

The complete blood count and cardiovascular disease: analyses across six cohorts of 23,370 adults

Sascha N. Goonewardena (1,2)*, Venkatesh L. Murthy(1)#

¹ Division of Cardiovascular Medicine, Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan, USA

² Division of Cardiovascular Medicine, VA Ann Arbor Health System, Ann Arbor, Michigan, USA.

*Primary contact: Sascha N. Goonewardena, MD. University of Michigan Frankel Cardiovascular Center, 1500 East Medical Center Drive, SPC 5853, Ann Arbor, MI 48109-5853. Email: sngoone@med.umich.edu

#Contact: Venkatesh L. Murthy, MD PhD. University of Michigan Frankel Cardiovascular Center, 1500 East Medical Center Drive, SPC 5873, Ann Arbor, MI 48109-5873. Email: vlmurthy@med.umich.edu

Keywords: cardiovascular disease; atherosclerosis; complete blood count; inflammation; lipoproteins; neutrophil; platelets; hemoglobin

Word Count: 4,350

Abbreviations:

ASCVD: atherosclerotic cardiovascular disease

BMP: basic metabolic panel

CAC: coronary artery calcification

CARDIA: Coronary Artery Risk Development in Young Adults

CBC: Complete blood count

- 1 CC: circulating cells
- 2 CM: circulating mediators
- 3 CVD: cardiovascular disease
- 4 CRIC: chronic renal insufficiency cohort
- 5 CRP: C-reactive protein
- 6 HbA1c: hemoglobin A1c
- 7 HDL: high-density lipoprotein
- 8 Hb: hemoglobin
- 9 LDL: low-density lipoprotein
- 10 MESA: Multi-Ethnic Study of Atherosclerosis
- 11 MI: myocardial infarction
- 12 NHANES: National Health and Nutrition Examination Survey
- 13 PCA: principal component analysis
- 14 PMN: neutrophil
- 15 RBC: red blood cell
- 16 WBC: white blood cell

Abstract

Background: The complete blood count (CBC) is one of the most commonly performed laboratory studies. Although studies have found associations between the CBC and cardiovascular disease (CVD), there is limited contemporary information regarding the relationship between the CBC and traditional risk factors and their joint association with CVD endpoints.

Objective: We sought to define the relationships between the CBC and traditional CVD risk factors and their joint association with CVD endpoints in diverse adult populations.

Methods: We first examined the relationships between the CBC variables (directly and their principal components), traditional CVD risk factors, and mortality in NHANES (n=7843). Next, we validated and extended these findings to more refined CVD endpoints in five additional cohorts (n=15,527).

Results: We first examined the variance accounted for by common laboratory studies (lipid panel, HbA1c, hs-CRP, and basic metabolic panel) by traditional risk factors in NHANES. With the exception of hemoglobin (Hb)-related components, we found that traditional risk factors accounted for less than 20% of the variance in CBC values, similar to that of lipid parameters. Additionally, in the clinically adjusted model, the CBC was more strongly associated with all-cause mortality than the lipid panel or CRP ($p<0.0001$). Next, we validated and extended these findings across five additional longitudinal cohorts with a mean follow-up of 16 years to evaluate the association of individual CBC parameters and their principal components with refined CVD endpoints. In the fully adjusted meta-analyses across the five cohorts, several CBC components including the white blood cell (WBC) count, neutrophil (PMN) count, hemoglobin (Hb) level, and an integrated immune cell score, were associated with individual CVD endpoints and a composite CV endpoint (MACE3: incident stroke, MI, and revascularization) with standardized hazard ratios of 1.13 ($p=0.002$), 1.15 ($p=0.0006$), 0.82 ($p<0.0001$), and 2.16 ($p<0.0001$) respectively.

Conclusion: This study represents the first systematic examination of the relationship between CBC features and established risk factors, as well as numerous CVD endpoints, in a diverse cohort

1 of 23,370 adults. The findings of this study underscore the potential utility of integrating CBC
2 features into CVD risk assessment and suggest important mechanistic insights into the
3 association between individual CBC components and the genesis of CVD.

1 Introduction

2 Some traditional risk factors for CVD include age, sex, and smoking.^{1,2} Building on demographic
3 characteristics, hemoglobin A1c (HbA1c) and creatinine (Cr) have been added to low-density
4 lipoprotein cholesterol (LDL-C) in CVD risk estimation instruments.^{3,4} Importantly, quantitative
5 molecular markers such as LDL-C and C-reactive protein (CRP) have not only advanced our risk
6 assessment but also have provided critical insights into the biological mechanisms of ASCVD and
7 have informed the development of cardiovascular therapies.^{5,6} Although the complete blood
8 count (CBC) is one of the most frequently performed laboratory studies in the United States, the
9 utility of the CBC and the individual CBC components for quantitative risk assessment of CVD is
10 understudied.⁷

11 Most laboratory studies focus on measuring small molecules or proteins that serve as circulating
12 molecular mediators (CMM) of CVD (e.g., Cr, CRP, HbA1c, and LDL-C). The CBC is the only
13 common laboratory study that profiles circulating cells (CC) that are known to contribute to CVD
14 through multiple mechanisms.⁸ However, little is known about the relationship between
15 traditional CVD risk factors and CBC components and how they together contribute to CVD risk.

16 Each of the three major aspects of the CBC (i.e., WBCs, RBCs, and platelets) has been associated
17 with CVD (primarily in the secondary prevention setting), including atherosclerotic cardiovascular
18 disease (ASCVD).⁹ Specifically, oxygen-carrying capacity (RBC parameters), thrombosis (platelet
19 parameters), and inflammation (WBC parameters) are all intimately connected to atherogenesis
20 and specific CVD phenotypes.¹⁰ With respect to oxygen-carrying capacity, several studies have
21 found associations between RBC counts and CVD. For example, Sabatine and colleagues found
22 that anemia was an independent predictor of MACE in acute coronary syndromes¹¹. Platelet
23 parameters have not only been associated with CVD risk but also are canonical cell targets to
24 reduce CVD risk.¹² Inflammation is associated with the initiation, progression, and clinical
25 manifestations of both ASCVD and CVD.¹³ More than simply an association, several randomized
26 control trials (RCT) of anti-inflammatory therapies have yielded promising results for ASCVD.^{14–16}

Although individual CBC components have been associated with CVD, the influence of traditional risk factors on the CBC and the effect of CBC components on incident CVD endpoints across diverse, stable outpatients remain largely unknown. To further elucidate these relationships, we first examined the associations between the CBC, traditional risk factors, and mortality in NHANES (n=7843). We grouped subsets of CBC parameters representing independent axes of biological variation in NHANES using principal component (PC) analysis. We then examined the relationship between individual CBC components and PC scores on incident CVD endpoints using adjusted Cox proportional hazards models across 15,527 individuals from five longitudinal cohorts (Framingham Offspring, Framingham 3rd Gen, MESA, CRIC, and CARDIA cohorts). We demonstrated that CBC-derived parameters are powerful predictors of incident CVD in diverse adult populations, providing novel insights into the contributions of the CBC to the pathobiology and risk of CVD.

