS). *Int J Cardiol*. 2015;201:113–118.

17. Wheeler JG, Mussolino ME, Gillum RF, Danesh J. Associations between differential leucocyte count and incident coronary heart disease: 1764 Incident cases from seven prospective studies of 30 374 individuals. *Eur Heart J*. 2004;25:1287–1292.
18. Shah AD, Denaxas S, Nicholas O, Hingorani AD, Hemingway H. Neutrophil Counts and Initial Presentation of 12 Cardiovascular Diseases: A CALIBER Cohort Study. *J Am Coll Cardiol*. 2017;69:1160–1169.
19. Sattar N, Preiss D. Reverse Causality in Cardiovascular Epidemiological Research: More Common Than Imagined? *Circulation*. 2017;135:2369–2372.
20. Döring Y, Soehnlein O, Weber C. Neutrophil Extracellular Traps in Atherosclerosis and Atherothrombosis. *Circ Res*. 2017;120:736–743.
21. Warnatsch A, Ioannou M, Wang Q, Papayannopoulos V. Inflammation. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. *Science*. 2015;349:316–20.
22. Decano JL, Mattson PC, Aikawa M. Macrophages in Vascular Inflammation: Origins and Functions. *Curr Atheroscler Rep*. 2016;18:34.
23. Torene R, Nirmala N, Obici L, Cattalini M, Tormey V, Caorsi R, et al. Canakinumab reverses overexpression of inflammatory response genes in tumour necrosis factor receptor-associated periodic syndrome. *Ann Rheum Dis*. 2016;76:303–309.
24. Ruperto N, Brunner HI, Quartier P, Constantin T, Wulffraat N, Horneff G, et al. Two Randomized Trials of Canakinumab in Systemic Juvenile Idiopathic Arthritis. *N Engl J Med*. 2012;367:2396–2406.
25. Ridker P, Everett BM, Thuren T et al. Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *N Engl J Med.* 2017;377:1119-1131.
26. George TI. Malignant or benign leukocytosis. Hematol Am Soc Hematol Educ Progr. 2012;2012:475–84.
27. Brass D, Mckay P, Scott F. Investigating an incidental finding of lymphopenia. *BMJ*. 2014;348:g1721–g1721.
28. Libby P, Nahrendorf M, Swirski FK. Leukocytes Link Local and Systemic Inflammation in Ischemic Cardiovascular Disease: An Expanded "Cardiovascular Continuum". *J Am Col Cardiol.* 2016; 67(9):1091-103.
29. Taleb S. Inflammation in atherosclerosis. *Arch Cardiovasc Dis*. 2016;109:708–715.
30. Gisterå A, Hansson GK. The immunology of atherosclerosis. *Nat Rev Nephrol*. 2017;13:368–380.
31. Hughes CE, Benson RA, Bedaj M, Maffia P. Antigen-Presenting Cells and Antigen Presentation in Tertiary Lymphoid Organs. *Front Immunol*. 2016;7:481.
32. Lafont A. Basic aspects of plaque vulnerability. *Heart*. 2003;89:1262–7.
33. Seneviratne A, Hulsmans M, Holvoet P, Monaco C. Biomechanical factors and macrophages in plaque stability. *Card Res.* 2013;2: 284-293.
34. Carbone F, Mach F, Montecucco F. Update on the role of neutrophils in atherosclerotic plaque vulnerability. *Curr Drug Targets.* 2015; 16(4): 312-33.
35. Gaul DS, Stein S, Matter CM. Neutrophils in cardiovascular disease. *Eur Heart J.* 2017; 38: 1702-1704.
36. Higuchi T, Omata F, Tsushihachi K, Higashioka K, Koyamada R, Okada S. Current cigarette smoking is a reversible cause of elevated white blood cell count: Cross-sectional and longitudinal studies. *Prev Med Rep*. 2016;417-422.
37. Nahrendorf M, Swirski FK. Immunology. Neutrophil-macrophage communication in inflammation and atherosclerosis. *Science*. 2015;349:237–8.
38. Sattar N, Murray HM, Welsh P, Blauw GJ, Buckley BM, Cobbe S, et al. Are markers of inflammation more strongly associated with risk for fatal than for nonfatal vascular events? *PLoS Med*. 2009;6:e1000099.
39. Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, Danesh J. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet*. 2010;375:132–140.

