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FULL RESEARCH PAPER
Prevention in Practice

Adding traditional and emerging biomarkers for risk assessment in secondary prevention: a prospective cohort study of 20 656 patients with cardiovascular disease

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Aims This study aims to explore whether conventional and emerging biomarkers could improve risk discrimination and calibration in the secondary prevention of recurrent atherosclerotic cardiovascular disease (ASCVD), based on a model using predictors from SMART2 (Secondary Manifestations of ARTerial Disease).

Methods and results In a cohort of 20 658 UK Biobank participants with medical history of ASCVD, we analysed any improvement in C indices and net reclassification index (NRI) for future ASCVD events, following addition of lipoprotein A (LP-a), apolipoprotein B, Cystatin C, Hemoglobin A1c (HbA1c), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase, and alkaline phosphatase (ALP), to a model with predictors used in SMART2 for the outcome of recurrent major cardiovascular event. We also examined any improvement in C indices and NRIs replacing creatinine-based estimated glomerular filtration rate (eGFR) with Cystatin C–based estimates. Calibration plots between different models were also compared. Compared with the baseline model (*C* index = 0.663), modest increments in *C* indices were observed when adding HbA1c ($\Delta C = 0.0064$, $P < 0.001$), Cystatin C ($\Delta C = 0.0037$, $P < 0.001$), GGT ($\Delta C = 0.0023$, $P < 0.001$), AST ($\Delta C = 0.0007$, $P < 0.005$) or ALP ($\Delta C = 0.0010$, $P < 0.001$) or replacing $eGFR_{Cr}$ with $eGFR_{CysC}$ ($\Delta C = 0.0036$, $P < 0.001$) or $eGFR_{Cr\text{-}CysC}$ ($\Delta C = 0.00336$, $P < 0.001$). Similarly, the strongest improvements in NRI were observed with the addition of HbA1c (NRI = 0.014) or Cystatin C (NRI = 0.006) or replacing $eGFR_{Cr}$ with $eGFR_{Cr\text{-}CysC}$ (NRI = 0.001) or $eGFR_{CysC}$ (NRI = 0.002). There was no evidence that adding biomarkers modified calibration.

Conclusion Adding several biomarkers, most notably Cystatin C and HbA1c, but not LP-a, in a model using SMART2 predictors modestly improved discrimination.

Lay summary This study aimed to determine whether incorporating additional blood-based biomarkers could enhance the prediction of recurrent cardiovascular events—such as myocardial infarction, stroke, or other major adverse cardiovascular events-in individuals with existing atherosclerotic cardiovascular disease (ASCVD). Using data from a cohort of 20,658 UK Biobank participants with a history of ASCVD, we evaluated the predictive performance of adding eight biomarkers—LP-a, ApoB, cystatin C, HbA1c, GGT, AST, ALT, and ALP—to an established risk prediction model, SMART2 (Secondary Manifestations of ARTerial Disease). Additionally, we assessed whether substituting the creatinine-based estimated glomerular filtration rate (eGFR) with a cystatin C-based eGFR could improve model accuracy. Our findings indicated that