Materials and Methods

NHANES

Cohort description

The National Health and Nutrition Examination Survey (NHANES) is a biennial weighted survey with sampling weights designed to be representative of non-institutionalized residents of the United States. We obtained data from the 2003-4, 2005-6, 2007-8, and 2009-10 cycles of NHANES and linked mortality outcomes from the National Center for Health Statistics (<https://www.cdc.gov/nchs/nhanes/index.htm>). NHANES participants provided informed consent and the overall study was approved by the NCHS Research Ethics Review Board. Because NHANES data are publicly available this analysis was exempt from local IRB review.

Sample selection

Starting with 41,156 individual participants, we excluded individuals who were aged <20 years (N=18,983), who did not attend a fasting examination (N=11,935), and who had incomplete key

covariates (N=959). We further excluded participants who did not have a complete blood count (CBC) (N=374) or differential (N=29), basic metabolic profile [BMP] (N=137), C-reactive protein (CRP) (N=3), hemoglobin A1c (HbA1c) (N=24), or lipid profile (N=214). We also excluded individuals with zero or missing weight in sampling weights computed for fasting participants (N=651) and individuals where the total of all components of the differential summed to <95% or 105% (N=4).

Laboratory measurements

We evaluated commonly obtained laboratory studies including fasting lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides), CBC with differential (hematocrit, hemoglobin, RBC count, WBC count, platelet count, red cell distribution width [RDW], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC], lymphocyte percentage, neutrophil percentage, monocyte percentage, basophil percentage, eosinophil percentage, lymphocyte count, neutrophil count, monocyte count, basophil count, and eosinophil count), BMP (sodium, potassium, glucose, chloride, bicarbonate, blood urea nitrogen [BUN], creatinine, and total calcium), CRP, and HbA1c. Laboratory values were hyperbolic arcsine transformed to improve normality and scaled to zero mean and unit variance.

Variance explained in laboratory measurements by clinical parameters

We first evaluated the extent of independent information in each lab parameter by computing the variance of each transformed and scaled lab variable explained by standard clinical risk factors (*i.e.* age, sex, race, smoking, body mass index, systolic and diastolic blood pressure, use of antihypertensive medications, self-reported diabetes status) using generalized linear models accounting for fasting survey weights and type I analysis of variance.

Outcome analyses

We then related each laboratory panel (with CRP and HbA1c treated as separate panels) to all-cause mortality, cardiac mortality and cardiovascular mortality using Cox proportional hazard models, accounting for fasting survey weights. We computed unadjusted models as well as

models adjusted for age, sex, and race as well as models adjusted for standard clinical risk factors (age, sex, race, smoking, body mass index, systolic and diastolic blood pressure, use of antihypertensive medications, and diabetes status).

Principal Component Analyses

Finally, we performed principal component analyses across all common laboratory panels as well as on each laboratory panel separately using varimax post-rotation, selecting the minimum number of components to explain at least 60% of total variance to group related measures within each panel and to reduce dimensionality. PCA scores were then computed for each participant and used as the key predictor in survival analysis repeated, analogous to above.

Repeatability of Laboratory Measures

To evaluate the stability of repeated laboratory measures over time, we leverage a subsample of the 2001-2 cycle of NHANES (N=551) who were asked to return within 8 days for repeat laboratory assessments. We computed within and between subjects coefficient of variation (CV) as well as two way intra-class correlation (ICC) to assess consistency.

Longitudinal Cohorts

Cohort descriptions

We then sought to validate the prognostic relevance of the CBC across multiple well-characterized longitudinal cohort studies: Coronary Artery Risk Development in Young Adults (CARDIA), Chronic Renal Insufficiency Cohort (CRIC), Framingham Heart Study (FHS) Offspring (OS) and 3rd Generation cohorts (3G), and the Multi-Ethnic Study of Atherosclerosis (MESA). Data for CARDIA, FHS OS, FHS 3G, and MESA were obtained from NHLBI BioLINCC (<https://biolincc.nhlbi.nih.gov/home/>) while data for CRIC were obtained from the NIDDK Central Repository (<https://repository.niddk.nih.gov/home/>). Participants in each study provided informed consent and each study was approved by the local IRBs at each recruitment site. This

secondary analysis was approved by the University of Michigan Institutional Review Board. Further details on cohorts are provided in the Supplemental methods.

Outcomes analyses

Within each cohort, we computed PCA scores for the CBC with differential variables using weights from the varimax-rotated PCA performed in NHANES. We then related the predictors (individual underlying CBC and differential variables and the PCA scores) to each outcome [incident myocardial infarction [MI], incident stroke, incident coronary revascularization, MACE3 (composite of mortality, incident MI, and incident stroke), and MACE4 (composite of mortality, incident MI, incident stroke, and incident coronary revascularization), and all-cause mortality] using Cox proportional hazards regression. We performed unadjusted, adjusted for age, sex, and race, and adjusted for clinical covariates (age, sex, race, smoking, body mass index, systolic and diastolic blood pressure, use of antihypertensive medications, diabetes status, total cholesterol, HDL, LDL, and triglycerides) regression analyses. Further details of exclusions in each cohort are provided in the Supplemental methods section.

Meta-analyses

We combined standardized hazard ratios (standardized to NHANES mean and variance) using random effects meta-analyses. We performed leave one out meta-analyses to evaluate impact of individual cohorts on estimates.

Statistical analyses

All statistical analyses were performed in R 4.4.1 (R Foundation for Statistical Research, Vienna Austria). Key modules included: *metafor* (for meta-analyses), *survival* (for survival analyses), *survey* (for survey weighted analyses), *srvyr* (for survey weighted analyses), and *irr* (for intra-class correlation). Modules were obtained from the Comprehensive R Archive Network (CRAN).