Highlights

- Among circulating leukocyte subpopulations, neutrophil count was most strongly, and linearly, associated with higher CVD mortality.
- Data were robust to a sensitivity analysis excluding those with pre-existing comorbidities and the first 2 years of follow-up.
- Circulating lymphocytes showed trends towards an inverse association with CVD mortality.
- Leukocyte subpopulations also showed similar associations with non-CVD mortality

Figure Legends

Figure A

Association of deciles of WBC with three mortality outcomes (**A** all-cause mortality, B fatal CVD, **C** non-CVD mortality) in all study participants, stratified by sex (blue= male, red=female).

Figure B

Association of deciles of neutrophil count with three mortality outcomes in all study participants (**A** all-cause mortality, **B** fatal CVD, **C** non-CVD mortality), stratified by sex (blue= male, red=female).

Figure C

Association of deciles of monocyte count in all study participants with three mortality outcomes (**A** all-cause mortality, **B** fatal CVD, **C** non-CVD mortality), stratified by sex (blue= male, red=female).

Figure D

Association of deciles of lymphocyte count in all study participants with three mortality outcomes (**A** all-cause mortality, **B** fatal CVD, **C** non-CVD mortality), stratified by sex (blue= male, red=female).

Table 1 Association of WBC count (by deciles) with classical risk factors

	≤4.87 (10^9/L)	4.87-5.41 (10^9/L)	5.41-5.88 (10^9/L)	5.88-6.29 (10^9/L)	6.29-6.69 (10^9/L)	6.69-7.10 (10^9/L)	7.10-7.60 (10^9/L)	7.60-8.20 (10^9/L)	8.20-9.18 (10^9/L)	≥9.18 (10^9/L)	P-value for trend
	n=48057	n=49251	n=46659	n=47508	n=47873	n=51802	n=44149	n=49550	n=45775	n=47635	
Age (years)	55.5 ± 7.9	56.2 ± 7.9	56.5 ± 8.0	56.7 ± 8.0	56.7 ± 8.0	56.9 ± 8.1	56.9 ± 8.1	56.8 ± 8.2	56.8 ± 8.2	56.6 ± 8.3	<0.0001
Male sex	21299 (44.3%)	22316 (45.3%)	21341 (45.7%)	21660 (45.6%)	21936 (45.8%)	23891 (46.1%)	20361 (46.1%)	22813 (46.0%)	21393 (46.7%)	22299 (46.8%)	<0.001
SBP (mmHg)	136.1 ± 19.1	137.8 ± 19.4	138.8 ± 19.5	139.4 ± 19.5	139.9 ± 19.7	140.5 ± 19.6	140.8 ± 19.8	141.3 ± 19.7	141.6 ± 19.8	141.8 ± 19.84	<0.0001
BMI (kg/m^2)	25.8 ± 4.1	26.4 ± 4.1	26.7 ± 4.3	27.0 ± 4.4	27.2 ± 4.5	27.6 ± 4.6	27.8 ± 4.8	28.1 ± 5.0	28.5 ± 5.2	29.0 ± 5.8	<0.0001
Deprivation (score)	-1.4 ± 3.1	-1.5 ± 3.0	-1.5 ± 3.0	-1.5 ± 3.0	-1.5 ± 3.0	-1.4 ± 3.0	-1.3 ± 3.1	-1.3 ± 3.1	-1.1 ± 3.2	-0.6 ± 3.3	<0.0001

Smoking											
Never	30236 (63.0%)	29896 (60.8%)	27647 (59.3%)	27703 (58.4%)	27413 (57.3%)	28737 (55.5%)	23886 (54.1%)	25599 (51.7%)	21999 (48.1%)	19188 (40.3%)	<0.0001
Previous	15810 (32.9%)	16980 (34.5%)	16406 (35.2%)	16785 (35.4%)	17003 (35.5%)	18492 (35.7%)	15792 (35.8%)	17635 (35.6%)	15789 (34.5%)	14607 (30.7%)	
Current	1962 (4.1%)	2331 (4.7%)	2550 (5.5%)	2966 (6.3%)	3418 (7.1%)	4509 (8.7%)	4437 (10.1%)	6257 (12.6%)	7937 (17.4%)	13792 (29.0%)	
Ethnicity											
White	42762 (89.4%)	45109 (92.0%)	42803 (92.2%)	43736 (92.5%)	44339 (93.0%)	47787 (92.7%)	40657 (92.5%)	45635 (92.5%)	41976 (92.1%)	43503 (91.8%)	<0.0001
South Asian	344 (0.7%)	491 (1.0%)	568 (1.2%)	657 (1.4%)	676 (1.4%)	871 (1.7%)	811 (1.8%)	972 (2.0%)	1014 (2.2%)	1090 (2.3%)	