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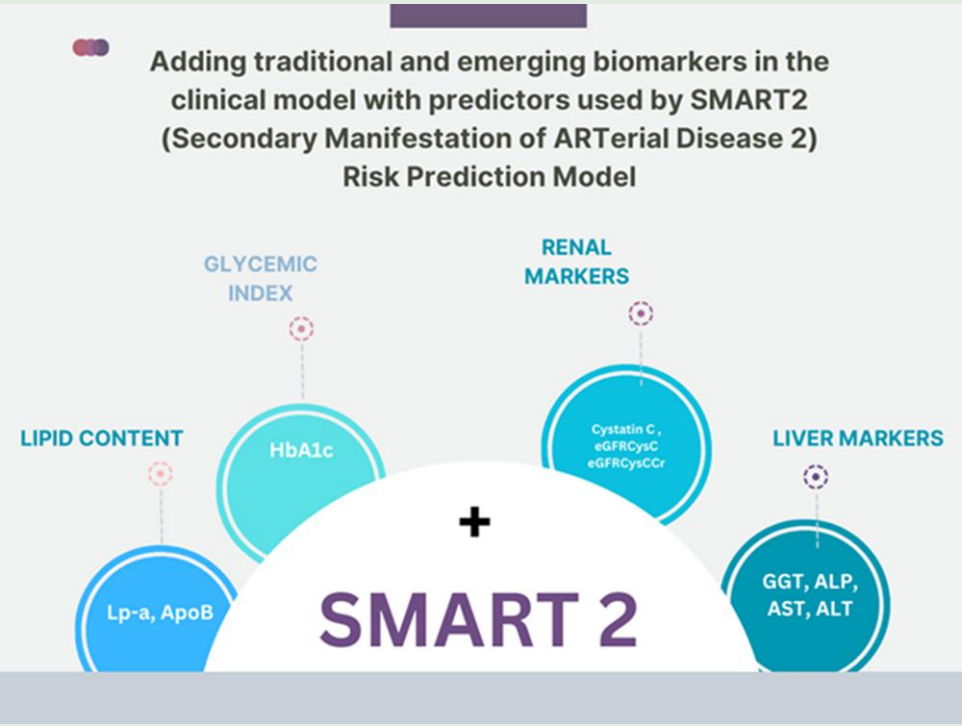
the inclusion of certain biomarkers, particularly cystatin C and HbA1c, modestly improved the model's discriminatory capacity for stratifying individuals into appropriate risk categories. However, other biomarkers, such as LP-a, did not significantly enhance risk prediction in this population.

Graphical Abstract



Keywords Cardiovascular disease • SMART2 • Risk prediction model • Secondary prevention

Introduction

Patients with established atherosclerotic cardiovascular disease (ASCVD) are categorized as very high risk for cardiovascular events. Their actual residual risk varies significantly depending on specific clinical factors.[1] The 2021 ESC Prevention guideline recommends adopting a personalized approach to assess cardiovascular disease (CVD) risk, taking into consideration of individual risk factors.[2] Risk stratification methods are useful for categorizing patients according to their clinical characteristics to identify those who can derive the most significant advantage. Several risk prediction models have been developed in the context of secondary prevention, with one notable example being SMART2 (Secondary Manifestations of ARTerial Disease).[3] SMART2 incorporates traditional clinical factors that are routinely assessed in daily practice, including age, sex, smoking status, diabetes mellitus, systolic blood pressure, non-HDL cholesterol (non-HDL-C) concentrations, presence of ASCVD, estimated glomerular filtration rate (eGFR), high-sensitivity C-reactive protein (hsCRP) levels, and time elapsed since the first clinical ASCVD event. The SMART2 model aims to predict the 10-year residual risk of ASCVD events in individuals who already have ASCVD.

One noteworthy aspect of SMART2 is its practicality, as all the variables used in the model are routinely measured in daily clinical practice. This facilitates the practical implementation of the risk score in real-world settings. SMART2 represents an updated version of a previous risk prediction model, with improvements such as geographic recalibration and external validation. These enhancements improved the accuracy and generalizability of the model, allowing for its more reliable application across diverse populations and healthcare settings.[3,4] However, previously studies showed a relatively modest discrimination with *C* statistics between 0.6 and 0.8 in most clinical cohorts.[3,5]

Hence, it is important to determine whether the accuracy of risk prediction in the secondary prevention of CVD can be further enhanced through the inclusion of additional emerging biomarkers that are widely used in clinical practice. Examples include the genetically determined lipoprotein particles lipoprotein A (LP-a) and apolipoprotein B, Cystatin C a biomarker of renal function, HbA1c a marker of longer term glycaemic control, and liver function tests. While Cystatin C is not currently routinely used in many clinical settings, there are trials of wider implementation.

