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***FAST*TRACK CLINICAL RESEARCH**
Epidemiology, prevention, and health care policies

Neutrophil counts and cardiovascular disease

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Abstract

Background and Aims Anti-inflammatory trials have shown considerable benefits for cardiovascular disease. High neutrophil counts, an easily accessible inflammation biomarker, are associated with atherosclerosis in experimental studies. This study aimed to investigate the associations between neutrophil counts and risk of nine cardiovascular endpoints using observational and genetic approaches.

Methods Observational studies were conducted in the Copenhagen General Population Study ($n = 101\,730$). Genetic studies were firstly performed using one-sample Mendelian randomization (MR) with individual-level data from the UK Biobank ($n = 365\,913$); secondly, two-sample MR analyses were performed using summary-level data from the Blood Cell Consortium ($n = 563\,085$). Outcomes included ischaemic heart disease, myocardial infarction, peripheral arterial disease, ischaemic cerebrovascular disease, ischaemic stroke, vascular-related dementia, vascular dementia, heart failure, and atrial fibrillation.

Results Observational analyses showed associations between high neutrophil counts with high risks of all outcomes. In the UK Biobank, odds ratios (95% confidence intervals) per 1-SD higher genetically predicted neutrophil counts were 1.15 (1.08, 1.21) for ischaemic heart disease, 1.22 (1.12, 1.34) for myocardial infarction, and 1.19 (1.04, 1.36) for peripheral arterial disease; similar results were observed in men and women separately. In two-sample MR, corresponding estimates were 1.14 (1.05, 1.23) for ischaemic heart disease and 1.11 (1.02, 1.20) for myocardial infarction; multiple sensitivity analyses showed consistent results. No robust associations in two-sample MR analyses were found for other types of leucocytes.

Conclusions Observational and genetically determined high neutrophil counts were associated with atherosclerotic cardiovascular disease, supporting that high blood neutrophil counts is a causal risk factor for atherosclerotic cardiovascular disease.

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Structured Graphical Abstract

Key Question

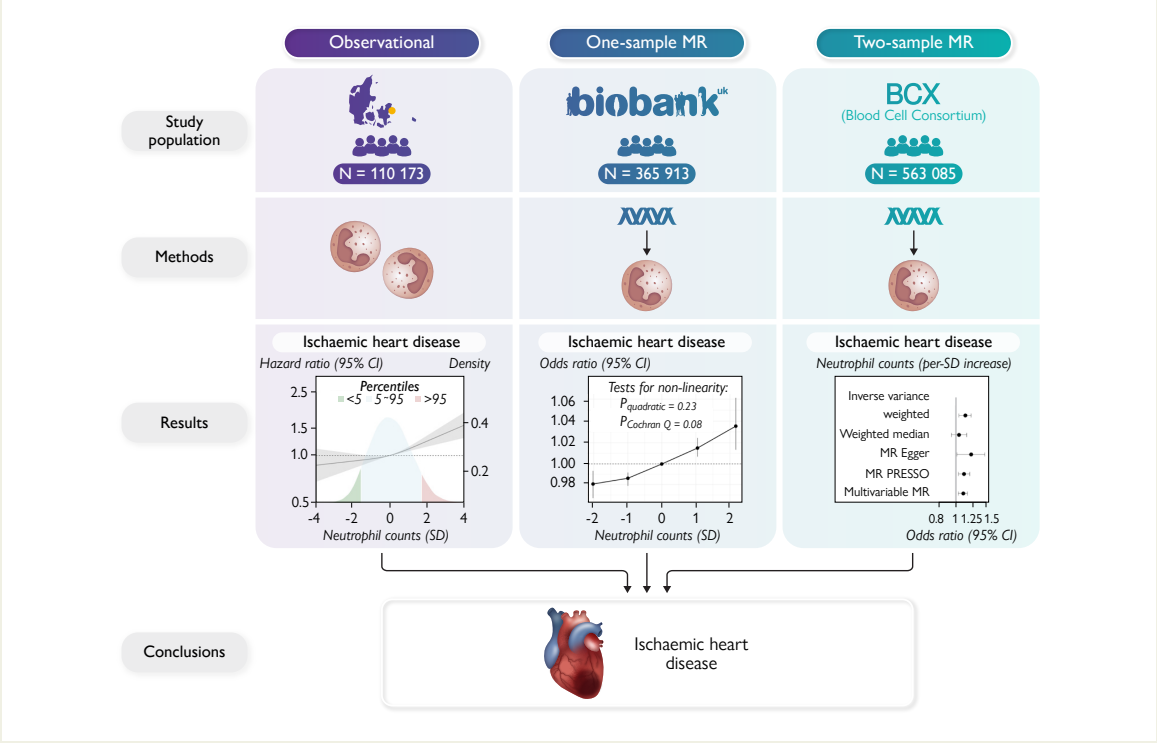
What are the causal associations between blood neutrophil counts and atherosclerotic cardiovascular disease in the general population?

Key Finding

Observational and genetically-determined higher neutrophil counts were associated with increased risk of ischaemic heart disease and peripheral artery disease.

Take Home Message

The association between blood neutrophil counts and risk of atherosclerotic cardiovascular disease may be causal.



Neutrophil counts and cardiovascular disease. CI, confidence interval; MR, Mendelian randomization; SD, standard deviation.

Keywords Inflammation • Neutrophil • Atherosclerosis • Cardiovascular disease

Introduction

Inflammation plays a crucial role in regulating the onset, progression, and outcomes of cardiovascular disease. Several trials implementing pharmaceutical anti-inflammatory therapies have yielded promising results, including canakinumab targeting interleukin (IL)-1β,[1] colchicine targeting microtubules,[2,3] and ziltivekimab targeting the IL-6 ligand.[4] These interventions showed remarkable reductions of pro-inflammatory mediators and improved clinical cardiovascular outcomes, highlighting inflammatory risk as an effective target for the prevention of cardiovascular disease, despite that ziltivekimab has not yet been tested in a Phase 3 outcome trial. Comprehensive observational and genetic studies are hence warranted to scrutinize key pro-inflammatory mediators—of which neutrophils stand central.

Beyond the innate immune frontline defence during infections and tissue injury, neutrophils also contribute to atherosclerosis in a stage-dependent manner in pre-clinical models.[5,6] Briefly, during atherogenesis, activated neutrophils degranulate to promote monocyte recruitment and secrete reactive oxygen species (ROS) and proteases leading to dysregulation of the endothelial cell layer, which enables LDL cholesterol extravasation. During atherosclerosis progression, neutrophils secrete myeloperoxidase (MPO), mediating the oxidation of LDL particles, further promoting foam cell formation.[5,6] In advanced atherosclerotic lesions, neutrophils can destabilize the plaque by secreting neutrophil extracellular traps (NETs), which perforate and lyse vascular smooth muscle cells, resulting in fibrous cap thinning and formation of vulnerable rupture-prone plaques. Uncertainty remains however in moving from animal experimental data to causal inference in humans.

The associations between leucocyte counts and atherosclerotic cardiovascular disease have been widely explored since the 1970s in epidemiological observational studies. Results from multiple large-scale cohorts worldwide have unequivocally identified an association between

high blood neutrophil counts and increased risk of cardiovascular disease,[7–19] whereas results of other leucocyte subpopulations remain inconsistent. Observational associations are however not equivalent to causality due to residual confounding and reverse causation—inherent limitations of observational study designs. Randomized clinical trials can address the question of causality, whereas genetic studies, such as the Mendelian randomization (MR) strategy, can suggest causal pathways of a biomarker. Particularly, for coronary artery disease, prior MR studies did not support C-reactive protein as a causal factor[20,21] but confirmed a potential causal role of the IL-6 receptor[22] in atherosclerotic cardiovascular disease. To our knowledge, no study has so far investigated the causal associations between high blood neutrophil counts and risk of a broad range of cardiovascular diseases.

We tested the hypothesis that high blood neutrophil counts are associated with increased risks of nine cardiovascular endpoints, including ischaemic heart disease, myocardial infarction, peripheral arterial disease, ischaemic cerebrovascular disease, ischaemic stroke, vascular-related dementia, vascular dementia, heart failure, and atrial fibrillation, using observational and genetic studies. Due to previously reported associations of other leucocyte types with cardiovascular disease, we also comprehensively evaluated lymphocytes, monocytes, basophils, and eosinophils.

Methods

A schematic overview of the study design is shown in Supplementary data online, *Figure S1*.

Observational study

Study population and outcomes

We included 101 730 White individuals of Danish descent from the Copenhagen General Population Study (CGPS). The study was approved by institutional review boards and Danish ethical committees and was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all participants. Exact inclusion numbers are given in Supplementary data online, *Figure S2*. Cause of death was obtained from the national Danish Causes of Death Registry, as reported by hospitals and general practitioners since 1977. Diagnoses of all disease endpoints were collected through the national Danish Patient Registry and were classified using the International Classification of Disease (ICD) codes (see Supplementary data online, *Supplementary Methods* and *Table S1*).