	Black	2291 (4.8%)	1148 (2.3%)	833 (1.8%)	727 (1.5%)	518 (1.1%)	517 (1.0%)	391 (0.9%)	354 (0.7%)	322 (0.7%)	302 (0.6%)	
	Other	2421 (5.1%)	2302 (4.7%)	2216 (4.8%)	2150 (4.5%)	2132 (4.5%)	2382 (4.6%)	2105 (4.8%)	2363 (4.8%)	2260 (5.0%)	2490 (5.3%)	
Baseline CVD		1602 (3.3%)	1947 (4.0%)	2168 (4.6%)	2288 (4.8%)	2620 (5.5%)	3154 (6.1%)	2854 (6.5%)	3475 (7.0%)	3736 (8.2%)	4667 (9.8%)	<0.0001
Baseline diabetes		1355 (2.8%)	1439 (2.9%)	1586 (3.4%)	1810 (3.8%)	2071 (4.3%)	2571 (5.0%)	2463 (5.6%)	3138 (6.3%)	3594 (7.9%)	4951 (10.4%)	<0.0001
Family history of CVD		25871 (53.8%)	27311 (55.5%)	26058 (55.8%)	26961 (56.8%)	27115 (56.6%)	29289 (56.5%)	25119 (56.9%)	28398 (57.3%)	26108 (57.0%)	27249 (57.2%)	<0.0001
Rheumatoid arthritis		499 (1.0%)	473 (1.0%)	479 (1.0%)	538 (1.1%)	470 (1.0%)	492 (0.9%)	469 (1.1%)	580 (1.2%)	574 (1.3%)	778 (1.6%)	<0.0001
Baseline CKD		53 (0.1%)	56 (0.1%)	63 (0.1%)	53 (0.1%)	73 (0.2%)	88 (0.2%)	77 (0.2%)	93 (0.2%)	101 (0.2%)	163 (0.3%)	<0.0001

Atrial fibrillation	260 (0.5%)	313 (0.6%)	282 (0.6%)	328 (0.7%)	355 (0.7%)	413 (0.8%)	350 (0.8%)	398 (0.8%)	414 (0.9%)	449 (0.9%)	<0.0001
Other baseline illness	27858 (58.0%)	29319 (59.5%)	28548 (61.2%)	29514 (62.1%)	30219 (63.1%)	33581 (64.8%)	29096 (65.9%)	33222 (67.0%)	31629 (69.1%)	34511 (72.4%)	<0.0001
Lymphocyte Count	1.4 [1.1, 1.6]	1.6 [1.3, 1.8]	1.7 [1.4, 2.0]	1.8 [1.5, 2.1]	1.9 [1.6, 2.3]	2.0 [1.6, 2.3]	2.0 [1.7, 2.4]	2.1 [1.8, 2.5]	2.3 [1.9, 2.7]	2.5 [2.1, 3.1]	<0.0001
Monocyte Count	0.3 [0.3, 0.4]	0.4 [0.3, 0.5]	0.4 [0.3, 0.5]	0.4 [0.3, 0.5]	0.4 [0.4, 0.5]	0.5 [0.4, 0.6]	0.5 [0.4, 0.6]	0.5 [0.4, 0.6]	0.6 [0.4, 0.7]	0.6 [0.5, 0.8]	<0.0001
Neutrophil Count	2.5 [2.1, 2.8]	3.0 [2.7, 3.3]	3.4 [3.1, 3.6]	3.6 [3.3, 3.9]	3.9 [3.6, 4.3]	4.2 [3.9, 4.6]	4.5 [4.2, 4.9]	4.9 [4.5, 5.4]	5.0 [5.0, 6.0]	6.7 [6.0, 7.5]	<0.0001
Eosinophil Count	0.1 [0.1, 0.1]	0.1 [0.1, 0.2]	0.1 [0.1, 0.2]	0.1 [0.1, 0.2]	0.1 [0.1, 0.2]	0.1 [0.1, 0.2]	0.2 [0.1, 0.2]	0.2 [0.1, 0.3]	0.2 [0.1, 0.3]	0.2 [0.1, 0.3]	<0.0001
Basophil Count	0.0 [0.0, 0.0]	0.0 [0.0, 0.0]	0.0 [0.0, 0.0]	0.0 [0.0, 0.0]	0.0 [0.0, 0.0]	0.0 [0.0, 0.0]	0.0]0.0, 0.1]	0.0]0.0, 0.1]	0.0]0.0, 0.1]	0.0]0.0, 0.1]	<0.0001