The aim of this study was to determine whether, and to what extent, additional clinical biomarkers could improve discrimination and calibration in a model using SMART2 predictors for the 10-year residual risk of recurrent ASCVD in patients with established ASCVD.

Methods

Study design

This study was a prospective cohort study based on UK Biobank. Over 500 000 people between the ages of 40 and 69 years were recruited into

UK Biobank between 2006 and 2010. An electronically signed consent form, a self-completed touch-screen questionnaire, a short computer-assisted interview, physical and functional tests, and the collection of blood, urine, and saliva were all included in the assessment visit.[6] Research ethics approval was granted for UK Biobank from North West Multi-centre Research Ethics Committee.

In this study, we only included established ASCVD patients with a previous hospital record of the following diagnoses at the baseline assessment: ischaemic heart disease (IHD; I20–I25), stroke (I60–I64), abdominal aortic aneurysm (AAA (I71)), or peripheral arterial disease (PAD; I70.2, I73).

Measurements

This study included all clinical variables used in the current SMART2-risk score, namely: age, sex, smoking status, diabetes mellitus, systolic blood pressure, non-HDL-C, presence of ASCVD, eGFR based on creatinine ($eGFR_{Cr}$), hsCRP, and time since first clinical ASCVD. Candidate additional biomarkers included: LP-a, apolipoprotein B (ApoB), Cystatin C, HbA1c, gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and eGFR based on Cystatin C ($eGFR_{CysC}$) and combined creatinine and Cystatin C ($eGFR_{Cr\text{-}CysC}$). Full details of the biochemistry sampling, handling, and quality control protocol for UK Biobank have been described and validated previously.[7,8] Systolic blood pressure was measured at the baseline assessment visit. Biochemistry measures were performed at a dedicated central laboratory between 2014 and 2017. Apolipoprotein B, total cholesterol, and CRP were analysed using the immune-turbidimetric method with a Beckman Coulter AU5800. Cystatin C was analysed using the immuno-turbidimetric method with Siemens Advia 1800. High-density lipoprotein cholesterol was analysed using the enzyme immune-inhibition method with the Beckman Coulter AU5800. Lipoprotein A was analysed using immuno-turbidimetric method with Beckman Coulter AU5800. Liver function tests (GGT, ALT, AST, and ALP) were analysed using enzymatic rate method with Beckman Coulter AU5800. All these tests were externally verified with 97% (for AST), 98% (for total cholesterol) to 100% (for ApoB, ALP, ALT, creatinine, Cystatin C, CRP, GGT, HDL-C, and LP-a) good or acceptable distribution.[9] Details of these measurements and assay performances can be found in the UK Biobank online showcase and protocol.[9] eGFR was calculated using the CKD-EPI 2021 creatinine formula ($eGFR_{Cr}$), CKD-EPI Cystatin C formula ($eGFR_{CysC}$), and CKD-EPI 2021 creatinine-cystatin formula ($eGFR_{Cr\text{-}CysC}$).[10] We excluded variables related to antithrombotic medication use from our analysis due to the lack of reliable data within the UK Biobank, since the available information on these medications was categorized broadly into drug groups based on interview responses.

The outcome measured in this study was recurrent ASCVD events defined as any of the following ICD-10 codes ascertained via linkage to hospital and death records: IHD, stroke, AAA, and PAD (see Supplementary material online, *Table S1*). Date and cause of death were obtained from death certificates held within the National Health Service Information Centre (England and Wales) and the National Health Service Central Register Scotland (Scotland). Date and cause of hospital admission were obtained from the Health Episode Statistics (England and Wales) and Scottish Morbidity Records (Scotland). Detailed information about the linkage procedures can be found at http://digital.nhs.uk/services. At the time of analysis, mortality data were available up to September 2021 in England and Wales and October 2021 in Scotland. Hospital admission data were available up to September 2021 in England, February 2018 in Wales, and July 2021 in Scotland.

