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Vascular aging biomarkers and mortality and cardiovascular outcomes: a systematic review and meta-analysis

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Abstract

Background

Vascular aging is associated with cardiovascular disease and mortality, but the evidence supporting candidate biomarkers varies. We assessed associations between vascular aging biomarkers and clinical outcomes across prospective studies.

Methods

We searched CENTRAL, MEDLINE and EMBASE and additional sources for prospective studies with at least six months of follow-up. We evaluated 16 biomarker categories and five outcomes using random-effects meta-analysis of risk estimates and event counts, trial sequential analysis, subgroup analyses and sensitivity analyses. The review was registered in PROSPERO (CRD42022377292).

Results

Across 223 studies involving 1,325,186 participants, a coronary artery calcium score above zero, a low ankle-brachial index and shorter leucocyte telomere length are associated with both mortality outcomes. Their pooled hazard ratios for all-cause mortality are 1.59 (95% confidence interval 1.35–1.87), 1.82 (1.66–2.01) and 1.13 (1.09–1.18), respectively; corresponding estimates for cardiovascular mortality are 1.13 (1.04–1.22), 2.28 (1.91–2.74) and 1.11 (1.04–1.18). Trial sequential analyses support these associations, and sensitivity analyses do not materially change the main findings. Associations with stroke, myocardial infarction and major adverse cardiovascular events vary by biomarker.

Conclusions

These findings identify candidate biomarkers for further vascular aging research. Heterogeneity and residual confounding limit clinical interpretation. Their combined predictive value and usefulness for guiding interventions require prospective validation.

Plain Language Summary

Blood vessels change as people age, but it remains unclear which measurements best indicate future health risks. We reviewed 223 studies involving more than 1.3 million people. Three measurements were associated with death from any cause and from cardiovascular disease: calcium deposits in heart arteries seen on scans, blood pressure at the ankle compared with the arm, and the length of protective DNA ends in white blood cells. These measurements may help researchers investigate vascular aging. However, existing illnesses and differences between studies may influence the findings. Further research must test whether combining these measurements improves risk assessment and helps guide care.

Introduction

Vascular aging is a primary trigger of systemic aging,[1–4] and is considered a pivotal mechanism in the pathogenesis of cardiovascular diseases (CVDs).[4,5] Given that CVD represents one of the leading causes of death globally,[6] assessment of vascular aging is crucial for furthering the understanding of CVD, better prediction, and earlier management of the aging process, from the deterioration of vascular health to the endpoints of disease onset and death. It holds promise for keeping older people healthy and reducing the burden of CVD in aging societies. However, the “gold standard” corresponding perfectly to vascular aging is still elusive, which hinders the development of a unified research framework and diagnostic and prognostic tools in this field.

Despite many advances, the validity and usefulness of aging biomarkers are still not widely acknowledged.[7] The paramount importance of aging biomarkers lies in their ability to predict the transition to pathological conditions or disease onset,[8] but the strong predictors of outcomes may lack a theoretical foundation supporting their role in the process of vascular aging.[9] To our knowledge, vascular aging involves early functional impairment and advanced structural changes of blood vessels,[4,5,10–12] however, the progression is not a stepwise and continuous as aging processes can develop in parallel and at different rates.[13,14] These complexities render it challenging to define a single molecule or method that measures “vascular aging” with good reliability and generalizability. Therefore, it is crucial to develop a composite metric using reliable biomarkers, which will reflect the comprehensive characterization of this process and offer increased accuracy and reliability.[15] Indeed, there exist many surrogate biomarkers available for consideration but few studies have quantitatively evaluated and compared them, make it hard to select reliable biomarkers from a wide range.[16–20] Even with existing large cohorts and biobanks, systematic cross-population validation and comparisons of predictive strength remain relatively limited and challenging.[7]

To address this gap, we conducted a large-scale systematic review and meta-analysis incorporating trial sequential analysis (TSA) to establish a practical framework to select reliable vascular aging biomarkers. This framework summarizes the predictive efficacy of candidate biomarkers for clinical outcomes across diverse populations. Notably, our approach aligns with the research process described earlier[7], and, as a pioneering application, it was implemented despite practical challenges. Emphasizing interpretability, we selectively included biomarkers that reflect aging-related vascular features or changes, rather than all potential outcome predictors. Rather than proposing novel biomarkers, this study synthesizing prospective cohort data aims to compare existing indicators and identify those that are most informative for aging and suitable for diagnosing vascular aging.

Methods

This systematic review and meta-analysis was prospectively registered in PROSPERO (CRD42022377292) and followed the PRISMA statement (PRISMA checklist in appendix pp 3–5).[21] Ethics approval and informed consent were not required due to the study design.

Search and Eligibility Criteria

Two reviewers (Y.S., D.L.) systematically searched CENTRAL, MEDLINE, and EMBASE from 2013 to 2025 (full strategies in appendix pp 6–13), supplemented by trial registries, grey literature database, and manual retrieval of the reference lists of identified relevant articles and previous reviews. Prospective studies were eligible for inclusion if they measured biomarkers at baseline and recorded at least one of the outcomes during ≥ 6 months of follow-up (detailed criteria in appendix p 14). Based on vascular aging-related features, we included 16 types of 15 candidate biomarkers from three distinct dimensions (structural, functional, and circulating biomarkers; appendix pp 15–16). Structural biomarkers included cIMT (carotid intima-media thickness), CP (carotid plaque), CACS (coronary artery calcium score), and carotid diameter. Functional biomarkers included baPWV (brachial-ankle pulse wave velocity), cfPWV (carotid-femoral pulse wave velocity), ABI (ankle-brachial index), CAVI (cardio-ankle vascular index), AIx (augmentation index), FMD (flow-mediated dilation), and RHI (reactive hyperaemia index), and ABI was categorized into 2 types (i.e. high ABI and low ABI). Circulating biomarkers included CEPCs (circulating endothelial progenitor cells), EMPs (endothelial-derived microparticles), LTL (leucocyte telomere length), and E-selectin. The primary outcomes were all-cause mortality and cardiovascular mortality. The secondary outcomes included stroke, myocardial infarction (MI), and major adverse cardiovascular events (MACEs) (appendix p 17).