Table 2 Correlations (r) of leukocyte variables with each other

	WBC	Lymphocyte count	Lymphocyte %	Monocyte count	Monocyte %	Neutrophil count	Neutrophil %	Eosinophil count	Eosinophil %	Basophil count
Lymphocyte count	0.561	-	-	-	-	-	-	-	-	-
Lymphocyte %	-0.252	0.604	-	-	-	-	-	-	-	-
Monocyte count	0.479	0.307	-0.094	-	-	-	-	-	-	-
Monocyte %	-0.248	-0.115	0.092	0.673	-	-	-	-	-	-
Neutrophil count	0.902	0.222	-0.578	0.328	-0.344	-	-	-	-	-
Neutrophil %	0.294	-0.506	-0.919	-0.107	-0.358	0.647	-	-	-	-
Eosinophil count	0.275	0.228	-0.003	0.255	0.061	0.139	-0.184	-	-	-
Eosinophil %	-0.068	0.030	0.088	0.092	0.160	-0.179	-0.305	0.900	-	-
Basophil count	0.266	0.205	-0.014	0.184	-0.015	0.197	-0.025	0.110	0.022	-
Basophil%	-0.027	0.059	0.088	0.070	0.097	-0.089	-0.161	0.066	0.080	0.665

All r values significant at $p<0.0001$, except correlation of Eosinophil count with lymphocyte % ($p=0.0243$).

Table 3 Association of classical cardiovascular risk factors and leukocyte measures with all cause, cardiovascular and non-cardiovascular death.

	Alive n=464797	Dead n=13482	P-value vs alive	CVD mortality n=1377	P-value vs alive	Non-CVD mortality n=8987	P-value vs alive
Age (years)	56.4 ± 8.1	61.3 ± 6.6	<0.0001	62.2 ± 6.1	<0.0001	61.2 ± 6.7	<0.0001
Male sex	211086 (45.4%)	8227 (61.0%)	<0.0001	949 (68.9%)	<0.0001	5005 (55.7%)	<0.0001
SBP (mmHg)	139.7 ± 19.6	143.9 ± 21.2	<0.0001	147.4 ± 22.7	<0.0001	143.2 ± 20.8	<0.0001
BMI (kg/m^2)	27.4 ± 4.8	28.1 ± 5.4	<0.0001	28.7 ± 5.5	<0.0001	27.6 ± 5.1	<0.0001
Deprivation (score)	-1.3 ± 3.1	-0.7 ± 3.4	<0.0001	-0.3 ± 3.5	<0.0001	-0.9 ± 3.3	<0.0001
Smoking			<0.0001		<0.0001		<0.0001
Non smoker	257211 (55.4%)	5103 (37.9%)		476 (34.6%)		3592 (40.0%)	
Ex-smoker	159641 (34.4%)	5665 (42.1%)		598 (43.5%)		3682 (41.0%)	
Current smoker	47464 (10.2%)	2696 (20.0%)		301 (21.9%)		1701 (19.0%)	
Ethnicity			<0.0001		0.27		<0.0001
White	425732 (92.0%)	12590 (94.0%)		1257 (92.1%)		8446 (94.5%)	
South Asian	7359 (1.6%)	135 (1.0%)		27 (2.0%)		56 (0.6%)	
Black	7296 (1.6%)	107 (0.8%)		14 (1.0%)		68 (0.8%)	
Other	22253 (4.8%)	568 (4.2%)		67 (4.9%)		368 (4.1%)	
Baseline CVD	26228 (5.6%)	2286 (17.0%)		440 (32.0%)	<0.0001	884 (9.8%)	<0.0001
Baseline diabetes	23454 (5.0%)	1786 (13.2%)	<0.0001	276 (20.0%)	<0.0001	855 (9.5%)	<0.0001
Family history of CVD	261530 (56.3%)	7958 (59.0%)	<0.0001	882 (64.1%)	<0.0001	5183 (57.7%)	0.0241
Rheumatoid arthritis	5064 (1.1%)	288 (2.1%)	<0.0001	40 (2.9%)	<0.0001	161 (1.8%)	<0.0001