Statistical analysis

Patient characteristics were presented as median and interquartile range (IQR) for quantitative variables, while categorical variables were presented as frequencies and percentages. Subjects with any missing data were excluded from analysis. Fine and Gray competing risk-adjusted sub-distribution hazard model was used to estimate sub-distribution hazard ratios (SHRs) and their 95% confidence intervals (CIs), using the *survival* package in R. Akaike information criterion was used to compare log-linear model fits to log transformations, squared transformations, or cubic splines. Based on this, cubic splines were used for Lp(a). ApoB, Cystatin C, GGT, AST, $eGFR_{CysC}$, $eGFR_{Cr\text{-}CysC}$, log transformations for ALP, and squared transformation for ALT. The SHR presented are assumed to be time weighted average over the follow-up period.[11] The proportional hazards assumption was assessed by visual inspection used Schoenfeld residual plots (see Supplementary material online, *Figure S1*).

Additional biomarkers were compared with a refitted version of SMART2 model in terms of the changes in the *C* index using the *compareC* package in R. Similarly, reclassification was assessed using the net reclassification index (NRI), by comparing the refitted version of SMART2 model and that with additional biomarkers. The continuous net reclassification index (NRI) for a risk difference of 0.05 was calculated to compare the new model with additional biomarkers and the original model using the *nricens* package in R. The categorical NRI for new models using residual risk thresholds 50% 10-year risk of ASCVD events, which was considered as the upper threshold for intensified treatment threshold in the original development of SMART2.[3] A sub-group analysis was conducted on patients with an eGFR of <45 mL/min/1.73 m^2 to compare the inclusion of the additional biomarkers Cystatin C, and replaced $eGFR_{CysC}$ and $eGFR_{Cr\text{-}CysC}$ into the model with a refitted version of SMART2, because these patients are of highly chance to have Cystatin C measurements available in routine clinical practice. The calibration plot and relevant metrics were derived using *CalibrationCurves* package in R. Statistical analyses were performed using R statistical software version 4.3.0.

Results

This study included 20 656 patients with established ASCVD. The mean and median (IQR) follow-up were 6.58 and 6.43 (3.12–10.16) years, respectively. The number of ASCVD events were 12 623, while events from other causes were 543.

The selection of participants included in this study is shown in *Figure 1*. The most common medical history was IHD. In general, patients exhibited normal lipid profile values, particularly in the case of non-HDL-C, LP-a, and ApoB. Median hepatic and renal function, blood pressure, and glucose levels are within the normal range, as indicated by the measurements. Slight elevations of CRP were observed. The patient characteristics of the study are shown in *Table 1*.

Table 2 presents the associations between candidate biomarkers and recurrent event adjusted for existing SMART2 variables. There were significant associations for LP-a, Apo B, Cystatin C, HbA1c, GGT, and ALP with recurrent ASCVD events.

In the baseline model, some variables like age, systolic blood pressure, and previous stroke violated proportional hazards assumption and the SHR should only be regarded as the weighted average of the time varying association. While in models with additional biomarkers, proportional hazards assumptions were met for most of the biomarkers (LP-a, ApoB, HbA1c, GGT, AST, ALT, and ALP) but some violations were found in Cystatin C, $eGFR_{CysC}$, and $eGFR_{CrCysC}$. Schoenfeld residuals are shown in Supplementary material online, *Figure S1*.