Results

CBC, traditional risk factors, and all-cause mortality

Because traditional risk factors can influence quantitative assessments of both CM and CC, we sought first to determine the variance explained in standard laboratory studies by traditional risk factors. We leveraged data from the 2003-2010 cycles of NHANES. 7,843 subjects had complete data and were included in this first analysis. The average age was 49, and 52% were female (**Supplemental Table 1**). We used principal component analysis to partition the total variance across all laboratory values, including individual components of the CBC. Eight principal components (PC) explained 63% of the total variance across all commonly performed laboratory studies; interestingly, many of the most highly weighted features were related to the CBC, including the lymphocyte, neutrophil (PMN), and monocyte counts (**Supplemental Figure 1**). Because of the importance and strong weighting of the CBC components in relation to all common laboratory studies, we sought to define the influence of traditional CVD risk factors on the values of all individual laboratory parameters (**Figure 1A**). With respect to total cholesterol and LDL-C, less than 10% of the total variance is explained by traditional CVD risk factors. In contrast, over 30% of the variance in the values of creatinine Cr and Hb can be accounted for by traditional CVD risk factors. Conversely, most components of the CBC (except red cell parameters) are more like LDL-C (with low variance explained by traditional risk factors) than they are like CRP (with moderate or greater variance explained by traditional risk factors). Based on these observations, it was clear that the CBC itself contained a large amount of independent information relative to the other laboratory studies of CM. To build on these observations, we examined the weighted association between all-cause mortality and each of the laboratory studies using incremental chi-squared (adjusted for the traditional risk factors detailed above) (**Figure 1B**). Intriguingly, we found that the CBC and CBC components have the strongest contribution to all-cause mortality in NHANES even when compared to the lipid panel, HbA1c, CRP, and basic metabolic panel (lipid panel $\Delta\chi^2=27.0$, $p = 0.0008$; HbA1c $\Delta\chi^2=6.0$, $p=0.01$; CRP $\Delta\chi^2=64.8$, $p<0.0001$; BMP $\Delta\chi^2=88.8$, $p<0.0001$; CBC $\Delta\chi^2=215.8$, $p<0.0001$; CBC with differential $\Delta\chi^2=258.9$, $p<0.0001$). Because the individual components of the CBC had distinct and

complementary information content, we performed PCA on the CBC components to derive independent groups of CBC measures. Approximately 63% of the variance in CBC components can be captured by 4 PCs (**Figure 2A**). Interestingly, the 4 major CBC PCs could be broadly categorized as 1) PMN/lymphocytes, 2) RBC quantity, 3) other inflammatory cells, and 4) RBC properties. Additionally, the CBC and its individual components had favorable stability and intra- and intersubject variability compared with other commonly used laboratory studies, considerations important when considering the clinical utility of any quantitative biomarker (**Supplemental Figure 2**).

CBC features are independently associated with refined CVD phenotypes in diverse adults

Building on our findings from the cross-sectional NHANES analyses, we wished to not only validate the mortality findings but also to extend them to more diverse cohorts with longitudinal follow-up leveraging more refined CVD endpoints including incident including myocardial infarction (MI), stroke, and coronary revascularization. We examined 15,527 subjects across the FHS offspring, FHS third generation, MESA, CARDIA, and CRIC cohorts. The average age in this longitudinal analysis was diverse, from young adults (CARDIA; 25 years) to older adults (FHS OS; 69 years). The baseline characteristics for individuals included from each cohort are shown in **Table 1**. Over a total of 249,098 follow-up person-years there were 697 incident MI, 458 incident strokes, 234 incident coronary revascularizations, and 2,459 deaths. To understand the relationships between the CBC and these CVD endpoints, we quantified the associations between the CBC and CVD endpoints using two broad approaches. First, we quantified CVD risk across the spectrum of individual CBC components and second, we quantified CVD risk based on the four CBC PC scores derived from the cross-sectional NHANES analysis (**Supplemental data file and Figure 3**). In the fully adjusted model, several observations are worth noting. All CBC components except basophil, eosinophil, and platelet counts were associated with all-cause mortality. WBC, PMN, lymphocyte, and monocyte counts were associated with the CVD composite endpoint (MACE3). Additionally, even after adjusting for hs-CRP (a marker of systemic inflammation), the CBC components remained related to all-cause mortality and several CVD

endpoints (**Supplemental Figure 3**). Building on the PCA of CBC values in NHANES, we applied the PC dimensions to the cohorts and examined their association with more refined CVD endpoints and all-cause mortality in FHS offspring, FHS 3G, MESA, CARDIA, and CRIC cohorts (n=15,527). Numerous strong associations were evident with all of the CVD endpoints and all-cause mortality with the CBC PC dimensions (**Supplemental Figure 4**). Of note, the PMN/lymphocyte (hazard ratio 2.45 [1.42-4.24]; p=0.001) and RBC quantity (hazard ratio 1.86 [1.34-2.59]; p=0.0002) PC scores were strongly associated with all-cause mortality in each cohort and when all cohorts were combined (**Figure 4**).

Discussion

In this study of 23,370 diverse adults across six cohorts, we defined the relationships between the CBC, traditional CV risk factors, CVD endpoints, and all-cause mortality. Specifically, we found that: 1) traditional CVD risk factors explain less than 10% of the variance in most CBC components; 2) CBC components were associated with all-cause mortality in all six cohorts examined; and 3) individual CBC components were strongly associated with MI, revascularization, and stroke and these effects were only modestly attenuated after adjustment for traditional risk factors and standard laboratory values including CRP. To our knowledge, this is the first study to systematically examine the association of the CBC and its components to traditional risk factors and other common laboratory studies with CVD endpoints across a large, diverse sample of adults.

The associations between CC and CVD were first described in a landmark case-control study in patients with MI.¹⁷ Subsequently, several cohort studies have validated and built on findings from this seminal study.^{18,19} For example, a large UK study from the CALIBER cohort demonstrated that high PMN counts and low lymphocyte counts had strong associations with myocardial infarction, peripheral arterial disease, and heart failure.²⁰ With respect to the effects of CV therapies on inflammation and WBC parameters, a recent analysis of five randomized control trials demonstrated that the NLR was associated with CV events and all-cause mortality.²¹ Intriguingly, this analysis found that specific CV therapies, including lipid-lowering and anti-

inflammatory therapies, had distinct effects on the NLR, reinforcing not only that specific circulating immune cell populations are associated with CVD but also that anti-inflammatory therapeutics have distinct effects on CC populations and the immune system in general. In addition to the immune components of the CBC, RBC features have also been associated with CVD.²² Sabatine and colleagues found that low values of RBC features were an independent predictor of MACE in acute coronary syndromes.¹¹ Studies have typically focused on anemia and elevated WBC counts; however, we found that even within normal ranges, WBC and RBC features are associated with CVD endpoints (**Supplemental Figure 5**). Our study builds on these prior studies, validating CBC associations with CVD even after correction for traditional ASCVD risk factors and inflammatory biomarkers.