Baseline CKD	676 (0.1%)	144 (1.1%)	<0.0001	28 (2.0%)	<0.0001	57 (0.6%)	<0.0001
Atrial fibrillation	3335 (0.7%)	227 (1.7%)	<0.0001	40 (2.9%)	<0.0001	83 (0.9%)	0.0429
Other baseline illness	306924 (66.0%)	11275 (83.6%)	<0.0001	1182 (85.8%)	<0.0001	7339 (81.7%)	<0.0001
WBC (10^9/L)	6.6 [5.6, 7.8]	7.1 [5.9, 8.6]	<0.0001	7.4 [6.2, 8.9]	<0.0001	7.0 [5.8, 8.4]	<0.0001
Lymphocyte %	28.6 [24.0, 33.6]	26.0 [20.8, 31.6]	<0.0001	25.7 [20.3, 31.6]	<0.0001	26.6 [21.2, 32.0]	<0.0001
Lymphocytes (10^9/L)	1.9 [1.5, 2.3]	1.8 [1.4, 2.3]	<0.0001	1.8 [1.5, 2.3]	0.071	1.8 [1.4, 2.3]	<0.0001
Monocyte%	6.8 [5.6, 8.2]	7.1 [5.7, 8.7]	<0.0001	7.3 [5.7, 88]	<0.0001	7.0 [5.6, 8.6]	<0.0001
Monocytes (10^9/L)	0.5 [0.4, 0.6]	0.5 [0.4, 0.6]	<0.0001	0.5 [0.4, 0.7]	<0.0001	0.5 [0.4, 0.6]	<0.0001
Neutrophil%	61.1 [55.6, 66.4]	63.2 [57.3, 69.2]	<0.0001	63.5 [56.9, 69.4]	<0.0001	62.9 [57.0, 68.8]	<0.0001
Neutrophils (10^9/L)	4.0 [3.3, 4.9]	4.5 [3.5, 5.6]	<0.0001	4.7 [3.7, 5.8]	<0.0001	4.4 [3.5, 5.4]	<0.0001
Eosinophil%	2.1 [1.4, 3.3]	2.1 [1.3, 3.2]	<0.0001	2.1 [1.3, 3.2]	0.51	2.0 [1.3, 3.1]	<0.0001
Eosinophils (10^9/L)	0.1 [0.1, 0.2]	0.1 [0.1, 0.2]	<0.0001	0.2 [0.1, 0.2]	<0.0001	0.1 [0.1, 0.2]	0.0158
Basophil%	0.4 [0.3, 0.7]	0.4 [0.3, 0.7]	0.0.15	0.4 [0.3, 0.7]	0.69	0.4 [0.3, 0.7]	0.0940
Basophils (10^9/L)	0.0 [0.0, 0.0]	0.0 [0.0, 0.1]	0.005	0.0 [0.0, 0.1]	0.45	0.0 [0.0, 0.1]	<0.0001