Cell counts measurement

All blood samples were analysed on standard hospital clinical haematology equipment to estimate leucocyte cell counts. Regular internal and external quality control programmes were performed. The samples were collected in standard vacutainers with EDTA as an anticoagulant agent and measured using Advia systems (Siemens, Munich, Germany). The distributions of each cell counts are presented in Supplementary data online, *Figure S3*.

Covariates

The selection of covariates is detailed in the Supplementary data online, *Supplementary Methods*. Age and sex were obtained from registries. Plasma total cholesterol, HDL cholesterol, and triglycerides were measured using standard hospital assays (Boehringer Mannheim GmbH, Mannheim, Germany; Konelab, Thermo Fischer Scientific, Waltheim, MA, USA). LDL cholesterol was calculated using the Friedewald equation[23] when plasma triglycerides were ≤4 mmol/L (≤352 mg/dL) and otherwise measured directly (Konelab). Plasma C-reactive protein levels were determined using high-sensitivity turbidimetry (Dako) or nephelometry (Dade Behring) assays according to the manufacturers' protocols, as described previously.[21] Body mass index (BMI, kg/m^2) was computed by measured weight in kilograms divided by height in metres squared. Education was categorized into three groups based on self-reported schooling years: <8, 8–12, and >12 years. Physical activity was categorized into two groups (≥4 or <4 hours per week). Smoking status included self-reported current, former, and never smoker. Alcohol consumption was grouped into high (>14/21 units per week for women/men), moderate (3–14/21 units per week for women/men), and low (<3 units). One unit was equivalent to 12 g of alcohol. Type 2 diabetes mellitus (yes/no) was defined as self-reported disease, use of insulin or oral hypoglycaemic agents, a non-fasting plasma glucose concentration of >11 mmol/L, and/or a registry diagnosis at baseline. Hypertension (yes/no) was defined as self-reported use of blood pressure-lowering medication, high systolic blood pressure (≥140 mmHg) or diastolic blood pressure (≥90 mmHg), or a registry diagnosis at baseline. Lipid-lowering therapy (yes/no) was self-reported, and >97% was statins.

Statistical analyses

Cause-specific Cox proportional hazard regression models were used to estimate hazard ratios (HRs) and corresponding 95% confidence intervals (CIs) for different outcomes based on cell counts percentile categories (corresponding absolute values in Supplementary data online, *Table S2*) and on a linear scale of standardized log-transformed cell counts using restricted cubic splines, adjusted for covariates. The proportionality of hazards over time was assessed by visual inspection of plotting -log[-log(survival)] vs. log(analysis time) and tested using Schoenfeld residuals. No major violation of proportionality was observed. Since neutrophils are indispensable for a healthy response to infection and inflammation, the pathogenic effects are likely to occur in the extreme groups. To this end, three knots located at the 5th, 50th, and 95th percentiles were selected as a reasonable compromise between flexibility and overfitting (cross-validated using Akaike's Information Criterion[24]); categorically, cell counts were divided into five groups by percentiles, 0–5th, 5–25th, 25–75th, 75–95th, and 95–100th to facilitate exploration of extreme cell counts. The 25–75th group was chosen as a robust reference group, within which neutrophils are considered predominantly essential for homeostatic functions. Three regression models were fitted, where age was adjusted for as time scale, i.e. subjects enter the analysis at their baseline age (delayed entry) and exit at their event/censoring/death age. The crude model (Model 1) was adjusted for age and sex. Multi-factorial regression models were additionally adjusted for education, BMI, smoking, alcohol consumption, physical activity, hypertension, and type 2 diabetes mellitus (Model 2) and additionally for HDL cholesterol, LDL cholesterol, triglycerides, and lipid-lowering therapy (Model 3).

Several sensitivity analyses were performed including: (i) sex-stratification analyses including test for interaction by sex; (ii) adjustment for C-reactive protein to account for systemic inflammatory status, use of aspirin as a common example of an anti-inflammatory drug, and use of other cardiovascular medications; and (iii) further breaking down of the highest percentile group (both by absolute levels and the 99th percentile) to rule out influence of acute inflammation or other clinical situations that extremely increase neutrophil counts. Furthermore, since the neutrophil-to-lymphocyte ratio (NLR) has been recognized as a good biomarker for risk prediction in cardiovascular disease, we evaluated predictions up to 10 years using Harrell's cumulative C-index[25] and time-dependent area under the receiver operating characteristic curve (AUC-ROC)[26] at 10 years adjusting for competing risks, comparing a model including neutrophils vs. a model including neutrophils plus NLR.

Missing data (<1%), considered as missing at random, were imputed using multiple imputations by chain equations. An in-depth description of the statistical analyses is available in the Supplementary data online.

Genetic study

Selection of genetic instruments

Single-nucleotide variants (SNVs) at a genome-wide significance level (P-value $<5 \times 10^{-8}$) were selected as genetic instrumental variables from the Blood Cell Consortium (BCX)—the largest genome-wide association studies (GWAS) on blood cell traits comprising 563 085 participants of European ancestry.[27] The final beta coefficient was per 1-standard deviation

(SD) higher cell counts per additional effect allele. The total variation explained by the instrumental variables was calculated based on the retrieved summary statistics for each cell type using the methods described previously.[28] F-statistics (beta/se)2 of all variants ranged from 30 to 2251 (see Supplementary data online, *Table S3*).

One-sample Mendelian randomization analyses using individual-level data

We included 365 913 non-related white British participants from the UK Biobank with available cell counts measurements. Nine endpoints were defined according to the ICD-based definition used in the CGPS cohort (see Supplementary data online, *Table S1*). We calculated a genetic risk score (GRS) for each participant weighted by the associations of the genetic instrumental variables identified from the BCX consortium. Subsequently, we categorized the GRS into five groups by percentiles aligned with the observational analyses. Mendelian randomization estimates were obtained dividing the GRS–outcome association by the GRS–cell counts association. Non-linear MR analyses were performed to assess non-linear associations between cell counts and different outcomes. This approach assessed how the association of cell counts with outcomes differs across different groups of instrument-free cell counts (residuals of cell counts regressing on their corresponding GRS).[29] Piecewise linear MR estimates within each stratum were generated, where the local average causal effect (LACE) of different types of cell counts on endpoints is estimated by dividing GRS–endpoint associations by GRS–cell counts associations. The assumption of the constant genetic effect on exposure was assessed using the doubly ranked method as developed recently,[30,31] and no violation was observed. *P*-values from two tests were generated, the quadratic test evaluating the trend between exposure and LACE values and Cochran's Q statistic assessing differences in MR estimates across groups.

Since all genetic instrumental variables for different cell counts from the BCX are generated in the combined sample of both sexes, we also used estimates from sex-stratified GWAS on neutrophil counts generated solely in the UK Biobank as made publicly available by the Neale Lab (http://www.nealelab.is/uk-biobank) to perform the main MR analyses for men and women separately.

Two-sample Mendelian randomization analyses using summary-level data

We performed two-sample MR analyses for five of nine endpoints with publicly available summary-level data from the to-date largest genomic consortia, including ischaemic heart disease,[32] myocardial infarction,[33] ischaemic stroke, heart failure,[34] and atrial fibrillation.[35] For a summarized description of the included consortia, see Supplementary data online, *Table S4* (detailed descriptions in the Supplementary data online, *Supplementary Methods*). We performed several analyses including the inverse-variance weighted (IVW),[36] weighted median estimator,[37] MR-Egger,[38] and MR-PRESSO.[39] Since participants from the UK Biobank contributed to both the exposure and outcome consortia, this sample overlap may lead to bias of the causal estimates towards the confounded associations. Therefore, we used a method (MRlap in R) described previously to account for sample overlap,[40] which uses cross-trait linkage disequilibrium-score regression to approximate the overlap relying only on GWAS summary statistics. We applied this method to adjust for significant associations between cell counts and different outcomes obtained through the IVW method.

All analyses were performed using R (v4.0.2) statistical software (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Observational analyses

Baseline characteristics for individuals in the CGPS are shown in Supplementary data online, *Table S5*. In a total of up to 995 010 follow-up person-years, 5888 ischaemic heart disease, 2090 myocardial infarction, 2314 peripheral arterial disease, 4821 ischaemic cerebrovascular disease, 2632 ischaemic stroke, 1375 vascular-related dementia, 256 vascular dementia, 2940 heart failure, and 6147 atrial fibrillation events occurred (see Supplementary data online, *Table S6*). Correlations between subtypes of leucocyte counts and age were low (see Supplementary data online, *Table S7*).