Study Selection

Duplicates were initially removed using Endnote with a systematic and rigorous deduplication method.[22] Two reviewers (Y.S., D.L.) independently screened titles and abstracts via Rayyan,[23] and then assessed full-text articles for eligibility. Any disagreements were resolved by a third reviewer (T.W.).

Data Extraction

Four authors (Y.S., D.L., X.Z., J.L.) independently extracted the following data from the eligible studies onto the predesigned form: title, author(s), publication year, study design, sample size, age, sex, medical history of participants, follow-up period, measurement of biomarker, outcome, analytical method, effect size with 95% confidence intervals (CIs), and the number of events and totality in comparable groups. When multiple effect sizes were identified, we adopted the one adjusted for the most confounding factors. If studies presented data from more than one independent cohort, we extracted the data separately and rechecked for overlapping study populations before formal analyses. We contacted the authors for missing information when necessary and resolved any discrepancies by consulting a third reviewer (T.W.).

Quality Assessment

The methodological quality of each included study was independently assessed by two reviewers (D.L. and Z.X.) using the Newcastle-Ottawa Scale (NOS),[24] with discrepancies resolved by a third author (Y.S.). A study of high quality was defined as a NOS score of 5–9.

Outcome Synthesis and Statistical Analysis

We conducted meta-analyses on the basis of (1) pooled risk estimates and (2) pooled event counts where at least three studies were available. For the included studies, hazard ratios (HRs), risk ratios (RRs), and odds ratios with their corresponding 95% CIs were collectively described as measures of estimated risk and pooled together on the same scale as possible after log-transformation to normalize distributions. The extracted risk estimates were statistically transformed in the same direction of risk change to allow comparability across studies (appendix pp 18–19), either on a continuous scale (e.g., per standard deviation increase) or a categorical scale (e.g., high versus low).[20] We adopted dichotomous definitions based on what the included studies set as the threshold. Additionally, event counts in both comparison groups were used to calculate the crude RRs. Because of anticipated heterogeneity among studies, we used a random-effects model with the inverse-variance method to reduce the imprecision of the pooled effect estimate.

The restricted maximum likelihood method was applied to estimate the between-study variance (τ^2),[25] and the confidence intervals were calculated via the *Q* profile method. We assessed study heterogeneity via the Cochran *Q* test and I^2 statistic, with I^2>75% considered to indicate substantial heterogeneity.[26] Given the diverse populations in the included studies, we conducted preplanned subgroup analyses to explore heterogeneity on the basis of sex, hypertension status, diabetes status, and type of study cohort, as well as additional NOS scores. Funnel plots were used to examine publication bias and Egger tests were used to detect asymmetry of funnel plot with at least ten studies. We performed multiple sensitivity analyses for primary outcomes to evaluate the robustness of our results: leave-one-out method, changing effect model, trim-and-fill method, and reanalysis with highest-quality studies (appendix p 20).

In addition, TSA was conducted to estimate the strength of evidence.[27,28] The main purpose of TSA is to minimize potential random statistical errors, which may happen when repetitive testing in cumulative meta-analyses. Thus, we first calculated the required information size (IS) (i.e. the number of participants needed in a meta-analysis to detect or reject a certain effect), and in our TSA the required IS was set for a power of 80%, a type I error rate of 5%, and a plausible RR reduction of 20%. A trial sequential monitoring boundary (TSMB) was further constructed to determine the statistical inference. In general, if the required IS was reached or the cumulative Z-curve crossed the monitoring boundary in a cumulative meta-analysis, we can infer that the firm evidence may have been established. Statistical analyses were performed via R (version 4.2.2) and TSA software (version 0.9.5.10 Beta). *P* value less than 0·05 was considered to indicate statistical significance.

Results

Study Characteristics

We identified 19,869 records in total and included 223 studies involving 1,325,186 participants (Fig. 1). There were 95,348 deaths from any cause and 17,246 deaths from cardiovascular causes. We conducted meta-analyses of 213 studies, 172 of which reported risk estimates adjusted for age (138/172), 107 of which provided detailed count data for each group. The main characteristics of studies are summarized in Table 1 (detailed information see Supplementary Table 1 in appendix pp 21–39). All of the NOS scores of the included studies were greater than six with most scoring ≥ 8, indicating the majority of included studies were high-quality (Supplementary Table 2 in appendix pp 40–49). Each excluded studies with reasons can be found in Supplementary Table 3,4 (appendix pp 50–112).

Table 1
Main characteristics of the 223 included studies.

Characteristics	No. of included studies
WHO Regions	
Africa	1
Americas	54
Eastern Mediterranean	1
Europe	92
South-East Asia	5
Western Pacific	64
Multi-regional	6
Sample size (1,325,186 in total)	
50–499	80
500–4999	109
≥ 5000	34
Publication period	
2013–2018	132
2019 to present	91
Mean or median age*, year	
≤ 49.9	14
50–59.9	77
60–69.9	100
70–79.9	23
≥ 80	5
Mean or median follow-up duration†, month	
MI indicates myocardial infarction; MACEs: major adverse cardiovascular events.	
* Four out of 223 studies did not report mean or median age of the study population.	
† Two out of 223 studies did not report mean or median follow-up duration.	
‡ Three studies did not report the number of events for all-cause mortality.	
§ One studies did not report the number of events for cardiovascular mortality.	