The quantitative risk assessment of CVD is the cornerstone of both primary and secondary prevention. It informs patient-centered diagnostic and therapeutic approaches. The current quantitative risk approaches combine patient demographic characteristics (including age and sex), biological variables (blood pressure and smoking), and laboratory studies (lipid and diabetes parameters). Our findings add to the discourse on CVD risk assessment, suggesting that readily available laboratory information from the CBC could complement existing components of quantitative CVD risk assessments.

Strengths and Limitations

Several limitations of our study warrant consideration. First, the observational nature of our study precludes any strict determinations of causality with respect to CC pathobiology; however, we believe that our findings are hypothesis generating and can help inform future mechanistic and translational studies and underscore the importance of further characterization of CC contributions to ASCVD. Second, despite the consistency of our findings across cohorts, there is heterogeneity in the characteristics of each cohort, making comparisons imperfect. However, this heterogeneity can also be viewed as a strength, because the main conclusions are consistent across diverse cohorts, strengthens them, and makes possible more generalizations. Importantly, the triangulation from complementary approaches yielded consistent results, largely strengthening the robustness and validity of the findings.

Conclusion

In summary, these contemporary analyses of 23,370 subjects across six cohorts systematically relate the CBC to both traditional risk factors and CVD endpoints. Our findings highlight the potential of incorporating the CBC into CVD risk assessment and suggest novel mechanistic insights into the role of CBC components in the pathobiology of CVD. This study adds further evidence linking CBC components to all dimensions of CVD and furthers our understanding of how CC can be used to identify individuals at risk of CVD.

Author Contributions

SNG and VLM contributed equally, and both designed the research, analyzed the data, and wrote the paper.

Funding and Declaration of Interests

Dr. Goonewardena is supported by VA MERIT grant 1I01CX002560, NIH/NHLBI grant R01HL150392, and the Taubman Medical Research Institute (Wolfe Scholarship). Dr. Murthy owns stock or stock options in General Electric, Amgen, Cardinal Health, Ionetix, Boston Scientific, Merck, Eli Lilly, Johnson and Johnson, Viatris, and Pfizer. He has received research grants and consulting fees from Siemens Medical Imaging. He has served on medical advisory boards for Ionetix. He is a consultant for INVIA Medical Imaging Solutions. Dr. Murthy is supported in part by grants from the National Institute of Diabetes, Digestive, and Kidney Diseases (U01DK123013-03); National Institute on Aging (R01 AG059729); National Heart, Lung and Blood Institute (R01 HL136685); American Heart Association Strategically Focused Research Network grant in Cardiometabolic Disease; and the Melvyn Rubenfire Professorship in Preventive Cardiology.

Data and Code Availability

Data for NHANES are freely available from the National Center for Health Statistics

1 Data for CARDIA, FHS OS, FHS 3G and MESA were obtained from NHLBI BioLINCC and can be
2 obtained by qualified investigators from NHLBI with an approved research proposal, data use
3 agreement and IRB approval.

4 Data for CRIC were obtained from the NIDDK Central Repository and can be obtained by qualified
5 investigators from NHLBI with an approved research proposal, data use agreement and IRB
6 approval.

7 No new software algorithms or tools were developed for this project.

8 **Acknowledgements**

9 Although not directly involved in the conduct of this study, we thank the research volunteers and
10 staff of the underlying studies for their contributions and commitment to research.

11 **References**

- 12 1. Artola Arita V, Beigrezaei S, Franco OH. Risk factors for cardiovascular disease: the known
13 unknown. *European Journal of Preventive Cardiology* 2024;31:e106–e107.
14 doi:10.1093/eurjpc/zwad392.
- 15 2. Bays HE, Taub PR, Epstein E, Michos ED, Ferraro RA, Bailey AL, et al. Ten things to know
16 about ten cardiovascular disease risk factors. *American Journal of Preventive Cardiology*
17 2021;5:100149. doi:10.1016/j.ajpc.2021.100149.
- 18 3. Goff DC, Lloyd-Jones DM, Bennett G, Coady S, D’Agostino RB, Gibbons R, et al. 2013
19 ACC/AHA Guideline on the Assessment of Cardiovascular Risk: A Report of the American
20 College of Cardiology/American Heart Association Task Force on Practice Guidelines.
21 *Circulation* 2014;129. doi:10.1161/01.cir.0000437741.48606.98.
- 22 4. Khan SS, Matsushita K, Sang Y, Ballew SH, Grams ME, Surapaneni A, et al. Development and
23 Validation of the American Heart Association’s PREVENT Equations. *Circulation*
24 2024;149:430–449. doi:10.1161/CIRCULATIONAHA.123.067626.
- 25 5. Nissen SE, Tuzcu EM, Schoenhagen P, Crowe T, Sasiela WJ, Tsai J, et al. Statin Therapy, LDL
26 Cholesterol, C-Reactive Protein, and Coronary Artery Disease. *N Engl J Med* 2005;352:29–
27 38. doi:10.1056/NEJMoa042000.
- 28 6. Ridker PM, Moorthy MV, Cook NR, Rifai N, Lee I-M, Buring JE. Inflammation, Cholesterol,
29 Lipoprotein(a), and 30-Year Cardiovascular Outcomes in Women. *N Engl J Med*
30 2024;NEJMoa2405182. doi:10.1056/NEJMoa2405182.
- 31 7. Medicare Part B Spending on Clinical Diagnostic Laboratory Tests in 2022 n.d.
- 32 8. Mallat Z, Tedgui A. Century of Milestones and Breakthroughs Related to the Immune