In multi-factorially adjusted Cox proportional hazard models, higher blood neutrophil counts were associated with increased risk of nine cardiovascular endpoints (*Figure 1*); no significant non-linear relationships were observed (all *P* for non-linearity >0.1). Categorically, in the age and sex-adjusted model (Model 1), individuals in the 95–100th vs. the 25–75th percentile group of blood neutrophil counts had increased risks of all outcomes (see Supplementary data online, *Table S8*). These associations attenuated stepwise, however, remained significant when additionally adjusting for education, BMI, smoking, alcohol consumption, physical activity, hypertension, and type 2 diabetes mellitus in Model 2 and additional LDL cholesterol, HDL cholesterol, triglycerides, and lipid-lowering therapy in Model 3 (*Figure 2*, left panel).

For other types of leucocytes, lower lymphocyte counts and higher monocyte counts were associated with higher risks of all outcomes except vascular dementia (see Supplementary data online, *Figures S4 and S5*), whereas higher basophil and eosinophil counts were associated with parts of the outcomes (see Supplementary data online, *Figures S6 and S7*). Categorical results generally showed similar results (see Supplementary data online, *Figures S8–S11*, left panel).

The analyses stratified by sex were largely similar to the main analyses; however, associations in women had larger effect sizes than in men (see Supplementary data online, *Figures S12–S21*). Additional adjustment for C-reactive protein, aspirin, and other cardiovascular drugs did not change the estimates substantially (see Supplementary data online, *Table S8*). When we broke down the 95–100th neutrophil percentile group, either by absolute cell counts or by the 99th percentile, estimates in each group compared with the reference group showed the same trend, although not all significant due to reduced statistical power (see Supplementary data online, *Figures S22 and S23*). Restricted cubic splines adding an extra knot at the 99th percentile showed similar results as those with three knots in the main analyses; the right tail was however not completely linear possibly due to very few cases (see Supplementary data online, *Figure S24*). The C-indices were 0.706 (95% CI: 0.699, 0.713) in the neutrophil prediction model and 0.707 (0.701, 0.713) in the neutrophil plus NLR model (*P* for difference = .96); corresponding AUC-ROC at 10 years were 0.811 (0.805, 0.815) and 0.812 (0.806, 0.817) (*P* for difference = .99), respectively.

Genetic studies

We identified 275 independent genetic variants at a genome-wide significance level for neutrophil counts, explaining ~4% of the total variation (see Supplementary data online, *Table S3*). The associations of genetic variants and types of leucocyte counts were subsequently used to calculate the weighted GRS in the UK Biobank, which were associated with the measured types of cell counts (see Supplementary data online, *Figure S25*). The baseline characteristics of the UK Biobank are quite comparable with the CGPS (see Supplementary data online, *Table S5*), except for some behavioural factors, possibly due to the well-acknowledged selection bias towards a healthier population of the UK Biobank.

For neutrophils, on a continuous scale, a 1-unit higher weighted GRS was associated with ischaemic heart disease, myocardial infarction, and

Figure 1 Multi-factorially adjusted hazard ratios between blood neutrophil counts and cardiovascular endpoints in the Copenhagen General Population Study. The *x*-axis represents the standardized neutrophil counts (per standard deviarion), and the *y*-axis represents the hazard ratio and the distribution of the population. The red and green colours indicate the lowest and highest fifth percentiles of neutrophil counts. The solid lines are hazard ratios from Model 3, adjusted for age, sex, education, body mass index, smoking, alcohol consumption, physical activity, hypertension, type 2 diabetes mellitus, LDL cholesterol, HDL cholesterol, triglycerides, and lipid-lowering therapy. The grey shaded areas are the corresponding confidence intervals from restricted cubic splines with three knots at the fifth, median, and 95th percentile of neutrophil counts. Specific numbers of individuals and numbers of incident cases are presented in Supplementary data online, *Table S6*. CI, confidence interval; HR, hazard ratio; SD, standard deviation

peripheral arterial disease, with odds ratios (ORs) (95% CI) of 1.13 (1.07, 1.19), 1.20 (1.11, 1.29), and 1.16 (1.03, 1.31), respectively (see Supplementary data online, *Table S9*). This was also reflected categorically across GRS percentile groups (*Figure 2*, right panel). *P*-values from non-linear analyses ranged from .20 to .97 for quadratic test and .08 to .96 for Cochran Q test, suggesting linear genetic relationships between neutrophil counts and outcomes (*Figure 3*). The LACE estimates supported associations between high neutrophil counts and modestly increased risk of ischaemic heart disease, myocardial infarction, and peripheral arterial disease (*Figure 3*). In one-sample MR analyses based on GRS, ORs for genetically determined per 1-SD higher neutrophil counts were 1.15 (1.08, 1.21) for ischaemic heart disease, 1.22 (1.12, 1.34) for myocardial infarction, and 1.19 (1.04, 1.36) for peripheral arterial disease, respectively (*Figure 4*). In the sex-stratified analyses, the estimates were similar between men and women for ischaemic heart disease, myocardial infarction, and peripheral arterial disease; however, for ischaemic stroke and vascular-related dementia, effect sizes were significant and were larger in men than women (see Supplementary data online, *Figure S26*).

In two-sample MR analyses, detailed information for included SNVs and their associations with neutrophil counts and outcomes are shown in Supplementary data online, *Table S10*. A 1-SD increase in genetically determined neutrophil counts was associated with higher risks of ischaemic heart disease and myocardial infarction [IVW-OR: 1.14 (1.05, 1.23) and 1.11 (1.02, 1.20)] (*Figure 5*). Results did not differ materially using other MR sensitivity methods; however, the point estimates were slightly attenuated upon correction for genetic outliers using MR-PRESSO or when adjusting for other types of leucocyte counts in multivariable MR. No significant pleiotropic association was detected using the MR-Egger intercept (all *P*-values >.1). After