Table 3 presents the *C* indices for the addition of some biomarkers into the baseline model. Discrimination was modestly improved on the addition of Cystatin C [ΔC (95% CI) 0.0037 (0.0027–0.0047)], HbA1c [ΔC (95% CI) 0.0064 (0.0050–0.0078)], GGT (ΔC (95% CI) 0.0023 (0.0015–0.0031)], AST [ΔC (95% CI) 0.0007 (0.0002–0.0011)], and ALP (ΔC (95% CI) 0.0010 (0.0005–0.0015)], and replacing $eGFR_{Cr}$ with $eGFR_{CysC}$ [ΔC (95% CI) 0.0036 (0.0026–0.0047)] and $eGFR_{CrCysC}$ [ΔC (95% CI) 0.0036 (0.0026–0.0047); *Figure 2*]. The

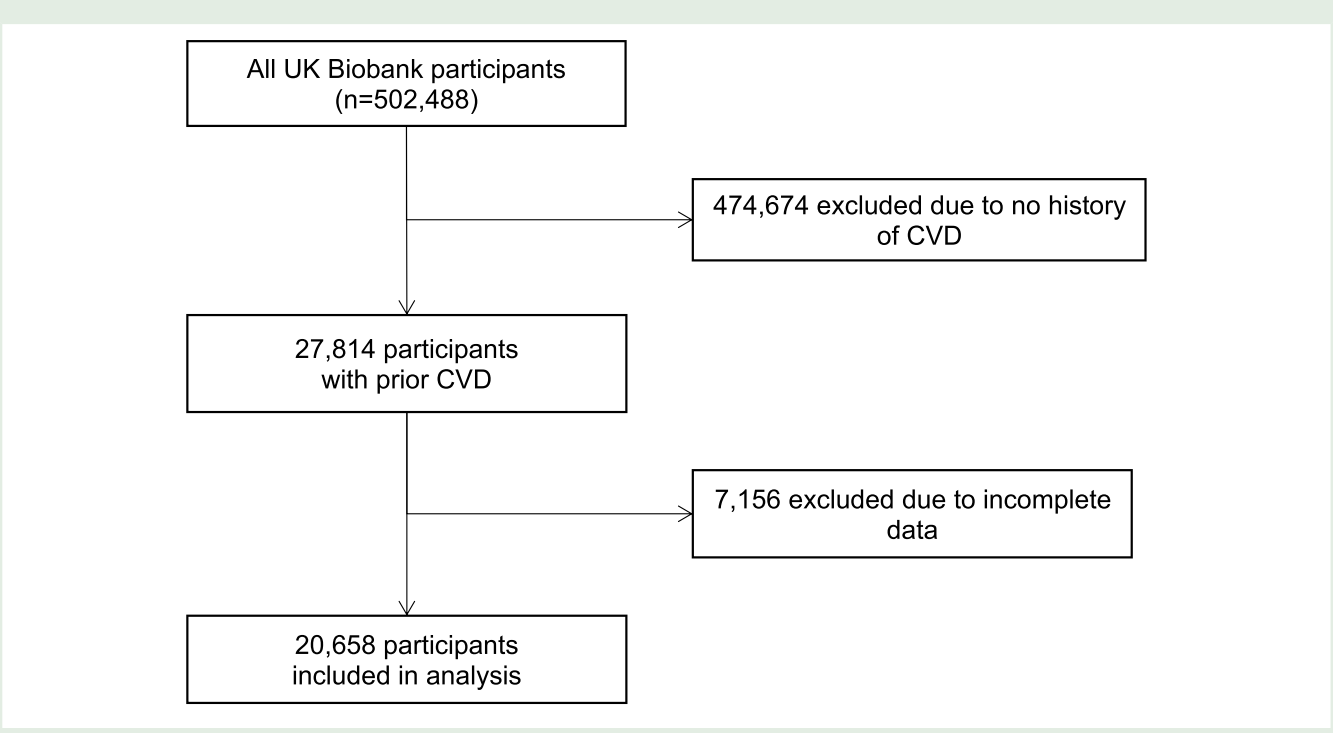


Figure 1 Flow chart of participants.

largest improvement seen in adding HbA1c into the baseline model [*C* index (95% CI) 0.6694 (0.6646–0.6742)]. Adding LP-a, ApoB, and ALT did not improve discrimination.