Characteristics	No. of included studies
6–59.9	105
60–119.9	78
120–239.9	37
≥ 240	1
Reported outcomes	
All-cause mortality (95348 events)‡	146
Cardiovascular mortality (17246 events)§	72
MACEs (19911 events)	51
Stroke (5995 events)	55
MI (3454 events)	36
For quantitative analysis (213 studies in total)	
Available risk estimates	172
Available event counts	107
MI indicates myocardial infarction; MACEs: major adverse cardiovascular events.	
* Four out of 223 studies did not report mean or median age of the study population.	
† Two out of 223 studies did not report mean or median follow-up duration.	
‡ Three studies did not report the number of events for all-cause mortality.	
§ One studies did not report the number of events for cardiovascular mortality.	

Biomarkers Predicting Primary Outcomes: Mortality

All included effect sizes were adjusted for as many confounders as possible (including demographic characteristics and cardiovascular risk factors). The meta-analyses of pooled risk estimates found that six biomarkers including the presence of CPs, the CACS > 0, high cfPWV, an abnormally low or high ABI, and short LTL, significantly predicted both all-cause mortality [HRs with 95% CIs: 1·52 (1·29, 1·79), 1·59 (1·35, 1·87), 1·18 (1·12, 1·25), 1·82 (1·66, 2·01), 1·26 (1·07, 1·49), and 1·13 (1·09, 1·18), respectively], and cardiovascular mortality [HRs: 2·33 (1·08, 5·04), 1·13 (1·04, 1·22), 1·18 (1·09, 1·27), 2·28 (1·91, 2·74), 1·43 (1·08, 1·89), and 1·11 (1·04, 1·18), respectively]. Two other biomarkers, high cIMT and high baPWV, were significantly associated with a high risk of all-cause mortality [HR: 1·45 (1·22, 1·73) and 1·23 (1·07, 1·41), respectively] but were not related to cardiovascular mortality (P > 0·05) (Fig. 2A and appendix pp 122–136).

Crude RRs were calculated from event counts to further ascertain the predictive ability of these biomarkers (Fig. 2B and appendix pp 137–148). Similar to the above results, the same set of vascular aging biomarkers (the presence of CPs, CACS > 0, high cfPWV, abnormally low or high ABI, and short LTL) demonstrated reliable predictive power with statistical significance for both all-cause mortality [RRs with 95% CIs: 3·68 (2·49, 5·43), 3·01 (2·59, 3·50), 2·86 (2·03, 4·05), 2·53 (2·20, 2·92), 1·55 (1·16, 2·09), and 1·40 (1·16, 1·70), respectively] and cardiovascular mortality [RRs: 5·64 (2·98, 10·68), 5·40 (4·43, 6·58), 3·27 (1·36, 7·87), 2·78 (2·13, 3·63), 2·05 (1·20, 3·53), and 2·42 (1·87, 3·14), respectively]. In addition to the prediction of all-cause mortality, significant associations of high cIMT and high baPWV with cardiovascular mortality were also discovered by pooling event counts.

Biomarkers Predicting Secondary Outcomes: Vascular Events

Different biomarker sets were determined to predict specific cardiovascular events in the meta-analyses for stroke, MI, and MACEs (Fig. 3). An increased risk of stroke was observed among subjects with the presence of CPs, high cIMT, CACS > 0, high baPWV, abnormally low ABI, high CAVI, and low RHI. Regarding the risk of MI, CACS > 0, and abnormally low or high ABI values had significant predictive effects. There were seven biomarkers of vascular aging with predictive ability for the occurrence of MACEs, of which six markers coincided with the risk of developing stroke or MI, including the cIMT, the presence of CPs, the CACS, the low ABI, the high ABI, and the CAVI. An additional biomarker, cfPWV, was significantly associated with the risk of both MACEs and death.

Trial Sequential Analysis

In addition, we conducted TSA for meta-analyses including dichotomous data. For primary outcomes, studies investigating the associations of the CACS and a low ABI with all-cause mortality and LTL with cardiovascular mortality reached the required IS, indicating that the test statistics were stable and reliable. In addition, the results conclusively indicated that LTL can be used to predict all-cause mortality and the CACS and a low ABI can be used to predict cardiovascular mortality, because the cumulative Z-curve exceeded the TSMB for statistical significance. Taken together, these findings suggest that a CACS > 0, a low ABI, and short LTL are effective biomarkers for predicting both all-cause and cardiovascular mortality (Fig. 4).

For secondary outcomes, on the basis of the cumulative Z-curve crossing the TSMB and the achievement of the required IS, the CACS was a robust predictor of developing stroke. Additionally, the strong association between a high ABI and the risk of MI was proven by the TSMB. For MACEs, studies on the high ABI reached the required IS, and for the presence of CPs, the CACS, and a low ABI, the cumulative Z-curve crossed the TSMB despite the estimated IS was not met. These results confirmed their usefulness for predicting the occurrence of MACEs (appendix pp 149–166).

Subgroup Analyses

We included diverse populations for the generalizability and explored variation across diverse demographic groups as planned. Moreover, the subgroup analysis by type of study cohort (patients vs. general population) could improve the accuracy by avoiding potential bias introduced by deaths. The heterogeneity in some meta-analyses could be partially explained (including the CP, CACS, baPWV, cfPWV, high ABI, CEPCs, and LTL for all-cause mortality; the CACS, baPWV, high ABI, and E-selectin levels for cardiovascular mortality; the baPWV and low ABI for stroke and MI; the low ABI for MACEs), and the predictive effect in subgroups remained robust with reduced heterogeneity (appendix pp 167–214). In the remaining subgroups, no definitive explanation for heterogeneity was identified because the test for subgroup differences indicated no statistically significant differences between groups ($P > 0{\cdot}05$).