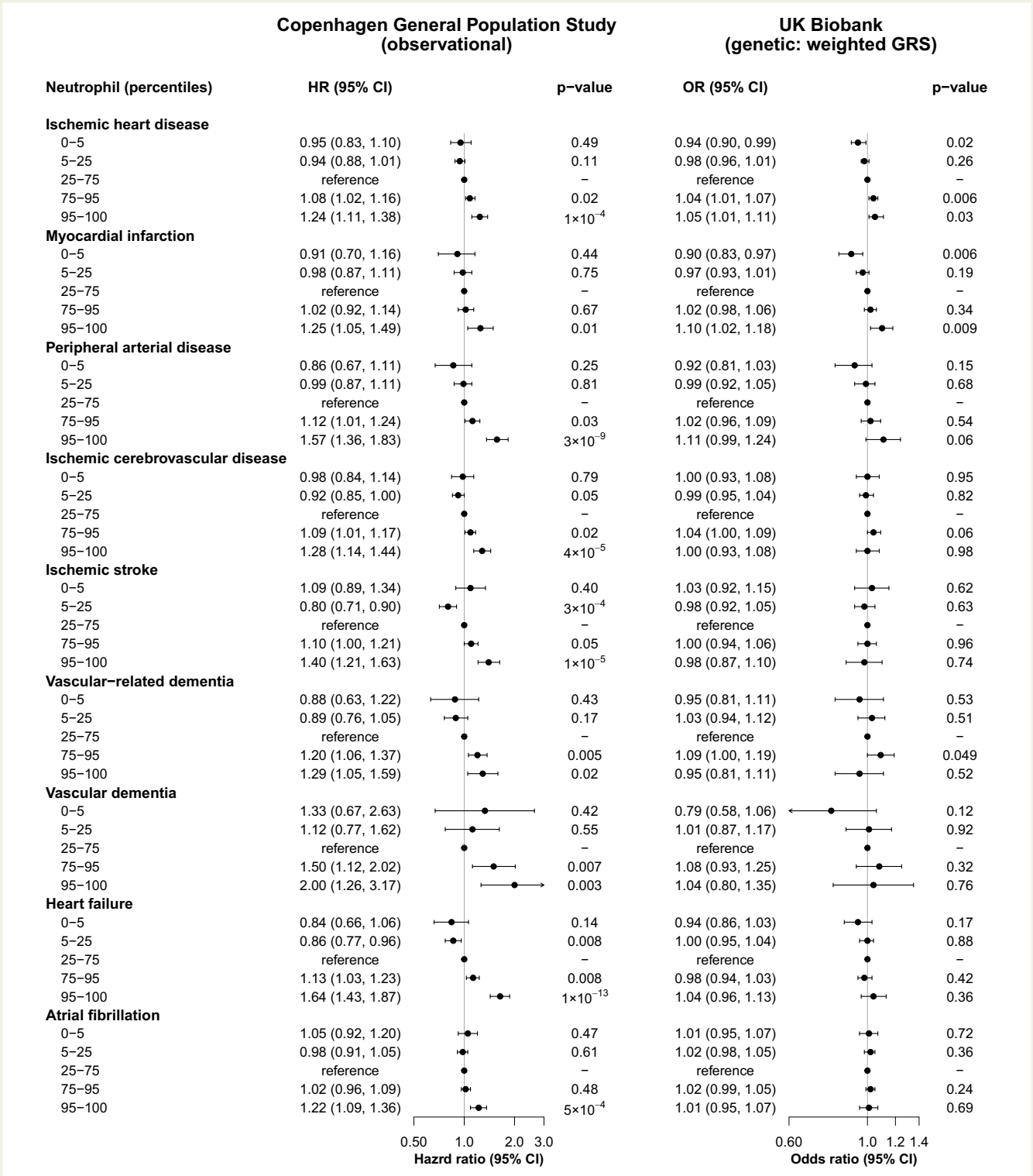


Figure 2 Associations between blood neutrophil counts percentile groups and cardiovascular endpoints in the Copenhagen General Population Study and the UK Biobank. The observational studies were performed in the Copenhagen General Population Study (left panel) where percentiles were based on blood neutrophil counts. Hazard ratios were from Model 3, adjusted for baseline age, sex, education, body mass index, smoking, alcohol consumption, physical activity, hypertension, type 2 diabetes mellitus, LDL cholesterol, HDL cholesterol, triglycerides, and lipid-lowering therapy. Genetic studies were conducted in the UK Biobank using individual-level data, where percentiles were based on the weighted genetic risk score of neutrophil counts. Odds ratios were adjusted for age at recruitment, sex, genotype batch, and first 10 principal components. CI, confidence interval; GRS, genetic risk score; HR, hazard ratio; SD, standard deviation

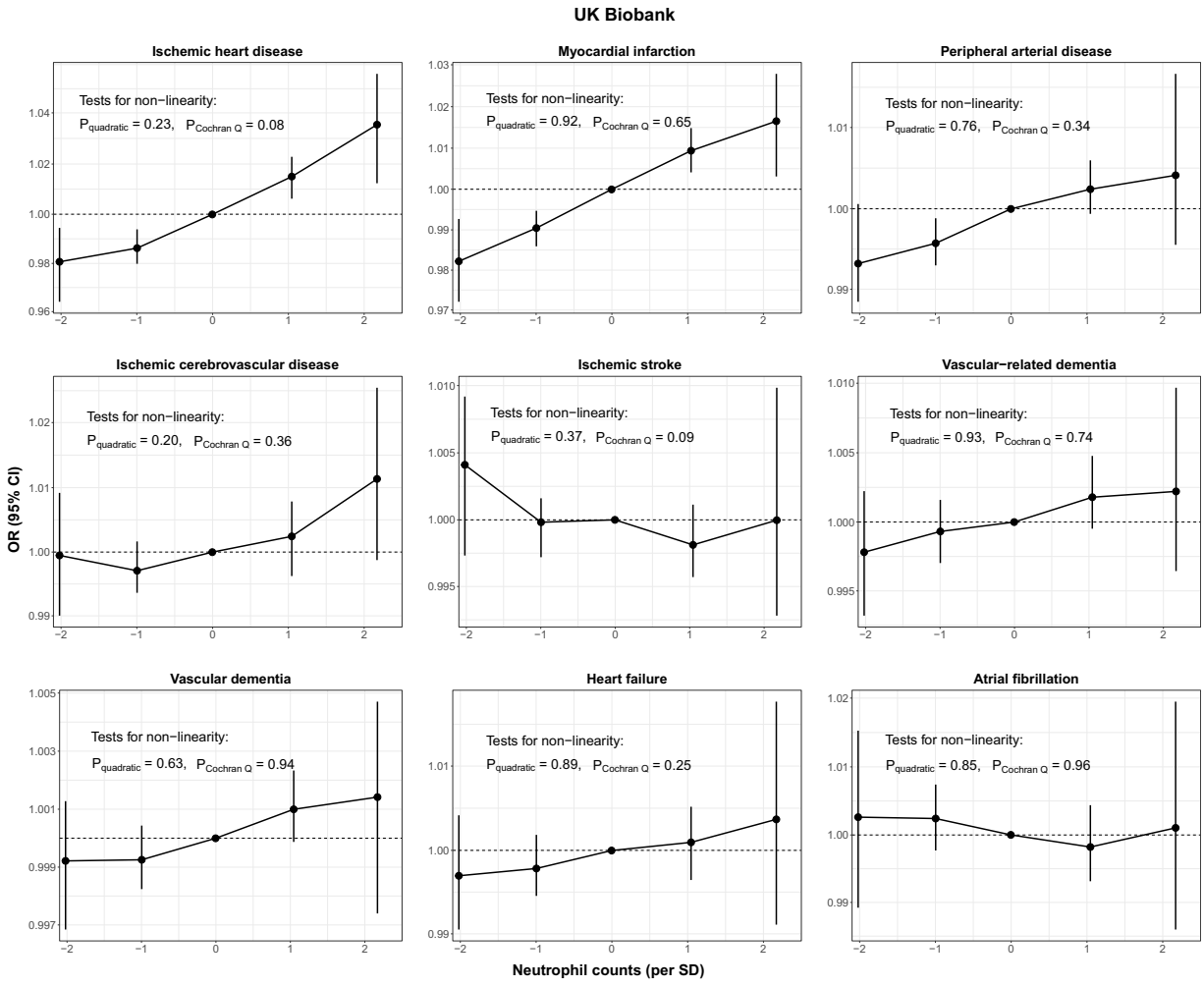


Figure 3 Non-linear Mendelian randomization estimates between blood neutrophil counts and cardiovascular endpoints in the UK Biobank. Localized average causal effects for nine cardiovascular endpoints were estimated using the piecewise linear method. Dots and corresponding whiskers from left to right represent the localized average causal effect (95% confidence interval) from five strata of 0–5th, 5–25th, 25–75th, 75–95th, and 95–100th, respectively. $P_{quadratic}$ and $P_{Cochran\ Q}$ are derived from the non-linear test and Cochran's Q statistic assesses differences in Mendelian randomization estimates across groups and quadratic test evaluates the trend between exposure and localized average causal effect values

accounting for sample overlap, the estimates for ischaemic heart disease and myocardial infarction were similar [IVW-ORs: 1.12 (1.01, 1.24) and 1.11 (0.99, 1.24)].

For monocytes and eosinophils, on a continuous scale, a 1-unit higher weighted GRS was associated with myocardial infarction, with ORs of 1.07 (1.02, 1.12) for both cell counts (see Supplementary data online, *Table S9*). Categorically (see Supplementary data online, *Figures S8–S11*, right panel), this was reflected for eosinophils for the 95–100th vs. the 25–75th GRS percentile group [OR 1.09 (1.01; 1.17)] (see Supplementary data online, *Figure S10*, right panel). *P*-values from non-linear analyses ranged from <.001 to .94 for Cochran Q tests and <.001 to .99 for quadratic tests (see Supplementary data online, *Figures S27–S30*), suggesting non-linear genetic relationships between eosinophils and ischaemic heart disease, myocardial infarction, ischaemic stroke, vascular dementia, heart failure, and atrial fibrillation (see Supplementary data online, *Figure S30*). In one-sample MR analyses based on GRS, ORs for genetically determined higher eosinophil and monocyte counts and myocardial infarction were 1.09 (1.03–1.15) and 1.08 (1.02–1.14), respectively (see Supplementary data online, *Figure S31*). Using the most powerful genetic approach, the two-sample MR assuming linear relationships, no associations were observed for monocyte, lymphocyte, basophil, and eosinophil counts (see Supplementary data online, *Figures S32–S35*). For lymphocytes and basophils, no robust associations across the spectrum of genetic analyses were observed.