- 1 Mechanisms of Atherosclerosis. *ATVB* 2024;44:1002–1006.
- 2 doi:10.1161/ATVBAHA.124.319397.
- 3 9. Horne BD, Anderson JL, John JM, Weaver A, Bair TL, Jensen KR, et al. Which White Blood
- 4 Cell Subtypes Predict Increased Cardiovascular Risk? *Journal of the American College of*
- 5 *Cardiology* 2005;45:1638–1643. doi:10.1016/j.jacc.2005.02.054.
- 6 10. Seo I-H, Lee Y-J. Usefulness of Complete Blood Count (CBC) to Assess Cardiovascular and
- 7 Metabolic Diseases in Clinical Settings: A Comprehensive Literature Review. *Biomedicines*
- 8 2022;10:2697. doi:10.3390/biomedicines10112697.
- 9 11. Sabatine MS, Morrow DA, Giugliano RP, Burton PBJ, Murphy SA, McCabe CH, et al.
- 10 Association of Hemoglobin Levels With Clinical Outcomes in Acute Coronary Syndromes.
- 11 *Circulation* 2005;111:2042–2049. doi:10.1161/01.CIR.0000162477.70955.5F.
- 12 12. Tsai M-T, Chen Y-T, Lin C-H, Huang T-P, Tarng D-C. U-shaped mortality curve associated with
- 13 platelet count among older people: a community-based cohort study. *Blood*
- 14 2015;126:1633–1635. doi:10.1182/blood-2015-06-654764.
- 15 13. Lawler PR, Bhatt DL, Godoy LC, Lüscher TF, Bonow RO, Verma S, et al. Targeting
- 16 cardiovascular inflammation: next steps in clinical translation. *European Heart Journal*
- 17 2021;42:113–131. doi:10.1093/eurheartj/ehaa099.
- 18 14. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, et al.
- 19 Antiinflammatory Therapy with Canakinumab for Atherosclerotic Disease. *N Engl J Med*
- 20 2017;377:1119–1131. doi:10.1056/NEJMoa1707914.
- 21 15. Tardif J-C, Kouz S, Waters DD, Bertrand OF, Diaz R, Maggioni AP, et al. Efficacy and Safety of
- 22 Low-Dose Colchicine after Myocardial Infarction. *N Engl J Med* 2019;381:2497–2505.
- 23 doi:10.1056/NEJMoa1912388.
- 24 16. Ridker PM, Devalaraja M, Baeres FMM, Engelmann MDM, Hovingh GK, Ivkovic M, et al. IL-6
- 25 inhibition with ziltivekimab in patients at high atherosclerotic risk (RESCUE): a double-blind,
- 26 randomised, placebo-controlled, phase 2 trial. *The Lancet* 2021;397:2060–2069.
- 27 doi:10.1016/S0140-6736(21)00520-1.
- 28 17. Leukocyte Count and Myocardial Infarction: Correction. *N Engl J Med* 1974;291:1361–1361.
- 29 doi:10.1056/NEJM197412192912517.
- 30 18. Horne BD, May HT, Muhlestein JB, Ronnow BS, Lappé DL, Renlund DG, et al. Exceptional
- 31 Mortality Prediction by Risk Scores from Common Laboratory Tests. *The American Journal*
- 32 *of Medicine* 2009;122:550–558. doi:10.1016/j.amjmed.2008.10.043.
- 33 19. Wheeler J. Associations between differential leucocyte count and incident coronary heart
- 34 disease: 1764 incident cases from seven prospective studies of 30 374 individuals. *European*
- 35 *Heart Journal* 2004;25:1287–1292. doi:10.1016/j.ehj.2004.05.002.
- 36 20. Shah AD, Denaxas S, Nicholas O, Hingorani AD, Hemingway H. Neutrophil Counts and Initial
- 37 Presentation of 12 Cardiovascular Diseases. *Journal of the American College of Cardiology*
- 38 2017;69:1160–1169. doi:10.1016/j.jacc.2016.12.022.
- 39 21. Adamstein NH, MacFadyen JG, Rose LM, Glynn RJ, Dey AK, Libby P, et al. The neutrophil–
- 40 lymphocyte ratio and incident atherosclerotic events: analyses from five contemporary
- 41 randomized trials. *European Heart Journal* 2021;42:896–903.
- 42 doi:10.1093/eurheartj/ehaa1034.
- 43 22. Lee WJ, Choi S, Park SM, Lee G, Chang J, Oh YH, et al. Association of hemoglobin variability
- 44 with the risk of cardiovascular disease: a nationally representative retrospective cohort

- 1 study from South Korea. Sci Rep 2023;13:2148. doi:10.1038/s41598-023-28029-w.
- 2
- 3

1 Tables

2 Table 1. Participant characteristics of longitudinal cohorts

3

Characteristic	FHS OS N = 1,816 ¹	FHS 3G N = 2,629 ¹	MESA N=2,598 ¹	CARDIA N = 4,768 ¹	CRIC N = 3,716 ¹
Age	69 (64, 75)	48 (41, 54)	68 (61, 76)	25.0 (22.0, 28.0)	59 (52, 66)
Female Sex	1,062 (58%)	1,379 (52%)	1,406 (54%)	2,607 (55%)	1,669 (45%)
Race					
White	1,731 (95%)	2,358 (90%)	1,118 (43%)	2,329 (49%)	1,736 (47%)
Non-White	15 (0.8%)	271 (10%)			
Black			724 (28%)	2,439 (51%)	1,489 (40%)
Chinese-American			24 (0.9%)		
Hispanic			732 (28%)		
American Indian/Alaska native					15 (0.4%)
Asian					105 (2.8%)
More than one race					78 (2.1%)
Native Hawaiian/Pacific Islands					6 (0.2%)
Unknown	70 (3.9%)				287 (7.7%)
Smoking					
current	111 (6.1%)	243 (9.2%)	211 (8.1%)	1,461 (31%)	488 (13%)
former	984 (54%)	819 (31%)	1095 (42%)	638 (13%)	1,542 (41%)
never	721 (40%)	1,567 (60%)	1,275 (49%)	2,669 (56%)	1,686 (45%)
Body Mass Index	27.7 (24.7, 31.1)	27.1 (24.1, 31.1)	28.5 (25.2, 32.4)	23.5 (21.1, 26.4)	31 (27, 36)
Total Cholesterol	187 (163, 210)	184 (163, 207)	181 (157, 206)	173 (153, 197)	178 (152, 208)
HDL Cholesterol	61 (49, 75)	57 (47, 71)	53 (44, 64)	52 (44, 61)	45 (37, 54)
LDL Cholesterol	99 (82, 121)	102 (83, 123)	104 (83, 126)	106 (87, 127)	98 (78, 123)
Triglycerides	99 (75, 137)	94 (68, 134)	93 (70, 130)	62 (45, 84)	128 (89, 185)