Sensitivity Analyses, Publication Bias and Qualitative Review

Multiple sensitivity analyses concerning mortality outcomes did not materially change the findings from the main analysis (Supplementary Table 5,6 in appendix pp 113–114; and appendix pp 215–227). Funnel plots of studies reporting mortality data indicated visible publication bias for some biomarkers (Supplementary Fig. 1,2 in appendix pp 120–121; and appendix pp 228–248). Furthermore, Egger tests revealed asymmetry in funnel plots for the CACS, baPWV, cfPWV and LTL for all-cause mortality based on pooled risk estimates (Supplementary Table 7 in appendix p 115). For the other two biomarkers not included in the meta-analysis, one study reported that carotid diameter was predictive of all-cause mortality as well as stroke, and relatively consistent results indicated significant associations of high levels of EMPs with the risk of experiencing all-cause mortality and cardiovascular events (Supplementary Table 8 in appendix pp 116–119).

Comparison and Selection of Biomarkers for Vascular Ageing

The optimal markers for assessing vascular aging were determined on the consideration of interpretability, accuracy for predicting clinical outcome, and usefulness for clinical practice. To a certain extent, one effect estimate using two methods for synthesis, pooling risk estimates and pooling event counts, served as another form of cross-population validation when different studies were included. They complemented and validated each other and provided more perspectives for evaluation, and their results were not entirely the same. Therefore, the consistency of both results might enhance the reliability of the selection. For fatal outcomes, a set of eight markers (the cIMT, CP, CACS, baPWV, cfPWV, low ABI, high ABI, and LTL) and a set of six markers (the CP, CACS, cfPWV, low ABI, high ABI, and LTL) possessed reliable and outstanding power for predicting all-cause mortality and cardiovascular mortality, respectively. Furthermore, on the basis of the TSA results, the evidence for the cIMT, CACS, cfPWV, low ABI, and LTL as predictors was more robust. Three biomarkers (the CACS, low ABI, and LTL) at the intersection of both mortality outcomes could be considered optimal choices for comprehensively assessing vascular aging and its related risk of death. Interestingly, two of the three markers (the CACS and low ABI) also exhibited predictive ability for all of the secondary outcomes. In addition, the results

revealed different biomarker sets for predicting the risk of stroke (the cIMT, CP, CACS, baPWV, and low ABI), MI (the CACS, low ABI, and high ABI), and MACEs (the CP, CACS, low ABI, high ABI, and CAVI).

Discussion

In this large-scale systematic review and meta-analysis with TSA of 223 prospective studies involving 1,325,186 participants, we developed a systematic evaluation framework to ascertain the associations between sixteen biomarkers and five clinical outcomes. Specifically, three biomarkers, the CACS, a low ABI, and LTL, have reliable and remarkable predictive power for mortality outcomes and could serve as the current optimal combination for comprehensively assessing vascular aging. Furthermore, the performance of some markers varies across different outcomes, which suggests that for specific CVDs, different sets of markers might be used to assess systemic or localized vascular aging more accurately. The results were robust across diverse populations.

Structural biomarker, coronary artery calcification reflects the extent of subclinical atherosclerosis, which could be a driver of clinical CVD.[29] The CACS has been shown to play a role in predicting CVD risk,[30,31] and it could add some incremental value beyond traditional CVD risk assessment equations.[32] Consistent with these findings, our study validated the predictive value of the CACS for all of five vascular aging-related clinical outcomes. Another promising biomarker, cIMT, was associated with death and the occurrence of stroke.[33–36] Increased cIMT reflects smooth muscle cell proliferation and collagen deposition during vascular aging.[37] Similarly, the presence of CPs is widely used to quantify the burden of atherosclerosis at the early stage.[38] The presence of CPs was associated with increased risk of stroke and the features of CP could add the predictive value.[39] Our findings revealed robust associations of cIMT and CPs with four clinical outcomes, especially emphasizing the ability of cIMT predicting any death and the presence of CPs predicting MACEs.

Functional biomarkers indicate arterial stiffness or endothelial dysfunction. PWV has long been regarded as the gold standard evaluation of arterial stiffness,[8] and the predictor for death or cardiovascular events among various participants.[18,33,40,41]. Our TSA results confirmed the reliability of cfPWV predicting any death. Usually, an abnormal ABI value indicates peripheral flow-limiting lesions, and it is widely employed as a noninvasive diagnostic tool for peripheral artery disease and a predictor for CVD.[42] Additionally, the modest decline in the ABI in older healthy individuals might be attributed to increasing central arterial stiffness with age.[43] In our study, both abnormally low and high ABI values were significantly associated with death and cardiovascular events, which implies that the process of vascular aging may be heterogeneous with a nonlinear trend. FMD and RHI, which measure peripheral macrovascular and microvascular endothelial function, respectively, have emerged in recent years as promising but controversial predictors for death and CVD.[8,44–46] However, our only significant finding was that a low RHI predicted a high risk of stroke. This could be attributed to the insufficient study population or the limited predictive ability of a single marker.

Circulating biomarkers, including CEPCs, E-selectin, and LTL, reflect cellular changes in the circulation during vascular aging, although the detailed mechanisms and clinical applications require further investigation.[47] LTL serves as an indicator of biological aging and an indirect marker of vascular aging, and individuals with the shortest LTL exhibited an increased risk of cardiovascular mortality or MI compared with those with the longest LTL.[48] In the present analysis, LTL was significantly associated with all-cause and cardiovascular death, as the conclusive evidence on the basis of TSA. Owing to the lack of relevant studies, it was difficult to explore correlations between circulating biomarkers and other cardiovascular outcomes.