Improvements in continuous NRI were found when all biomarkers, except ApoB, were added to the baseline model, with the strongest improvement in HbA1c (NRI = 0.0072), Cystatin C (NRI = 0.00349), and replacing $eGFR_{Cr}$ with $eGFR_{CysC}$ (NRI = 0.382) and $eGFR_{Cr\text{-}CysC}$ (NRI = 0.0355) as shown as in *Table 4*). Similarly, improvement was found in categorical NRI with 50% threshold with HbA1c was the strongest improvement (NRI = 0.0144), Cystatin C (NRI = 0.0144), and replacing $eGFR_{Cr}$ with $eGFR_{CysC}$ (NRI = 0.0072) and $eGFR_{Cr\text{-}CysC}$ (NRI = 0.0058) as shown in *Table 5*).

The baseline model had a good calibration with calibration slope 1.05 (95% CI 1.01–1.09; *Figure 3*). The addition of biomarkers did not alter the calibration.

The sub-group analyses for patients with eGFR <45 mL/min/1.73 m^2 ($n = 339$) were shown in Supplementary material online, *Table S3*. Adding Cystatin C [Δ*C* (95% CI) 0.0046 (−0.0055, 0.0148)], and replacing $eGFR_{Cr}$ with $eGFR_{CysC}$ [Δ*C* (95% CI) 0.0045 (−0.0032, 0.0123)] and $eGFR_{Cr\text{-}CysC}$ [Δ*C* (95% CI) 0.0073 (−0.0019, 0.0163)] showed greater improvement in *C* statistics in the point estimates than in the primary analysis, but were not statistically significant possibly due to lower sample size. Improvements in continuous and categorical reclassification were seen in either adding Cystatin C or replacing $eGFR_{Cr}$ with $eGFR_{CysC}$ and $eGFR_{Cr\text{-}CysC}$.

Discussion

This study used UK Biobank data to investigate whether additional variables improved risk discrimination of a model with the predictors used by SMART2 for the 10-year risk of recurrent ASCVD in patients with established ASCVD. To our knowledge, this is the first study to examine whether a wide range of clinical biomarkers improve a SMART2-based model. The findings suggested modest improvements in discrimination when Cystatin C, HbA1c, GGT, AST, and ALP were added to the refitted version of SMART 2, as well as when $eGFR_{CysC}$ was replaced by $eGFR_{CysC}$ and $eGFR_{Cr\text{-}CysC}$.

Cystatin C was one of the strongest candidate predictors in the study findings. It has previously been linked in several research investigations to cardiovascular outcomes. Cystatin C is a protein, which is freely filtered by the glomerulus, can be a marker for kidney function, and used to estimate GFR. Some earlier investigations have examined the use of raw Cystatin C in risk prediction models. Cystatin C concentrations as a single marker are strongly associated with the risk of cardiovascular morbidity and mortality. The association between Cystatin C and cardiovascular events has been firmly established in both acute coronary syndromes (ACSs) and chronic coronary syndromes. Cystatin C has a positive association with atherosclerosis severity, independent of eGFR, and other cardiovascular risk factors.[12] A meta-analysis has shown that elevated serum Cystatin C concentrations are strongly associated with increased risk of major adverse cardiovascular events (MACEs) and mortality in patients experiencing acute myocardial infarction (MI) after percutaneous coronary intervention (PCI).[13] In a prospective study of acute MI patients, it was reported that Cystatin C could predict MACE in individuals with normal renal function and those without cardiogenic shock.[14] Similarly, it was reported that Cystatin C concentrations were associated with long-term mortality in patients undergoing late PCI following ACS.[15] Due Cystatin C's positive association with ACS, it has been recommended for inclusion in risk stratification for treatment of high-risk patients.[16] In critically ill patients, Cystatin C, both in its own right and as part of eGFR assessment, has been demonstrated to significantly improve prediction of long-term cardiovascular mortality.[17] The significant impact of Cystatin C on individuals diagnosed with stable coronary artery disease is widely recognized as a prognostic indicator for cardiovascular events and the