Discussion

The principal findings of the present study were that observational and genetically determined higher neutrophil counts were associated with increased risk of ischaemic heart disease, myocardial infarction, and peripheral arterial disease in the overall analysis and when men and women were analysed separately (*Structured Graphical Abstract*). These findings were obtained from large cohorts of the general population and from the to-date largest genomic consortia. The consistent and biologically plausible observational and genetic findings suggest that a

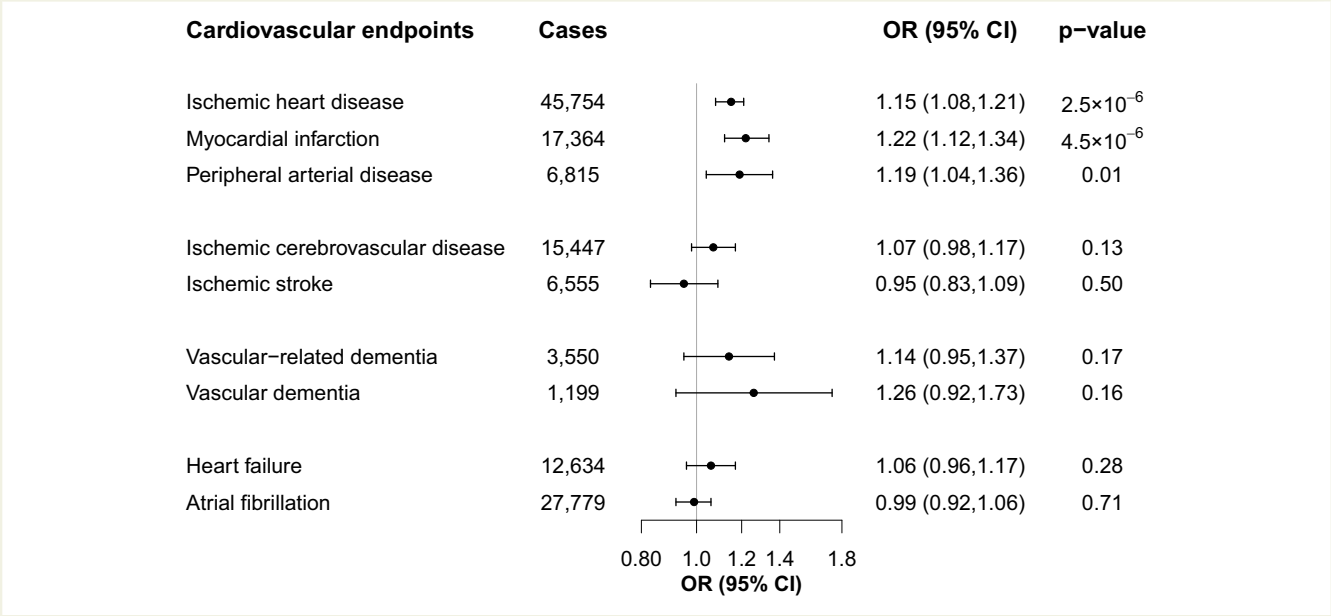


Figure 4 Mendelian randomization estimates between blood neutrophil counts and cardiovascular endpoints in the UK Biobank. Estimates were obtained through dividing the genetic association with outcomes by the genetic association with neutrophil counts, where logistic regression and linear regression were used for disease outcomes and cell counts, respectively. CI, confidence interval; OR, odds ratio

high neutrophil counts is a causal risk factor for atherosclerotic cardiovascular disease.

The associations between leucocyte counts and cardiovascular disease were first described in 1974 by Friedman *et al.*,[7] who observed that high leucocyte counts were associated with myocardial infarction using a case-control design. Subsequently, several studies from different large cohorts have been carried out worldwide. In the NHANES-I follow-up study, individuals aged 25–74 years with a neutrophil count in the third vs. first tertile had increased risk of coronary heart disease and cardiovascular mortality,[8,9] while no corresponding associations were identified for lymphocyte and monocyte counts. In the CALIBER cohort that included about 4% of the population of UK ($n = 775\,231$), high neutrophil counts and low lymphocyte counts within the normal clinical range had strong linear associations with a broad range of cardiovascular outcomes, including myocardial infarction, peripheral arterial disease, and heart failure.[10,11] Using the UK Biobank data ($n = 478\,259$), individuals in the highest decile of neutrophil counts were at higher risk of non-fatal cardiovascular disease, and the estimates were similar between men and women.[12] In the EPIC-NL cohort study of 14 362 individuals, comparing the highest with the lowest tertile, increased total neutrophil, lymphocyte, and monocyte counts were associated with higher risk of cardiovascular disease.[13] Other large-scale cohorts from Asia also found similar results, e.g. higher neutrophil and monocyte counts, but not lymphocyte counts, were associated with higher risk of cardiovascular risk in the Chinese Dongfeng–Tongji cohort ($n = 26\,655$),[14] whereas individuals with higher lymphocytes and monocytes were at an increased risk of cardiovascular disease in a Korean cohort ($n = 12\,752$).[15] The observed conflicting associations for lymphocytes, monocytes, and other granulocytes may attribute to heterogeneities among study populations, disease status, and different confounding factors adjusted for in the analytic models. Yet, these findings, in line with our results, imply positive associations between high neutrophil counts and increased risk of cardiovascular disease in individuals of different ages, sexes, and ethnicities. The consistent findings of neutrophils in the global literature are now further supported by the present robust genetic observations.

Current anti-inflammatory intervention trials on cardiovascular disease are focusing on inhibiting nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3 (NLRP3) function or altering its downstream IL-6 signalling,[1–4] with remarkable beneficial effects. A recent study comprising over 60 000 participants from five randomized clinical trials showed that a decreased NLR independently predicted decreased cardiovascular risk and advocated NLR as a potential biomarker in cardiovascular risk assessment.[41] The ratio reduction might however be due to a decrease in neutrophils, an increase in lymphocytes, or a combination of both. Interestingly, canakinumab (targeting IL-1β), which reduced NLR in a dose-dependent manner and only decreased neutrophil counts but did not influence lymphocyte counts, showed a significant reduction in cardiovascular disease. In contrast, methotrexate increasing NLR due to a decrease in lymphocytes failed to show any benefits.[41] In our sensitivity analyses, when we additionally added NLR to the neutrophil prediction model, the AUC and Harrell's C-index did not differ significantly between the models, indicating that NLR does not confer additional predictive value beyond the neutrophil counts. The differences between the C-index and the AUC are likely due to the large fraction of censored individuals in the cohort, which may have led to an under-estimation of the C-index for estimation of the 10-year risk of cardiovascular disease.[26] Moreover, a recent two-sample MR study using instrumental variables for NLR from the BCX consortium did not provide any evidence supporting NLR as a causal risk factor for coronary artery disease.[42] Collectively with our findings, it seems that the beneficial effects are driven, or at least partly mediated, by neutrophil reduction.

Mechanistically, studies during the past decade have elucidated important functions of neutrophils in cardiovascular inflammation throughout various stages. At the site of vascular inflammation, activated neutrophils