To bridge the gap in diagnostic standards for vascular aging, a significant contribution of our study is the proposal of reliable and effective biomarkers from a wide range by estimating their abilities to predict death and cardiovascular events. Undertaking large-scale cross-population studies of multiple biomarkers is still imperative but challenging and will require considerable funding and necessitate substantial coordination efforts; moreover, these challenges and requirements will continue because of the constant development of new markers.[7] Therefore, this study is a critical step towards comprehending and addressing vascular aging-related diseases and provides guidance for future research in the field of vascular aging.

The composite of three clinically validated biomarkers (the CACS, the ABI, and LTL) is promising for the comprehensive assessment of vascular aging and can be used to concretize the abstract concept of vascular aging. The three markers are feasible for use in clinical practice and are relatively easy to detect and implement with a Doppler probe, a sphygmomanometer, and a computed tomography scan during physical examination. By applying these markers within the context of specific outcomes, individual cardiovascular risk prediction, patient stratification, and tailored interventions for precision medicine can be facilitated. Although the combined effects of the three vascular aging biomarkers remain to be validated in the future, we believe that the suite of biomarkers will continue to evolve and be refined as researchers delve deeper into the nature of vascular aging. This iterative process of discovery and validation will enhance our understanding and promote the development of more precise diagnostic and predictive tools. Indeed, a diverse range of novel biomarkers have emerged for evaluating vascular aging, such as deep learning-based retinal age,[49] and magnetic resonance imaging-based left ventricular remodelling signatures.[50] There is growing interest in building biological aging clocks (e.g., epigenetic clocks) via multi-omics analysis combined with machine learning.[51,52] However, establishing a preliminary and concrete definition of vascular aging remains the cornerstone.

Our study has several limitations. Some restrictions in the inclusion criteria for ensuring high-quality evidence and uniform comparisons potentially resulted in a certain degree of data loss, such as a narrow definition of MACEs and the exclusion of reports with small sample sizes or published too early. However, most of our results were consistent with those of previous meta-analyses,[16–20,40,44,45,48,53–56] suggesting that the robustness of the conclusions was hardly compromised by the inclusion criteria. Moreover, we observed marked heterogeneity in the meta-analyses of several included biomarkers,

which can be partly attributed to the broad spectrum of study populations, where differences in the measurement methods of biomarkers may have further contributed to the heterogeneity.[57] Additionally, the further exploration was limited in this study because we had no access to all the raw data. Future individual participant data meta-analyses or large-scale cohorts will be necessary to confirm these findings and make more contribution.

In conclusion, several biomarkers of vascular aging are significantly associated with all-cause mortality and adverse cardiovascular outcomes. Among them, a coronary artery calcium score > 0, a low ankle-brachial index, and short leucocyte telomere length emerged as the most consistent and robust predictors across diverse populations. These findings support the clinical utility and generalizability of integrating these indices as surrogate markers of vascular aging. Conceptualizing vascular aging through this composite biomarker profile may offer a par practical framework for individualized cardiovascular risk stratification and inform the development of targeted interventions aimed at delaying vascular aging and its associated pathologies.

Abbreviations

CVD: cardiovascular disease

TSA: Trial sequential analysis

cIMT: carotid intima-media thickness

CPs: carotid plaques

CACS: coronary artery calcium score

FMD: flow-mediated dilation

RHI: reactive hyperaemia index

baPWV: brachial-ankle pulse wave velocity

cfPWV: carotid-femoral pulse wave velocity

ABI: ankle-brachial index

CAVI: cardio-ankle vascular index

AIx: augmentation index

LTL: leucocyte telomere length

CEPCs: circulating endothelial progenitor cells

EMPs: endothelial-derived microparticles

MI: myocardial infarction

MACEs: major adverse cardiovascular events

NOS: Newcastle-Ottawa Scale

HR: hazard ratio

RR: risk ratio

CI: confidence interval

IS: information size

TSMB: trial sequential monitoring boundary

Declarations

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Author contributions

L.J., T.W., and Y.S. conceived and supervised the project. Y.S. and T.W. developed the study design. Y.S., D.L., X.Z., J.L, Z.X., S.C., and X.L. carried out the investigations. Y.S., D.L., X.Z., J.L., Z.Q., X.Chen, X.Cai, and Z.L. performed testing and data curation. Y.S. and D.L. conducted the formal analysis and drafted the manuscript. Y.S. and X.Z. improved the visualization and interpretability of results. All authors reviewed the manuscript and provided critical revisions, participated sufficiently in the work and approved the final version of the paper for submission. The corresponding authors had full access to all data and final responsibility to submit for publication.

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Declaration of interests

All authors declare no competing interests.

Data availability

All data needed to evaluate the conclusions are analyzed and reported in Results section and Supplementary Material. The original data are publicly available within the databases and search strategies described in Supplementary Material. The raw output data and all R language codes for data analysis in this study can be accessed at https://github.com/Sun-Yi-Xin/Vascular_Aging_Biomarkers_Metaanalysis. For further questions, please contact the corresponding author.