Table 1 Participant characteristics

	Overall	IHD		Stroke		PAD		AAA	
		Yes	No	Yes	No	Yes	No	Yes	No
n	**20 656**	**13 480**	**7176**	**3818**	**16 838**	**5062**	**15 594**	**383**	**20 273**
Age	63 (58–66)	63 (59–67)	61 (55–65)	62 (57–66)	63 (58–66)	61 (55–65)	63 (59–66)	65 (62–68)	63 (58–66)
Sex, *n* (%)									
Female	7675 (37)	4068 (30)	3607 (50)	1483 (39)	6192 (37)	2618 (52)	5057 (32)	49 (13)	7626 (38)
Male	12 981 (63)	9412 (70)	3569 (50)	2335 (61)	10 646 (63)	2444 (48)	10 537 (68)	334 (87)	12 647 (62)
Smoking, *n* (%)	2641 (13)	1607 (12)	1034 (14)	550 (14)	2091 (12)	806 (16)	1835 (12)	60 (16)	2581 (13)
Medical history, *n* (%)									
IHD	13 480 (65)	13 480	7176	602 (16)	12 878 (76)	908 (18)	12 572 (81)	121 (32)	1 335 966)
PVD	5062 (25)	908 (6.7)	4154 (58)	289 (7.6)	4773 (28)	5062	15 594	375 (98)	4687 (23)
AAA	383 (1.9)	121 (0.9)	262 (3.7)	30 (0.8)	353 (2.1)	375 (7.4)	8 (<0.1)	383	20 273
Stroke	3818 (18)	602 (4.5)	3.216 (45)	3818	16 838	289 (5.7)	3529 (23)	30 (7.8)	3788 (19)
Years since first ASCVD	4 (1–8)	4 (2–9)	3 (−2 to 8)	3 (0–8)	4 (1–9)	3 (−1 to 9)	4 (1–8)	3 (1–7)	4 (1–8)
SBP (mmHg)	138 (126–151)	138 (126–151)	138 (126–152)	139 (127–152)	138 (126–151)	138 (126–152)	138 (126–151)	140 (128–152)	138 (126–151)
Non-HDL-C (mmol/L)	3.37 (2.79–4.12)	3.23 (2.70–3.88)	3.69 (3.01–4.53)	3.38 (2.78–4.13)	3.37 (2.79–4.11)	3.76 (3.06–4.59)	3.26 (2.72–3.94)	3.31 (2.79–4.02)	3.37 (2.79. 4.12)
Creatinine (μmol/L)	76 (65–88)	78 (68–89)	72 (62–84)	76 (65–88)	76 (66–88)	72 (62–84)	77 (67–89)	84 (74–96)	76 (65–88)
$eGFR_{cr}$ (mL/min/1.73 m^2)	88 (76–96)	87 (75–94)	90 (78–98)	88 (75–96)	88 (76–95)	90 (78–98)	88 (75–95)	82 (70–92)	88 (76–96)
$eGFR_{cys}$ (mL/min/1.73 m^2)	80 (68–93)	79 (67–91)	83 (70–96)	80 (67–93)	80 (68–93)	82 (68–96)	79 (68–91)	70 (58–83)	80 (68–93)
$eGFR_{cyscr}$ (mL/min/1.73 m^2)	89 (77–99)	88 (76–97)	91 (80–102)	88 (76–100)	89 (78–99)	91 (78–102)	88 (77–98)	81 (68–92)	89 (78–99)
CRP (mg/L)	1.5 (0.7–3.2)	1.5 (0.8–3.2)	1.5 (0.7–3.3)	1.7 (0.8–3.4)	1.5 (0.7–3.2)	1.5 (0.7–3.4)	1.5 (0.8–3.2)	1.8 (0.9–40)	1.5 (0.7–3.2)
LP-a (nmol/L)	25 (8–109)	27 (9–120)	21 (8–85)	22 (8–97)	25 (9–111)	23 (8–91)	25 (8–114)	30 (10–104)	25 (8–109)
ApoB (g/L)	0.87 (0.74–1.02)	0.84 (0.72–0.98)	0.92 (0.77–1.10)	0.86 (0.73–1.03)	0.87 (0.74–1.02)	0.93 (0.79–1.11)	0.84 (0.72–0.99)	0.85 (0.74–1.01)	0.87 (0.73–1.02)
Cystatin C (mg/L)	0.96 (0.86–1.08)	0.97 (0.88–1.09)	0.93 (0.83–1.05)	0.96 (0.86–1.09)	0.96 (0.86–1.07)	0.93 (0.83–1.07)	0.96 (0.87–1.08)	1.06 (0.93–1.23)	0.96 (0.86–1.07)
HbA1c (mmol/mol)	37 (34–41)	38 (35–42)	36 (33–39)	37 (34–40)	37 (34–41)	36 (33–40)	38 (35–41)	37 (34–40)	37 (34–41)
GGT (U/L)	32 (22–50)	34 (24–52)	29 (20–46)	31 (22–51)	32 (22–50)	29 (20–46)	33 (23–52)	33 (24–53)	32 (22–50)
AST (IU/L)	26 (22–31)	26 (22–31)	25 (21–29)	25 (22–30)	26 (22–31)	25 (21–29)	26 (22–31)	26 (22–32)	26 (22–31)
ALT (IU/L)	22 (17–29)	23 (18–31)	20 (16–27)	22 (16–28)	22 (17–30)	20 (15–27)	23 (18–30)	21 (16–29)	22 (17–29)
ALP (IU/L)	83 (70–100)	83 (70–101)	83 (69–100)	84 (70–102)	83 (69–100)	83 (69–101)	83 (70–100)	84 (69–101)	83 (70–100)