Figure A

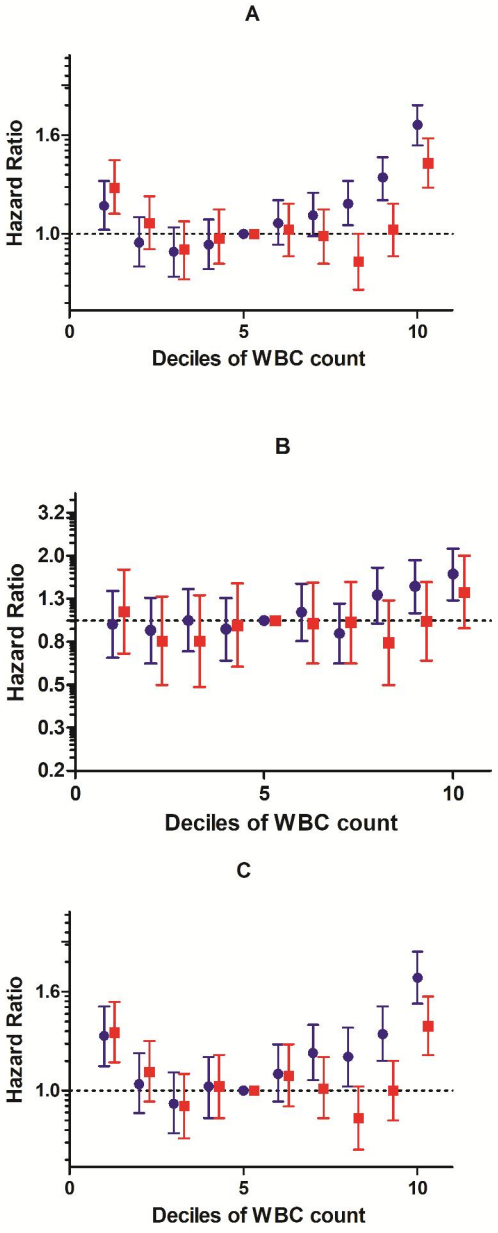

A
Hazard Ratio
1.6
1.0
0
5
10
Deciles of WBC count
B
Hazard Ratio
3.2
2.0
1.3
0.8
0.5
0.3
0.2
0
5
10
Deciles of WBC count
C
Hazard Ratio
1.6
1.0
0
5
10
Deciles of WBC count

Figure B

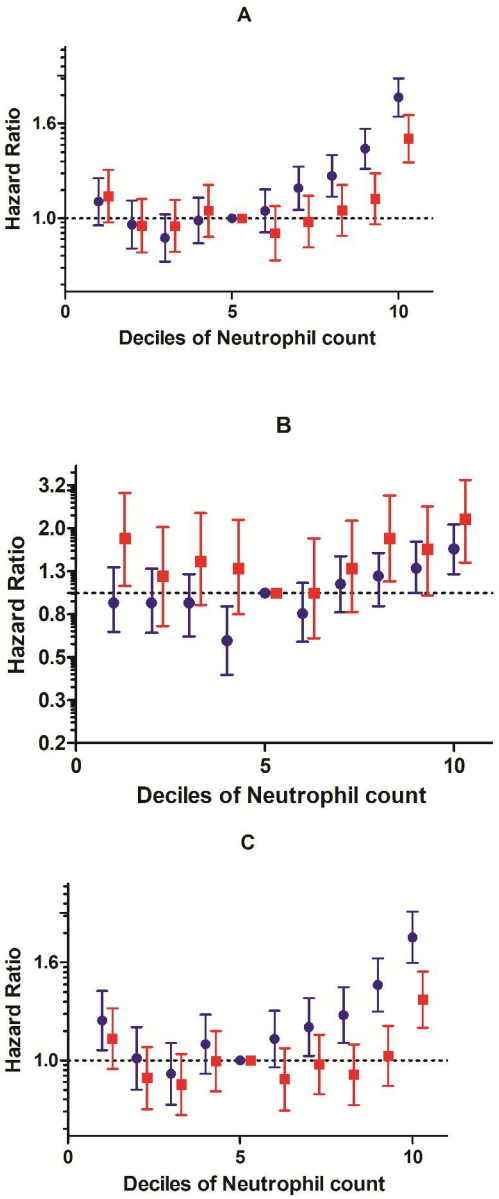

A
Hazard Ratio
1.6
1.0
0
5
10
Deciles of Neutrophil count
B
Hazard Ratio
3.2
2.0
1.3
0.8
0.5
0.3
0.2
0
5
10
Deciles of Neutrophil count
C
Hazard Ratio
1.6
1.0
0
5
10
Deciles of Neutrophil count