Systolic Blood Pressure	126 (115, 137)	116 (107, 126)	120 (110, 136)	110 (103, 117)	125 (113, 141)
Diastolic Blood Pressure	72 (66, 79)	75 (68, 81)	68 (61, 75)	68 (62, 75)	71 (62, 80)
BP Medication Use	949 (52%)	488 (19%)	1,433 (55%)	107 (2.2%)	3,608 (97%)
Diabetes					
Diabetic	246 (14%)	128 (4.9%)	511 (20%)	40 (0.8%)	1,800 (48%)
Non-Diabetic	1,570 (86%)	2,501 (95%)	2,087 (80%)		1,916 (52%)
High Sensitivity C-Reactive Protein	1.54 (0.77, 3.21)	1.27 (0.61, 2.95)	2.1 (0.9, 4.4)		3 (1, 6)
Hematocrit	40.0 (37.7, 42.5)	40.8 (38.4, 43.7)	40.0 (37.5, 42.7)	42.0 (39.0, 45.5)	37.4 (34.2, 40.9)
Hemoglobin	13.70 (12.90, 14.50)	14.00 (13.10, 15.00)	13.40 (12.40, 14.40)	14.20 (13.20, 15.30)	12.55 (11.40, 13.80)
RBC Count	4.33 (4.07, 4.63)	4.51 (4.23, 4.84)	4.48 (4.15, 4.79)	4.78 (4.41, 5.17)	4.26 (3.86, 4.66)
WBC Count	5.75 (4.80, 6.90)	5.80 (4.90, 6.80)	5.80 (4.80, 6.90)	5.80 (4.80, 7.10)	6.30 (5.20, 7.70)
Mean Corpuscular Hemoglobin	31.60 (30.50, 32.50)	31.10 (30.10, 32.00)	30.20 (28.90, 31.40)	29.93 (28.66, 31.07)	30.00 (28.40, 31.00)
Mean Corpuscular Hemoglobin Concentration	34.20 (33.60, 34.70)	34.20 (33.70, 34.60)	33.40 (32.80, 34.10)	33.76 (33.18, 34.30)	33.70 (33.00, 34.20)
Mean Corpuscular Volume	92.5 (89.8, 95.2)	90.8 (88.2, 93.5)	90.0 (87.0, 93.0)		88.8 (85.0, 92.0)
Red Cell Distribution Width	13.00 (12.60, 13.50)	12.70 (12.40, 13.20)	13.90 (13.40, 14.60)		
Platelet Count	229 (197, 268)	241 (207, 278)	224 (187, 266)	267 (227, 313)	237 (195, 284)
Platelet Volume	8.80 (8.20, 9.40)	8.40 (7.90, 9.00)			
Lymphocytes (%)	26 (21, 31)	28 (24, 33)	30 (24, 36)	37 (30, 43)	27 (21, 33)
Neutrophils (%)	61 (55, 66)	59 (54, 64)	59 (53, 65)	53 (46, 60)	62 (55, 68)
Monocytes (%)	8.80 (7.50, 10.20)	8.40 (7.20, 9.80)	8.00 (6.00, 9.00)	5.00 (4.00, 7.00)	7.30 (6.00, 8.94)
Basophils (%)	0.60 (0.40, 0.80)	0.80 (0.60, 1.00)	1.00 (0.00, 1.00)	0.00 (0.00, 1.00)	0.40 (0.00, 0.90)
Eosinophils (%)	2.90 (2.00, 4.20)	2.60 (1.80, 3.90)	2.00 (1.00, 4.00)	2.00 (1.00, 3.00)	2.78 (1.72, 4.12)
Lymphocyte Count	1.50 (1.20, 1.80)	1.60 (1.30, 1.90)	1.70 (1.35, 2.10)	2.10 (1.73, 2.54)	1.65 (1.30, 2.09)

Neutrophil Count	3.50 (2.80, 4.30)	3.40 (2.70, 4.20)	3.40 (2.70, 4.30)	3.02 (2.30, 4.02)	3.83 (2.95, 4.96)
Monocyte Count	0.50 (0.40, 0.60)	0.50 (0.40, 0.60)	0.40 (0.31, 0.57)	0.30 (0.20, 0.41)	0.46 (0.36, 0.60)
Basophil Count	0.00 (0.00, 0.00)	0.00 (0.00, 0.10)	0.00 (0.00, 0.05)	0.00 (0.00, 0.06)	0.03 (0.00, 0.05)
Eosinophil Count	0.20 (0.10, 0.30)	0.20 (0.10, 0.20)	0.10 (0.10, 0.20)	0.12 (0.06, 0.20)	0.18 (0.10, 0.27)
Neutrophil:Lymphocyte Ratio	2.35 (1.77, 3.10)	2.10 (1.65, 2.64)	1.97 (1.46, 2.69)	1.45 (1.07, 1.97)	2.32 (1.73, 3.18)
Incident MACE3	267 (15%)	78 (3.0%)	214 (8.2%)	620 (13%)	1,793 (48%)
Incident MACE4	285 (16%)	99 (3.8%)	239 (9.2%)	651 (14%)	
Incident MI	33 (1.8%)	24 (0.9%)	41 (1.6%)	104 (2.2%)	495 (13%)
Incident Stroke	43 (2.4%)	14 (0.5%)	52 (2.0%)	109 (2.3%)	240 (6.5%)
Incident Coronary Revascularization	42 (2.3%)	40 (1.5%)	46 (1.8%)	106 (2.2%)	
All Cause Mortality	218 (12%)	43 (1.6%)	148 (5.7%)	470 (9.9%)	1,580 (43%)

1 1 Median (Q1, Q3); n (%)

Figures

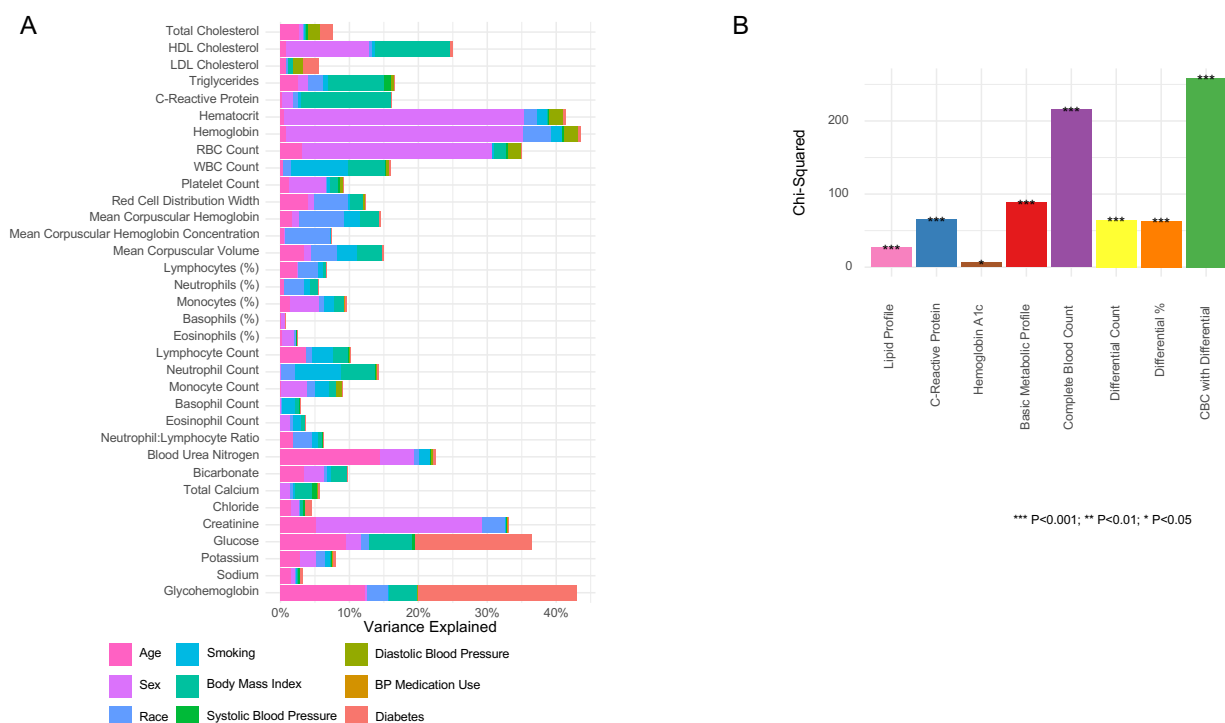


Figure 1. The variance explained of common laboratory studies by traditional risk factors and information content for all-cause mortality.