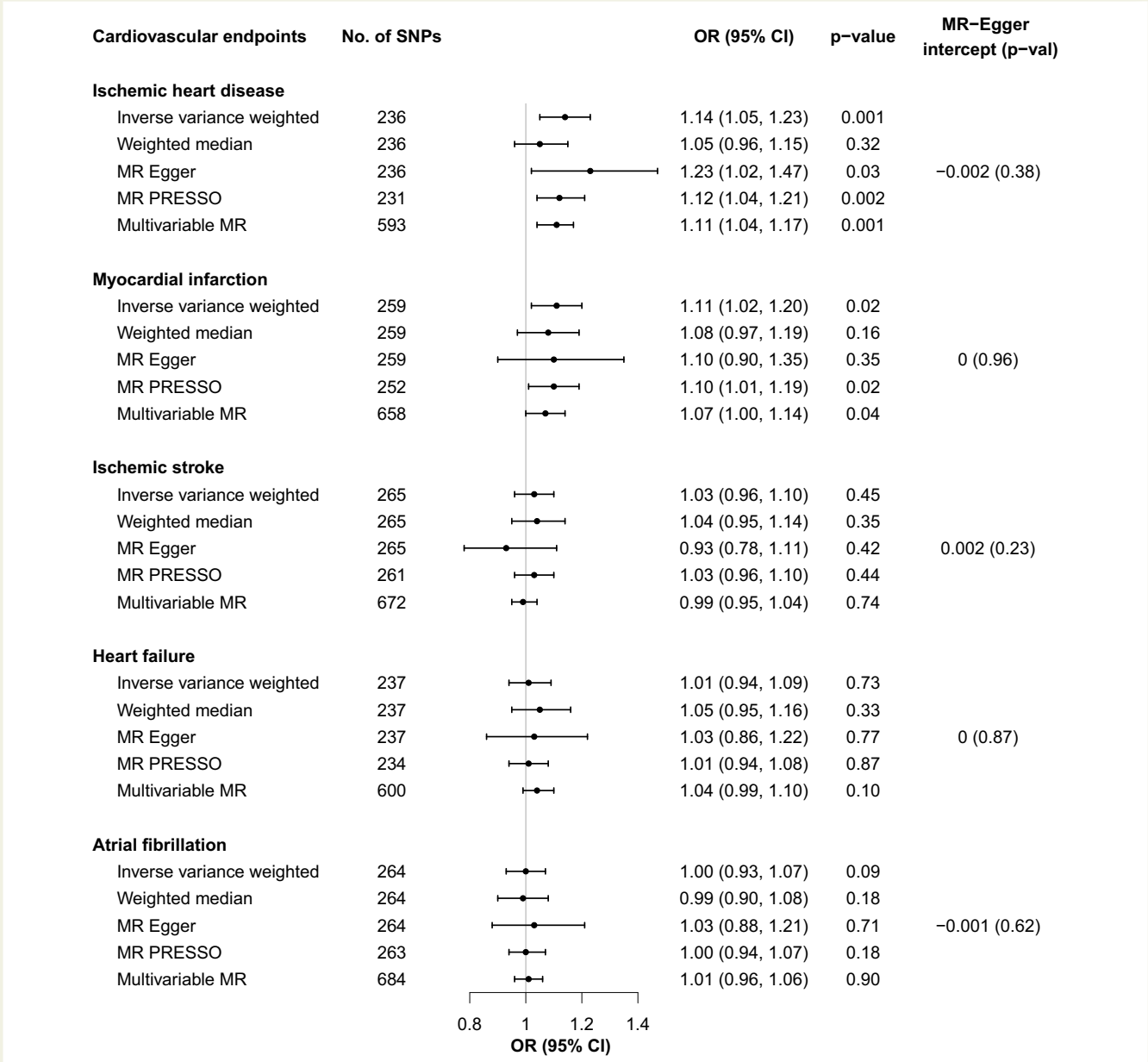


Figure 5 Two-sample Mendelian randomization estimates between blood neutrophil counts and cardiovascular endpoints using publicly available summary-level data. Estimated odds ratios represent the effects of per 1-SD increase in neutrophil counts on risk of five cardiovascular endpoints, obtained from different methods. Multivariable Mendelian randomization was adjusted for the other four types of leucocytes. OR, odds ratio; CI, confidence interval; MR, Mendelian randomization

develop several synergistic strategies to accomplish their functions, including the release of ROS, the release of proteolytic enzymes through degranulation, including several pro-inflammatory alarmins (such as S100A8/A9) and proteases (such as cathepsin G, neutrophil elastase, and MPO), and the formation of NETs. Secreted excessive ROS dysregulate and activate the endothelium and disintegrate the underlying extracellular matrix and further enable enhanced adhesion and recruitment of monocytes and the transfer of LDL from the lumen to the arterial intima.[43] Reactive oxygen species mediate the formation of oxidized LDL and could also lead to matrix metalloproteinases activation, resulting in plaque rupture.[44] Extracellular S100A8/A9 binds to the receptor for advanced glycation end products on myeloid progenitor cells and acts as a potent activator of the innate immune response, subsequently induces the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells signalling pathway, which in turn stimulates granulopoiesis in the bone marrow leading to increased accumulation of neutrophils.[45,46] Moreover, S100A8/A9 has been shown to directly suppress mitochondrial function under hypoxic conditions, leading to cardiomyocyte death,[47] and short although not long-term S100A9 blockade also improves cardiac function after myocardial infarction.[48–50] Myeloperoxidase catalyzes the conversion of hydrogen peroxide to hypochlorous acid, which oxidizes LDL and consequently accelerates foam cell formation.[51] Myeloperoxidase also impedes adenosine triphosphate-binding cassette transporter A1-dependent cholesterol efflux from macrophages as well

as dysfunctional HDL that competes with native HDL in lipid uptake via scavenger receptors on macrophages.[52] Myeloperoxidase indirectly causes endothelial dysfunction by interfering with nitric oxide metabolism, one of the key elements for endothelium and vasculature maintenance.[53] Neutrophil extracellular traps lead to interferon responses and the secretion of IL-1β and IL-18 by macrophages through the NLRP3 inflammasome, which acts as a positive feedback loop to induce NETs formation.[54] Finally, pro-inflammatory responses, including secretion of MPO, ROS, and cytotoxic histone H4 driven by NETs, cause an inflammatory environment that favours plaque destabilization and rupture and stimulates the death of vascular smooth muscle cells.

In addition to the findings for ischaemic heart disease and peripheral arterial disease, we also found an observational association between higher neutrophil counts and all other endpoints, and genetic associations in the UK Biobank for cerebrovascular disease and vascular-related dementia, particularly in men. These associations are likely to be mediated mechanistically by atherosclerosis. The integrity and functional damage of blood vessels supplying the brain caused by atherosclerosis reduce the cerebral blood flow and break down neurovascular coupling[55,56]—hallmarks of ischaemic cerebrovascular disease and vascular dementia. Population-based studies have supported an association between atherosclerosis and ischaemic stroke and vascular dementia[57–59]; however, more powerful future studies are needed to assess whether neutrophils play roles in their pathogenesis. The potential association between neutrophil counts and heart failure, an important complication resulting from ischaemic heart disease, may be attributed to the development of ischaemic heart disease through the synergistic influence of multiple risk factors. Consequently, genetic studies have not replicated this association as a distinct independent causal risk factor. Additionally, inflammation plays a critical role in the structural remodelling of the atria, perpetuating the development of atrial fibrillation.[60] A high neutrophil counts is commonly associated with a pro-inflammatory status, characterized by increased biomarkers. Therefore, the observed association may be linked to inflammation rather than specifically to neutrophils.

For other types of leucocytes, previous results are conflicting.[8–10,12–14,16–19] The present genetic studies of individual-level data from the UK Biobank suggested associations between high monocyte and eosinophil counts and myocardial infarction and found a non-linear relationship for eosinophils. The association between eosinophils and myocardial infarction is likely driven by the extremely high eosinophil group. A recent study highlighted that eosinophil cells interact with platelets to promote atherosclerosis by stabilizing thrombosis through eosinophil extracellular traps.[61] The present most powerful two-sample MR did not support these individual-level data—this strategy however cannot evaluate potential non-linear relationships. Thus, more powerful individual level data are warranted to scrutinize the role of high eosinophil counts in myocardial infarction. Monocytes are recruited into and accumulate at the intima, where they may differentiate into lesional macrophages and further proliferate to foam cells—the key component of the atherosclerotic plaque, promoting plaque formation, progression, rupture, and thrombosis[62,63] and ultimately myocardial infarction. The relative inconsistent results on monocytes in the present analyses may however be due to the heterogeneity of monocytes, in which the Ly6C^{high} but not the Ly6C^{low} subtype is the major contributor to atherosclerosis.[62] More experimental evidence is warranted to clarify the potential roles of monocytes and eosinophils in cardiovascular pathogenesis.

Our study has several strengths. The two independent prospective cohorts with large size of individual-level data, comprehensive confounding information, and a wide spectrum of cardiovascular disease were exploited for observational and genetic studies, respectively. Furthermore, we conducted two-sample MR with different sensitivity analyses in the largest genomic consortia currently available, ensuring sufficient statistical power. The triangulation from three different compensatory approaches yielded converged results, largely strengthening the robustness and validity of the findings. However, there are also important limitations. To minimize the confounding by population stratification, all analyses were restricted to participants of European ancestry wherever possible. Therefore, the extrapolation of our findings to other populations with different ethnicities is limited. In addition, there is sample overlap in the two-sample MR analyses, predominantly derived from the inclusion of the UK Biobank data in both BCX and genomic consortia of cardiovascular disease. Due to the construction of the publicly available summary statistics in the consortia, we are not able to perform all the analyses using completely independent datasets; however, the corrected estimates using the most novel statistical method for correction of sample overlap were similar to the main analyses, indicating that our study is unlikely to suffer from severe bias from sample overlap. Lastly, although the GRS for cell counts were generated based on the genetic associations identified by the BCX Consortium, this consortium integrated data from UK Biobank, and thus the GRSs are not completely externally weighted.

Our findings indicate that observational and genetically determined higher neutrophil counts are associated with atherosclerotic cardiovascular disease, implying that a high neutrophil counts is a causal risk factor for atherosclerotic cardiovascular disease. These novel findings may provide potential implications for future disease prevention and anti-inflammatory trial design.

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Supplementary data

Supplementary data are available at *European Heart Journal* online.

Declarations

Disclosure of Interest

J.L. and J.Q.T. have nothing to declare. B.G.N. reports consultancies or talks sponsored by AstraZeneca, Sanofi, Regeneron, Akcea, Amgen, Kowa, Denka, Amarin, Novartis, Novo Nordisk, Esperion, and Silence Therapeutics. A.T.-H. reports consultancies or talks sponsored by Amgen, Akcea, AstraZeneca, Draupnir Bio, Novartis, Regeneron, Sanofi, and Silence Therapeutics. R.F.-S. reports consultancies or talks sponsored by Novo Nordisk and Siemens.