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Figures

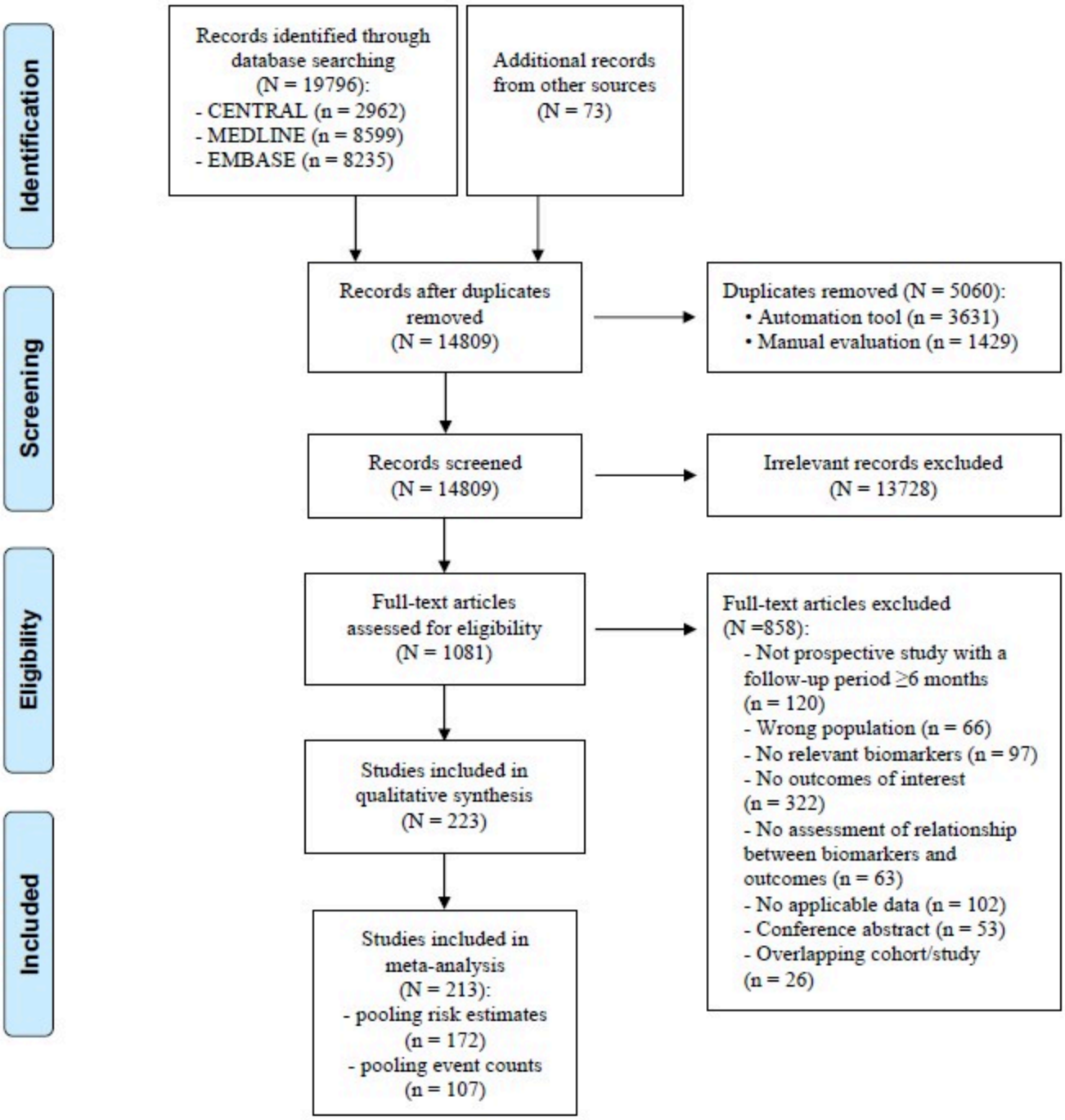


Figure 1

Study retrieval and selection. A flow diagram displaying the process for study inclusion and exclusion in the systematic review and meta-analysis of following the PRISMA guidelines.

A

Biomarker	N	No.	All cause mortality	HR (95% CI)	p	I^2 (%)	N	No.	CV mortality	HR (95% CI)	p	I^2 (%)
Structural												
cIMT	11	8213		1.45 [1.22, 1.73]	<0.01	0	3	2995		0.72 [0.09, 6.11]	0.76	72
CP	8	13368		1.52 [1.29, 1.79]	<0.01	5	4	26375		2.33 [1.08, 5.04]	0.03	71
CACS	23	122320		1.59 [1.35, 1.87]	<0.01	96	5	53938		1.13 [1.04, 1.22]	<0.01	84
Functional												
baPWV	12	128730		1.23 [1.07, 1.41]	<0.01	75	7	15706		1.00 [1.00, 1.01]	0.37	67
cfPWV	16	6506		1.18 [1.12, 1.25]	<0.01	56	8	2894		1.18 [1.09, 1.27]	<0.01	79
low ABI	19	41684		1.82 [1.66, 2.01]	<0.01	21	8	24828		2.28 [1.91, 2.74]	<0.01	25
high ABI	12	17544		1.26 [1.07, 1.49]	<0.01	0	5	13424		1.43 [1.08, 1.89]	0.01	0
AIx	8	2641		1.02 [0.99, 1.06]	0.16	72	3	1427		1.66 [0.99, 2.78]	0.06	71
FMD	3	15230		1.05 [0.95, 1.15]	0.33	38	3	15694		1.06 [0.82, 1.37]	0.66	0
Circulating												
CEPCs	5	1788		1.34 [0.86, 2.08]	0.2	91	–					
E−selectin	7	2562		1.06 [0.92, 1.23]	0.39	66	5	1926		0.97 [0.73, 1.28]	0.82	81
LTL	26	607709		1.13 [1.09, 1.18]	<0.01	88	8	560360		1.11 [1.04, 1.18]	<0.01	81