Figure C

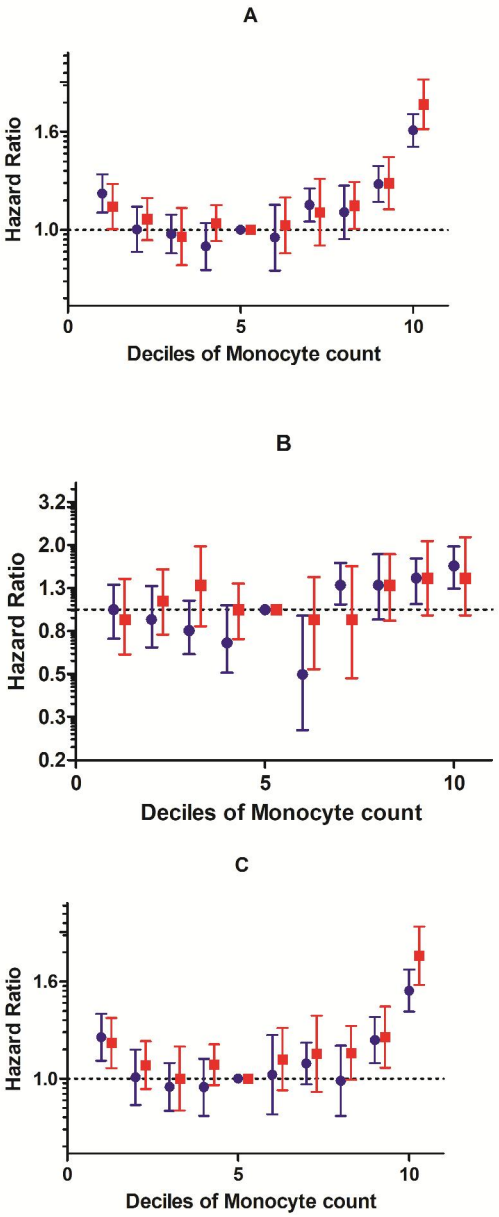
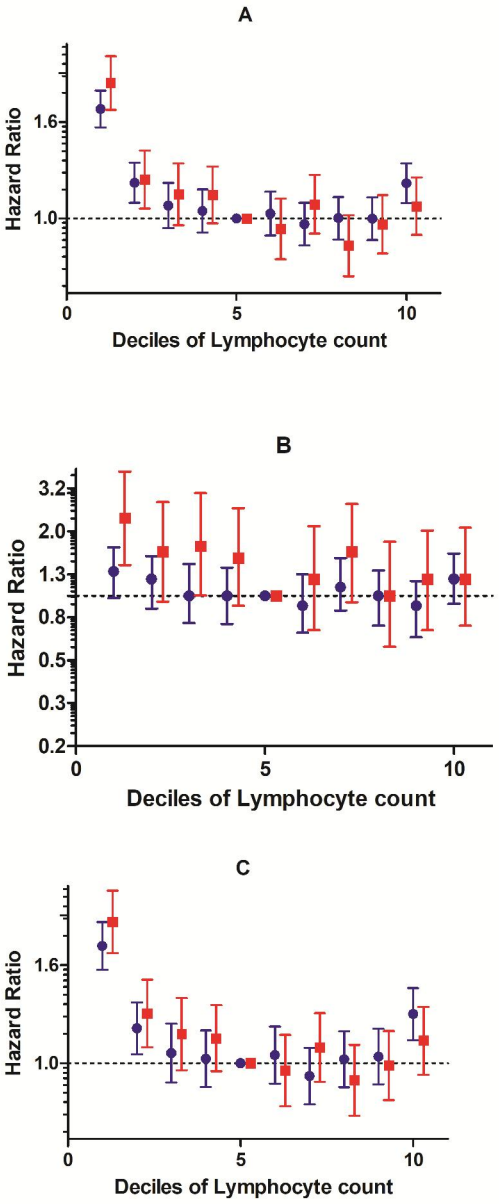

A
Hazard Ratio
1.6
1.0
0
5
10
Deciles of Monocyte count
B
Hazard Ratio
3.2
2.0
1.3
0.8
0.5
0.3
0.2
0
5
10
Deciles of Monocyte count
C
Hazard Ratio
1.6
1.0
0
5
10
Deciles of Monocyte count

Figure D


A
Hazard Ratio
1.6
1.0
0
5
10
Deciles of Lymphocyte count
B
Hazard Ratio
3.2
2.0
1.3
0.8
0.5
0.3
0.2
0
5
10
Deciles of Lymphocyte count
C
Hazard Ratio
1.6
1.0
0
5
10
Deciles of Lymphocyte count