Data Availability

Data from the Copenhagen General Population Study are not available to share due to privacy restrictions of participants. Data from the UK Biobank could be obtained upon request to the board. Consortia data on exposures and endpoints are available on https://www.ebi.ac.uk/gwas/.

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Ethical Approval

The Copenhagen General Population Study was approved by institutional review boards and Danish ethical committees and was conducted according to the Declaration of Helsinki. The UK Biobank has approval from the North West Multi-centre Research Ethics Committee (MREC).

Pre-registered Clinical Trial Number

Not applicable.

References

1. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, *et al.* Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med* 2017;**377**:1119–31. https://doi.org/10.1056/NEJMoa1707914
2. Tardif JC, Kouz S, Waters DD, Bertrand OF, Diaz R, Maggioni AP, *et al.* Efficacy and safety of low-dose colchicine after myocardial infarction. *N Engl J Med* 2019;**381**:2497–505. https://doi.org/10.1056/NEJMoa1912388
3. Nidorf SM, Fiolet ATL, Mosterd A, Eikelboom JW, Schut A, Opstal TSJ, *et al.* Colchicine in patients with chronic coronary disease. *N Engl J Med* 2020;**383**:1838–47. https://doi.org/10.1056/NEJMoa2021372
4. Ridker PM, Devalaraja M, Baeres FMM, Engelmann MDM, Hovingh GK, Ivkovic M, *et al.* IL-6 inhibition with ziltivekimab in patients at high atherosclerotic risk (RESCUE): a double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet* 2021;**397**:2060–9. https://doi.org/10.1016/s0140-6736(21)00520-1
5. Silvestre-Roig C, Braster Q, Ortega-Gomez A, Soehnlein O. Neutrophils as regulators of cardiovascular inflammation. *Nat Rev Cardiol* 2020;**17**:327–40. https://doi.org/10.1038/s41569-019-0326-7
6. Sreejit G, Johnson J, Jaggers RM, Dahdah A, Murphy AJ, Hanssen NMJ, *et al.* Neutrophils in cardiovascular disease: warmongers, peacemakers, or both? *Cardiovasc Res* 2022;**118**:2596–609. https://doi.org/10.1093/cvr/cvab302
7. Friedman GD, Klatsky AL, Siegelaub AB. The leukocyte count as a predictor of myocardial infarction. *N Engl J Med* 1974;**290**:1275–8. https://doi.org/10.1056/nejm197406062902302
8. 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**:1287–92. https://doi.org/10.1016/j.ehj.2004.05.002
9. Gillum RF, Mussolino ME, Madans JH. Counts of neutrophils, lymphocytes, and monocytes, cause-specific mortality and coronary heart disease: the NHANES-I epidemiologic follow-up study. *Ann Epidemiol* 2005;**15**:266–71. https://doi.org/10.1016/j.annepidem.2004.08.009
10. 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**:1160–9. https://doi.org/10.1016/j.jacc.2016.12.022
11. Shah AD, Denaxas S, Nicholas O, Hingorani AD, Hemingway H. Low eosinophil and low lymphocyte counts and the incidence of 12 cardiovascular diseases: a CALIBER cohort study. *Open Heart* 2016;**3**:e000477. https://doi.org/10.1136/openhrt-2016-000477
12. Welsh C, Welsh P, Mark PB, Celis-Morales CA, Lewsey J, Gray SR, *et al.* Association of total and differential leukocyte counts with cardiovascular disease and mortality in the UK Biobank. *Arterioscler Thromb Vasc Biol* 2018;**38**:1415–23. https://doi.org/10.1161/atvbaha.118.310945
13. Lassale C, Curtis A, Abete I, van der Schouw YT, Verschuren WMM, Lu Y, *et al.* Elements of the complete blood count associated with cardiovascular disease incidence: findings from the EPIC-NL cohort study. *Sci Rep* 2018;**8**:3290. https://doi.org/10.1038/s41598-018-21661-x
14. Wang Q, Guo Q, Zhou L, Li W, Yuan Y, Lei W, *et al.* Associations of baseline and changes in leukocyte counts with incident cardiovascular events: the Dongfeng-Tongji cohort study. *J Atheroscler Thromb* 2022;**29**:1040–58. https://doi.org/10.5551/jat.62970
15. Kim JH, Lim S, Park KS, Jang HC, Choi SH. Total and differential WBC counts are related with coronary artery atherosclerosis and increase the risk for cardiovascular disease in Koreans. *PLoS One* 2017;**12**:e0180332. https://doi.org/10.1371/journal.pone.0180332
16. 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**:1638–43. https://doi.org/10.1016/j.jacc.2005.02.054
17. Pinto EM, Huppert FA, Morgan K, Mrc C, Brayne C. Neutrophil counts, monocyte counts and cardiovascular disease in the elderly. *Exp Gerontol* 2004;**39**:615–9. https://doi.org/10.1016/j.exger.2003.12.011
18. Guasti L, Dentali F, Castiglioni L, Maroni L, Marino F, Squizzato A, *et al.* Neutrophils and clinical outcomes in patients with acute coronary syndromes and/or cardiac revascularisation. A systematic review on more than 34,000 subjects. *Thromb Haemost* 2011;**106**:591–9. https://doi.org/10.1160/th11-02-0096
19. Hu ZB, Lu ZX, Zhu F, Jiang CQ, Zhang WS, Pan J, *et al.* Higher total white blood cell and neutrophil counts are associated with an increased risk of fatal stroke occurrence: the Guangzhou biobank cohort study. *BMC Neurol* 2021;**21**:470. https://doi.org/10.1186/s12883-021-02495-z
20. Wensley F, Gao P, Burgess S, Kaptoge S, Di Angelantonio E, Shah T, *et al.* Association between C reactive protein and coronary heart disease: Mendelian randomisation analysis based on individual participant data. *BMJ* 2011;**342**:d548. https://doi.org/10.1136/bmj.d548
21. Zacho J, Tybjaerg-Hansen A, Jensen JS, Grande P, Sillesen H, Nordestgaard BG. Genetically elevated C-reactive protein and ischemic vascular disease. *N Engl J Med* 2008;**359**:1897–908. https://doi.org/10.1056/NEJMoa0707402
22. Swerdlow DI, Holmes MV, Kuchenbaecker KB, Engmann JE, Shah T, Sofat R, *et al.* The interleukin-6 receptor as a target for prevention of coronary heart disease: a Mendelian randomisation analysis. *Lancet* 2012;**379**:1214–24. https://doi.org/10.1016/s0140-6736(12)60110-x
23. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;**18**:499–502. https://doi.org/10.1093/clinchem/18.6.499
24. Harrell F Jr. *Springer Series in Statistics: Regression Modeling Strategies with Applications to Linear Models, Logistic and Ordinal Regression, and Survival Analysis*. Switzerland: Springer International Publishing, 2015.
25. Wolbers M, Koller MT, Witteman JC, Steyerberg EW. Prognostic models with competing risks: methods and application to coronary risk prediction. *Epidemiology* 2009;**20**:555–61. https://doi.org/10.1097/EDE.0b013e3181a39056
26. Blanche P, Dartigues JF, Jacqmin-Gadda H. Estimating and comparing time-dependent areas under receiver operating characteristic curves for censored event times with competing risks. *Stat Med* 2013;**32**:5381–97. https://doi.org/10.1002/sim.5958
27. Vuckovic D, Bao EL, Akbari P, Lareau CA, Mousas A, Jiang T, *et al.* The polygenic and monogenic basis of blood traits and diseases. *Cell* 2020;**182**:1214–1231.e11. https://doi.org/10.1016/j.cell.2020.08.008
28. Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, *et al.* Biological, clinical and population relevance of 95 loci for blood lipids. *Nature* 2010;**466**:707–13. https://doi.org/10.1038/nature09270
29. Staley JR, Burgess S. Semiparametric methods for estimation of a nonlinear exposure-outcome relationship using instrumental variables with application to Mendelian randomization. *Genet Epidemiol* 2017;**41**:341–52. https://doi.org/10.1002/gepi.22041
30. Burgess S. Violation of the constant genetic effect assumption can result in biased estimates for non-linear Mendelian randomization. *Hum Hered* 2023 Aug 31 [Epub ahead of print]. https://doi.org/10.1159/000531659
31. Tian H, Mason AM, Liu C, Burgess S. Relaxing parametric assumptions for non-linear Mendelian randomization using a doubly-ranked stratification method. *Genet* 2023;19:e1010823. https://doi.org/10.1371/journal.pgen.1010823
32. van der Harst P, Verweij N. Identification of 64 novel genetic loci provides an expanded view on the genetic architecture of coronary artery disease. *Circ Res* 2018;**122**:433–43. https://doi.org/10.1161/circresaha.117.312086
33. Hartiala JA, Han Y, Jia Q, Hilser JR, Huang P, Gukasyan J, *et al.* Genome-wide analysis identifies novel susceptibility loci for myocardial infarction. *Eur Heart J* 2021;**42**:919–33. https://doi.org/10.1093/eurheartj/ehaa1040
34. Shah S, Henry A, Roselli C, Lin H, Sveinbjörnsson G, Fatemifar G, *et al.* Genome-wide association and Mendelian randomisation analysis provide insights into the pathogenesis of heart failure. *Nat Commun* 2020;**11**:163. https://doi.org/10.1038/s41467-019-13690-5
35. Roselli C, Chaffin MD, Weng LC, Aeschbacher S, Ahlberg G, Albert CM, *et al.* Multi-ethnic genome-wide association study for atrial fibrillation. *Nat Genet* 2018;**50**:1225–33. https://doi.org/10.1038/s41588-018-0133-9
36. Burgess S, Butterworth A, Thompson SG. Mendelian Randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol* 2013;**37**:658–65. https://doi.org/10.1002/gepi.21758
37. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol* 2016;**40**:304–14. https://doi.org/10.1002/gepi.21965
38. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol* 2015;**44**:512–25. https://doi.org/10.1093/ije/dyv080
39. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet* 2018;**50**:693–8. https://doi.org/10.1038/s41588-018-0099-7