0.5 1 2 0.5 1 2

B

Biomarker	N	No. of total	No. of events	All cause mortality	RR (95% CI)	p	I^2 (%)	N	No. of total	No. of events	CV mortality	RR (95% CI)	p	I^2 (%)
Structural														
cIMT	5	1675	269		1.96 [1.46, 2.61]	<0.01	11	4	1418	118		2.43 [1.58, 3.74]	<0.01	0
CP	5	12474	613		3.68 [2.49, 5.43]	<0.01	69	3	3011	71		5.64 [2.98, 10.68]	<0.01	13
CACS	8	93303	4447		3.01 [2.59, 3.50]	<0.01	54	4	70656	766		5.40 [4.43, 6.58]	<0.01	15
Functional														
baPWV	5	17735	833		3.05 [1.69, 5.50]	<0.01	89	4	7130	197		10.27 [2.78, 37.92]	<0.01	59
cfPWV	6	3183	308		2.86 [2.03, 4.05]	<0.01	17	3	2443	133		3.27 [1.36, 7.87]	<0.01	65
low ABI	19	46725	6589		2.53 [2.20, 2.92]	<0.01	78	8	26512	1516		2.78 [2.13, 3.63]	<0.01	80
high ABI	10	33314	2899		1.55 [1.16, 2.09]	<0.01	77	5	21377	685		2.05 [1.20, 3.53]	<0.01	75
FMD	–							3	975	18		1.17 [0.38, 3.66]	0.78	0
Circulating														
CEPCs	4	632	107		0.42 [0.32, 0.55]	<0.01	0	–						
LTL	11	579245	51694		1.40 [1.16, 1.70]	<0.01	98	3	74381	2899		2.42 [1.87, 3.14]	<0.01	16

0.1 1 5 0.1 1 5

Figure 2

Composited forest plots of the predictive effects of vascular aging biomarkers for mortality outcomes. Three categories of vascular aging biomarkers are showed with at least three studies available for meta-analyses. The meta-analyses using two methods of effect synthesis were based on pooled risk estimates (**A**) and pooled event counts (**B**) respectively. Forest plots illustrate their respective associations with all-cause mortality (left) and cardiovascular (CV) mortality (right) in the random effects model with the inverse-variance method. CV mortality indicates cardiovascular mortality; HR: hazard ratio; RR: risk ratio; CI: confidence interval; I^2, the variation attributable to heterogeneity; cIMT: carotid intima-media thickness; CP: carotid plaque; CACS: coronary artery calcium score; baPWV: brachial-ankle pulse wave velocity; cfPWV: carotid-femoral pulse wave velocity; ABI: ankle-brachial index; AIx: augmentation index; FMD: flow-mediated dilation; CEPCs: circulating endothelial progenitor cells; LTL: leucocyte telomere length.