A) We leveraged data from the 2003-2010 cycles of NHANES to assess the variance explained of common laboratory measures by traditional risk factors using 6,162 subjects with complete data and fasting status. Type I analysis of variance accounting for survey weights was performed to estimate percent variance explained after hyperbolic arcsine transformation. These results illustrate that values from the CBC had comparable independent information content beyond traditional risk factors and comparable to that of lipid and basic metabolic panels. B) Using incremental chi-squared, we examined the survey-weighted association between common laboratory panels and all-cause mortality. Adjustments were made for age, sex, race, smoking, BMI, blood pressure, use of antihypertensive medications, and self-reported diabetes. Higher χ^2 indicates greater information content.

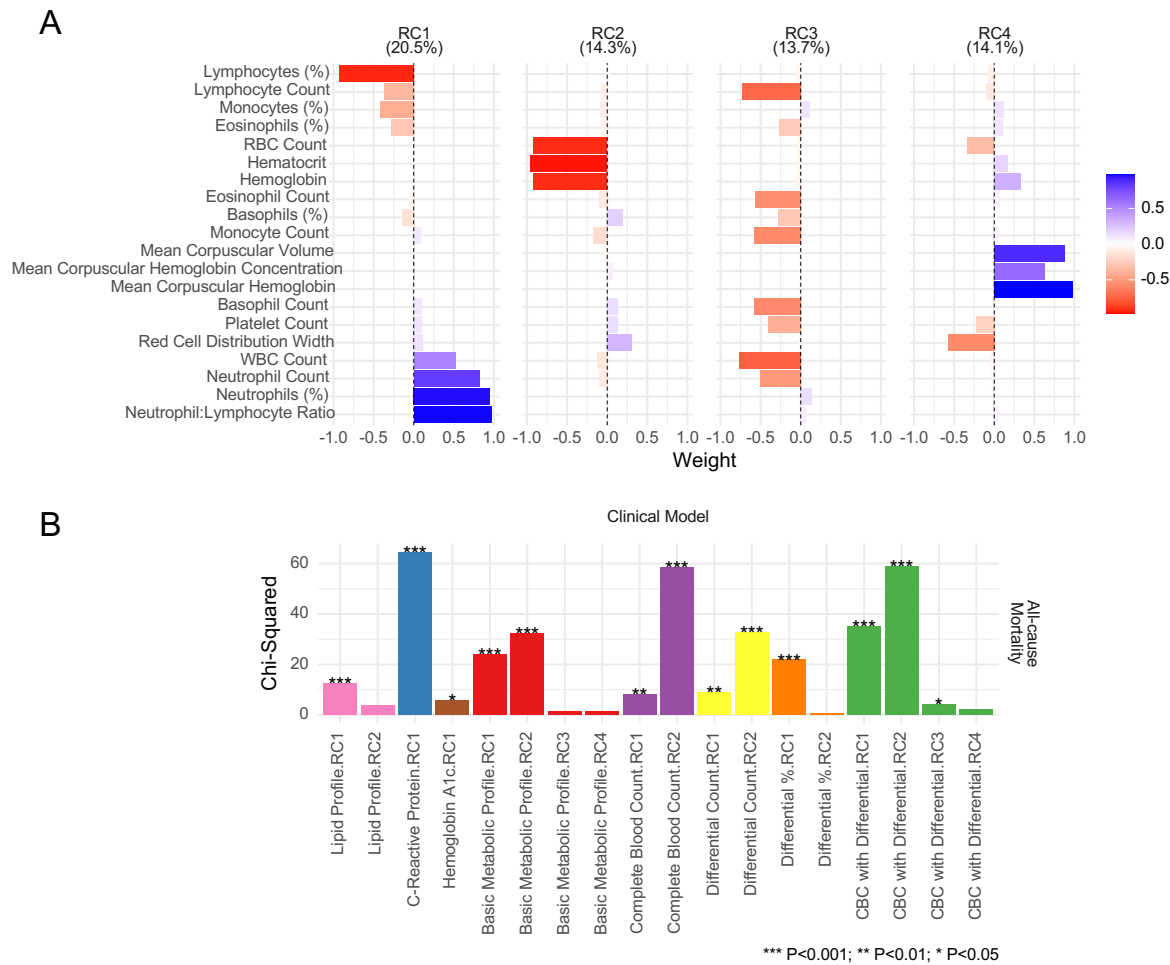


Figure 2. Dimension reduction of the CBC and information content for all-cause mortality.

A) Principal component analysis (PCA) of the CBC with differential demonstrated that four components explain 62.9% of the total variance in the CBC with differential study. PCA was performed with varimax post-rotation and accounted for survey sampling weights for the fasting subpopulation in NHANES. The first component (explaining 20.5% of the total variance) was positively loaded on total WBC count, neutrophils, and NLR and negatively loaded on lymphocytes. The second component (explaining for 14.3% of the total variance) was loaded on red blood cell quantity measures, negatively loaded on RBC count, hematocrit, and hemoglobin. The third component (explaining 13.7% of total variance) was negatively loaded with multiple inflammatory cell parameters including total WBC count, lymphocytes, and eosinophils. Finally,

the fourth component (explaining 14.1% of total variance) was loaded on red blood cell characteristics with positive loadings for MCH, MCHC, and MCV and negative loading for RDW. B) Analogously to Figure 2, we computed incremental χ^2 for Cox proportional hazards models for all-cause mortality adding rotated principal components of common laboratory panels to clinical variables (*i.e.* age, sex, race, smoking, body mass index, systolic and diastolic blood pressure, use of antihypertensive medications, self-reported diabetes status). Higher χ^2 indicates greater information content. Models accounted for survey sampling weights for the fasting subpopulation in NHANES.