40. Mounier N, Kutalik Z. Bias correction for inverse variance weighting Mendelian randomization. *Genet Epidemiol* 2023;47:314–31. https://doi.org/10.1002/gepi.22522
41. Adamstein NH, MacFadyen JG, Rose LM, Glynn RJ, Dey AK, Libby P, *et al.* The neutrophil-lymphocyte ratio and incident atherosclerotic events: analyses from five contemporary randomized trials. *Eur Heart J* 2021;**42**:896–903. https://doi.org/10.1093/eurheartj/ehaa1034
42. Cupido AJ, Kraaijenhof JM, Burgess S, Asselbergs FW, Hovingh GK, Gill D. Genetically predicted neutrophil-to-lymphocyte ratio and coronary artery disease: evidence from Mendelian randomization. *Circ Genom Precis Med* 2022;**15**:e003553. https://doi.org/10.1161/circgen.121.003553
43. Sugamura K, Keaney JF Jr. Reactive oxygen species in cardiovascular disease. *Free Radic Biol Med* 2011;**51**:978–92. https://doi.org/10.1016/j.freeradbiomed.2011.05.004
44. Moris D, Spartalis M, Spartalis E, Karachaliou GS, Karaolanis GI, Tsourouflis G, *et al.* The role of reactive oxygen species in the pathophysiology of cardiovascular diseases and the clinical significance of myocardial redox. *Ann Transl Med* 2017;**5**:326. https://doi.org/10.21037/atm.2017.06.27
45. Nagareddy PR, Murphy AJ, Stirzaker RA, Hu Y, Yu S, Miller RG, *et al.* Hyperglycemia promotes myelopoiesis and impairs the resolution of atherosclerosis. *Cell Metab* 2013;**17**:695–708. https://doi.org/10.1016/j.cmet.2013.04.001
46. Volz HC, Laohachewin D, Seidel C, Lasitschka F, Keilbach K, Wienbrandt AR, *et al.* S100a8/A9 aggravates post-ischemic heart failure through activation of RAGE-dependent NF-κB signaling. *Basic Res Cardiol* 2012;**107**:250. https://doi.org/10.1007/s00395-012-0250-z
47. Li Y, Chen B, Yang X, Zhang C, Jiao Y, Li P, *et al.* S100a8/a9 signaling causes mitochondrial dysfunction and cardiomyocyte death in response to ischemic/reperfusion injury. *Circulation* 2019;**140**:751–64. https://doi.org/10.1161/circulationaha.118.039262
48. Bergt C, Pennathur S, Fu X, Byun J, O'Brien K, McDonald TO, *et al.* The myeloperoxidase product hypochlorous acid oxidizes HDL in the human artery wall and impairs ABCA1-dependent cholesterol transport. *Proc Natl Acad Sci U S A* 2004;**101**:13032–7. https://doi.org/10.1073/pnas.0405292101
49. Marinković G, Grauen Larsen H, Yndigegn T, Szabo IA, Mares RG, de Camp L, *et al.* Inhibition of pro-inflammatory myeloid cell responses by short-term S100A9 blockade improves cardiac function after myocardial infarction. *Eur Heart J* 2019;**40**:2713–23. https://doi.org/10.1093/eurheartj/ehz461
50. Marinković G, Koenis DS, de Camp L, Jablonowski R, Graber N, de Waard V, *et al.* S100a9 links inflammation and repair in myocardial infarction. *Circ Res* 2020;**127**:664–76. https://doi.org/10.1161/circresaha.120.315865
51. Malle E, Marsche G, Arnhold J, Davies MJ. Modification of low-density lipoprotein by myeloperoxidase-derived oxidants and reagent hypochlorous acid. *Biochim Biophys Acta* 2006;**1761**:392–415. https://doi.org/10.1016/j.bbalip.2006.03.024
52. Zheng L, Nukuna B, Brennan ML, Sun M, Goormastic M, Settle M, *et al.* Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. *J Clin Invest* 2004;**114**:529–41. https://doi.org/10.1172/jci21109
53. Abdo AI, Rayner BS, van Reyk DM, Hawkins CL. Low-density lipoprotein modified by myeloperoxidase oxidants induces endothelial dysfunction. *Redox Biol* 2017;**13**:623–32. https://doi.org/10.1016/j.redox.2017.08.004
54. Döring Y, Libby P, Soehnlein O. Neutrophil extracellular traps participate in cardiovascular diseases: recent experimental and clinical insights. *Circ Res* 2020;**126**:1228–41. https://doi.org/10.1161/circresaha.120.315931
55. Shabir O, Berwick J, Francis SE. Neurovascular dysfunction in vascular dementia, Alzheimer's and atherosclerosis. *BMC Neurosci* 2018;**19**:62. https://doi.org/10.1186/s12868-018-0465-5
56. Huang YT, Hong FF, Yang SL. Atherosclerosis: the culprit and co-victim of vascular dementia. *Front Neurosci* 2021;**15**:673440. https://doi.org/10.3389/fnins.2021.673440
57. Gustavsson AM, van Westen D, Stomrud E, Engström G, Nägga K, Hansson O. Midlife atherosclerosis and development of Alzheimer or vascular dementia. *Ann Neurol* 2020;**87**:52–62. https://doi.org/10.1002/ana.25645
58. Hofman A, Ott A, Breteler MM, Bots ML, Slooter AJ, van Harskamp F, *et al.* Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer's disease in the Rotterdam Study. *Lancet* 1997;**349**:151–4. https://doi.org/10.1016/s0140-6736(96)09328-2
59. van Oijen M, de Jong FJ, Witteman JC, Hofman A, Koudstaal PJ, Breteler MM. Atherosclerosis and risk for dementia. *Ann Neurol* 2007;**61**:403–10. https://doi.org/10.1002/ana.21073
60. Korantzopoulos P, Letsas KP, Tse G, Fragakis N, Goudis CA, Liu T. Inflammation and atrial fibrillation: a comprehensive review. *J Arrhythm* 2018;**34**:394–401. https://doi.org/10.1002/joa3.12077
61. Marx C, Novotny J, Salbeck D, Zellner KR, Nicolai L, Pekayvaz K, *et al.* Eosinophil-platelet interactions promote atherosclerosis and stabilize thrombosis with eosinophil extracellular traps. *Blood* 2019;**134**:1859–72. https://doi.org/10.1182/blood.2019000518
62. Woollard KJ, Geissmann F. Monocytes in atherosclerosis: subsets and functions. *Nat Rev Cardiol* 2010;**7**:77–86. https://doi.org/10.1038/nrcardio.2009.228
63. Tabas I, Lichtman AH. Monocyte-macrophages and T cells in atherosclerosis. *Immunity* 2017;**47**:621–34. https://doi.org/10.1016/j.immuni.2017.09.008

Correction

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Correction to: Unravelling the heart's comic drama: can TLRs and hyaluronan metabolism stoke neutrophil rage in acute coronary syndrome?

This is a correction to: Grégory Franck, Unravelling the heart's comic drama: can TLRs and hyaluronan metabolism stoke neutrophil rage in acute coronary syndrome?, *European Heart Journal* 2023;**44**:3908–10, https://doi.org/10.1093/eurheartj/ehad455

In the originally published version of this editorial, in-text references to article 'Toll-like receptor 2, hyaluronan, and neutrophils play a key role in plaque erosion: the OPTICO-ACS study', by D. Meteva *et al.*, were incorrectly given as "Laggerbauer *et al.*".

This has been corrected.