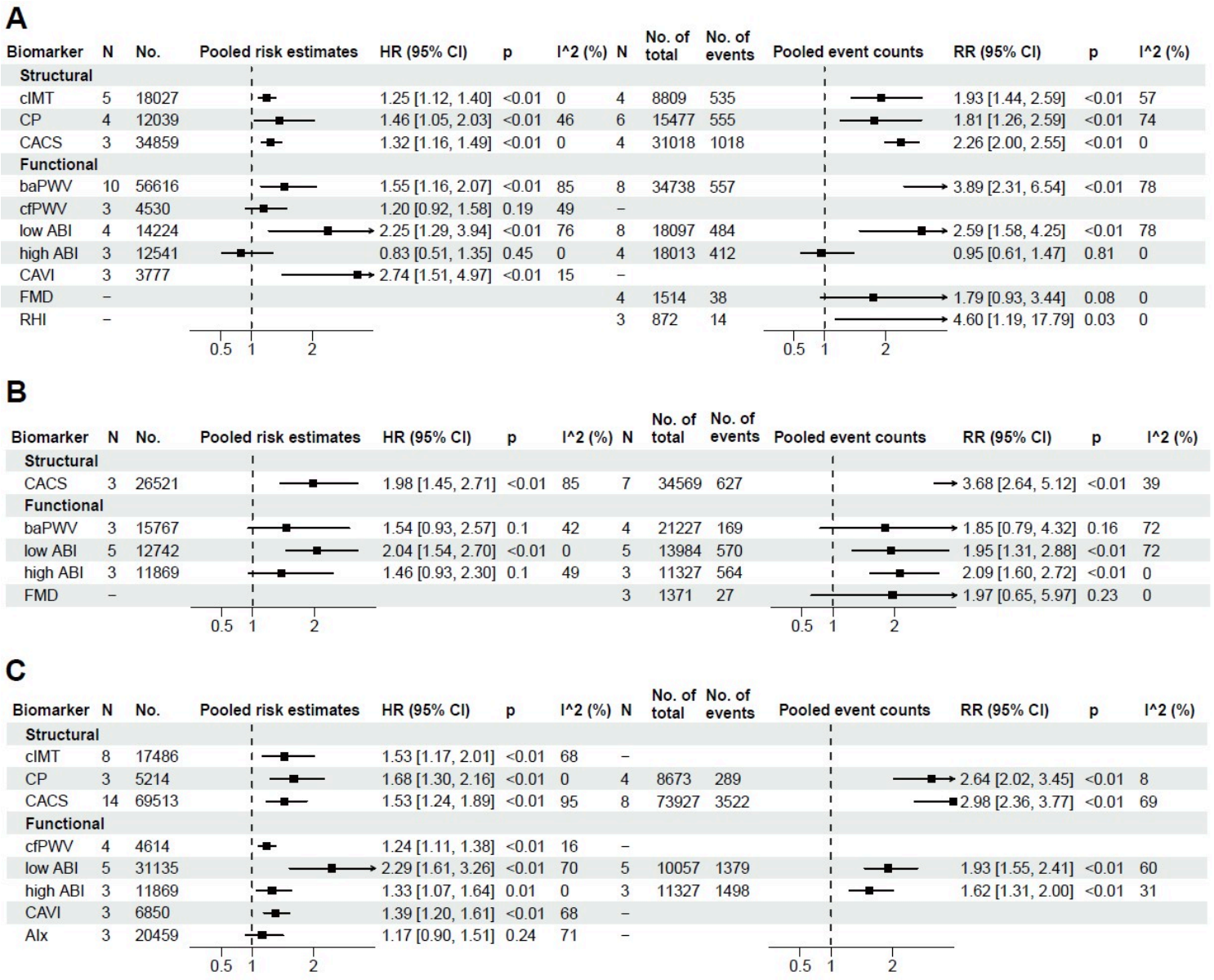


Figure 3

Composited forest plots of the predictive effects of vascular aging biomarkers for cardiovascular events. Two categories of vascular aging biomarkers are showed with at least three studies available for meta-analyses, including structural biomarkers and functional biomarkers. For stroke (**A**), myocardial infarction (**B**) and major adverse cardiovascular events (**C**), forest plots illustrate the results using two methods of effect synthesis: pooled risk estimates (left, HR) and pooled event counts (right, crude RR). *P* values are two-sided in the random effects models with the inverse-variance method. HR indicates hazard ratio; RR: risk ratio; CI: confidence interval; I^2, the variation attributable to heterogeneity; cIMT: carotid intima-media thickness; CP: carotid plaque; CACS: coronary artery calcium score; baPWV: brachial-ankle pulse wave velocity; cfPWV: carotid-femoral pulse wave velocity; ABI: ankle-brachial index; CAVI: cardio-ankle vascular index; AIx: augmentation index; FMD: flow-mediated dilation; RHI: reactive hyperaemia index.

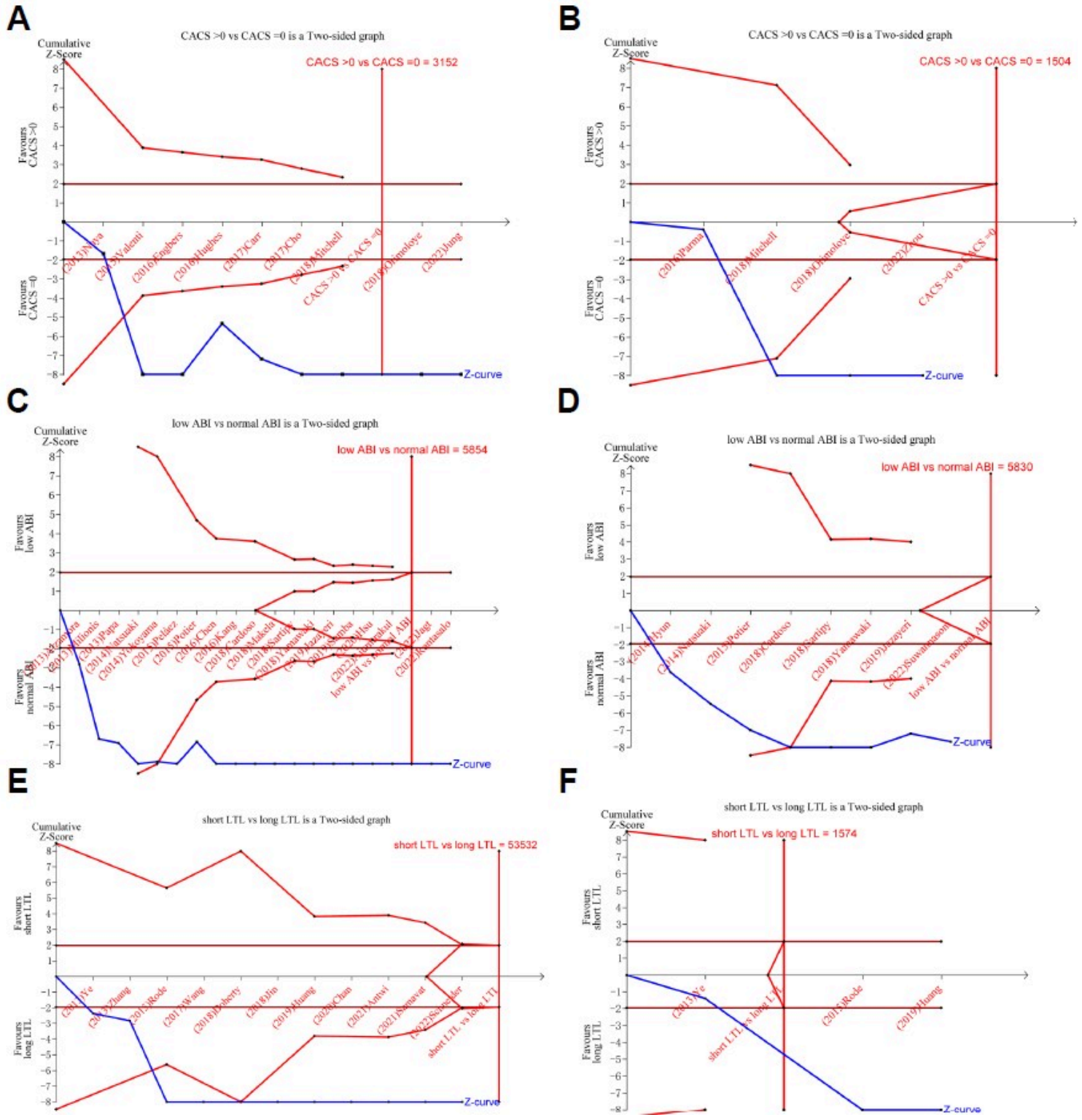


Figure 4

Trial sequential analysis for further confirmation of the role of three vascular aging biomarkers predicting mortality. A and C and E, All-cause mortality. **B and D and F**, Cardiovascular mortality. Three vascular aging biomarkers are the coronary artery calcium score>0 (**A** and **B**), low ankle-brachial index (**C** and **D**) and short leucocyte telomere length (**E** and **F**). The cumulative Z-curves (blue) exceeding the trial sequential monitoring boundaries (red curves, symmetrical above and below) for statistical significance

in **A–E**and the achievement of the required information size (red vertical line) in **A** and **C** and **F,** indicate the reliable effect estimates. The required information size is calculated by a power of 80%, a type I error rate of 5%, and a relative risk reduction of 20%.

Supplementary Files

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