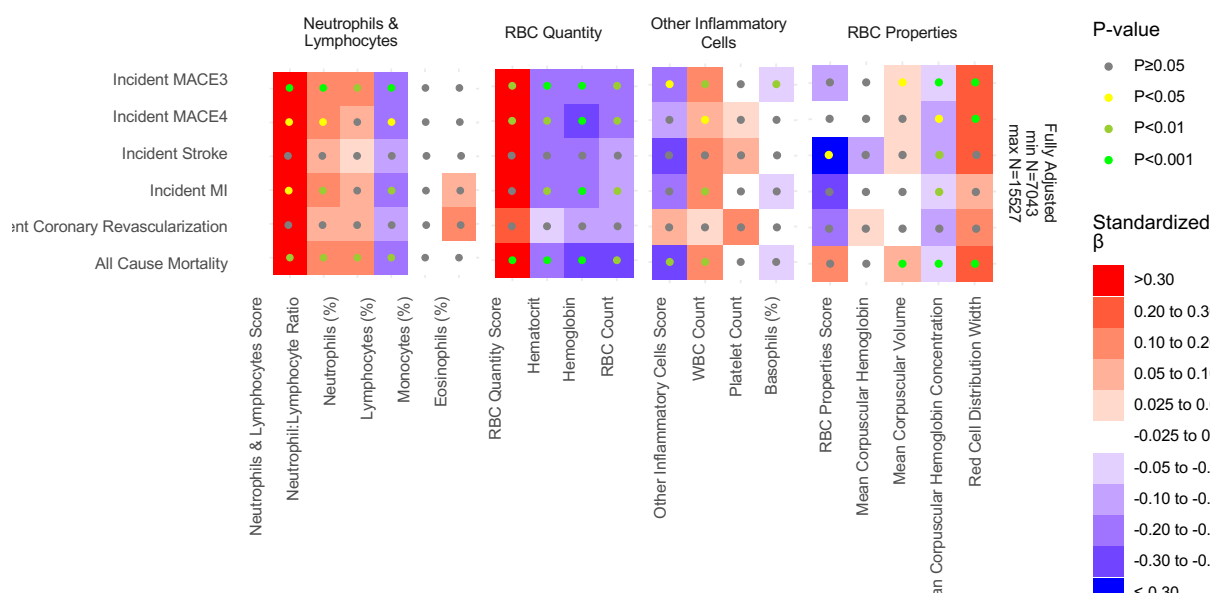


Figure 3. CBC components are associated with refined CV endpoints across five diverse, longitudinal cohorts.

Heatmap of standardized β -coefficients from Cox proportional hazards models relating CBC components to CV endpoints. Values of standardized β -coefficients directionality (color), magnitude (color gradient), and statistical significance (color of circle) are depicted. All models are fully adjusted for age, sex, race, smoking, body mass index, systolic and diastolic blood pressure, use of antihypertensive medications, diabetes status, total cholesterol, HDL, LDL, and triglycerides.

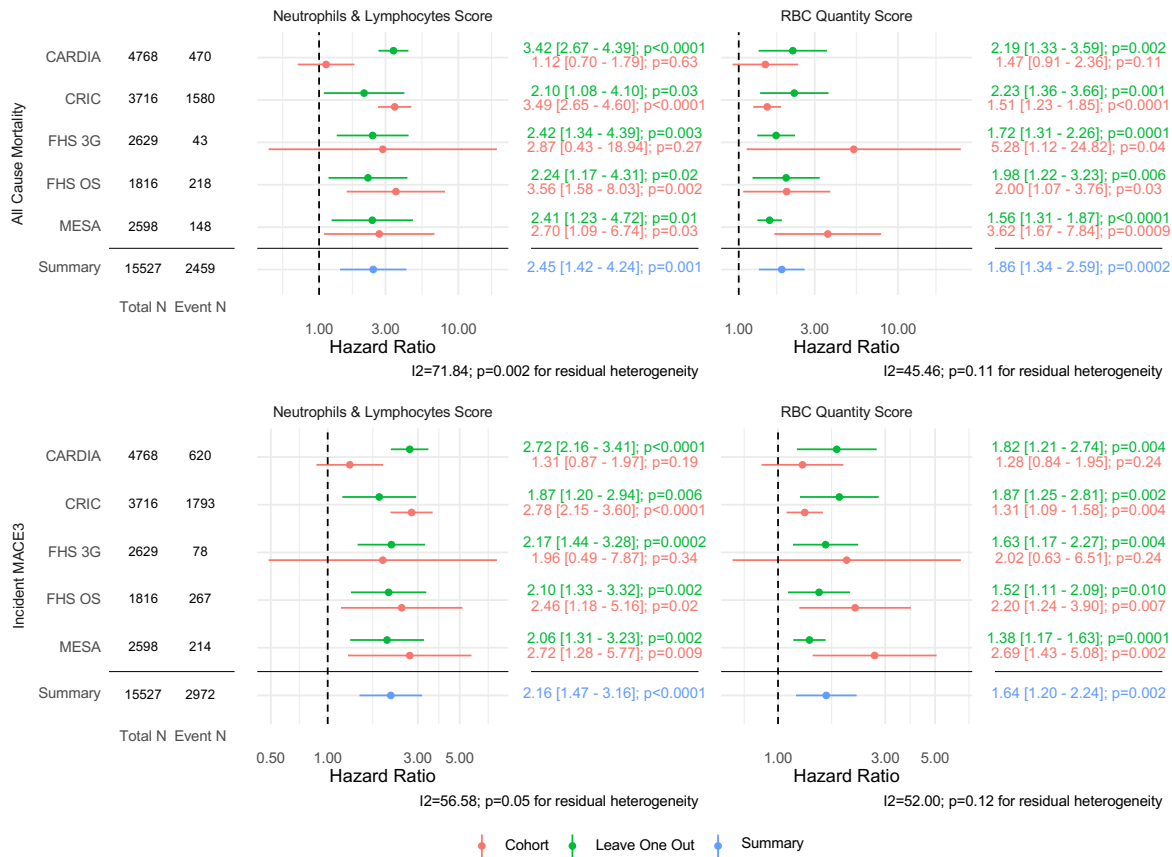


Figure 4. Key CBC-derived scores for all-cause mortality and MACE3 across five longitudinal cohorts.

Forest plots of standardized β -coefficients of key CBC-derived scores for all-cause mortality and MACE3. Tables on the left indicate the number of total subjects and events in each cohort. Hazard ratios (circles) and their 95% confidence intervals (bars) are indicated in different colors for individual cohort estimates (coral), summary meta-analysis (blue), and leave one cohort analysis sensitivity analyses (green). Cox models in each cohort were adjusted for age, sex, race, smoking, body mass index, systolic and diastolic blood pressure, use of antihypertensive medications, diabetes status, total cholesterol, HDL, LDL, and triglycerides.