Numbers shown are median (1st quartile–3rd quartile) except otherwise stated.

AAA, abdominal aortic aneurysm; ALP, alkaline phosphatase; ASCVD, atherosclerotic cardiovascular disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; GGT, gamma-glutamyl transferase; HDL-C, HDL cholesterol; IHD, ischaemic heart disease; PAD, peripheral arterial disease; SBP, systolic blood pressure.

onset of chronic kidney disease during long-term monitoring.[18] Cystatin C is conventionally used to estimate kidney function, and either as $eGFR_{CysC}$ or in combination with serum creatinine as $eGFR_{Cr\text{-}CysC}$, has been shown to provide better predictive discrimination for future ASCVD events than $eGFR_{Cr}$. The Kidney Disease: Improving Global Outcomes guideline identify specific clinical conditions in which eGFR may be less reliable, potentially impacting clinical decision-making. These include conditions affecting muscle mass, as well as certain diet and medication.[19] In this study's sub-group analysis with patients with eGFR <45 mL/min/1.73 m^2, we detected a potentially strong risk discrimination of adding Cystatin C, and replacing eGFR with $eGFR_{CysC}$ and $eGFR_{Cr\text{-}CysC}$ in predicting ASCVD risk even though the estimates were of low precision likely due to sample size. This finding was consistent with another study which found $eGFR_{CysC}$ provided more accurate prediction of all-cause mortality and fatal/non-fatal CVD.[20] The difference might be due to smaller sample size in this study.

However, it is worth to note that some characteristics, such as older age, male gender, current cigarette smoking, inflammatory process (higher CRP levels), and obesity, can alter Cystatin C as a single marker—these factors independently influence renal function.[21] While increasing evidence supports the wider adoption of Cystatin C testing for routine kidney function assessment and its integration into cardiovascular risk stratification, several issues have emerged, including its cost, accessibility, and the clinical awareness, and understanding of its results.[22] A study in primary care settings found that utilizing $eGFR_{CysC}$ did not enhance risk prediction models for chronic kidney disease progression and all-cause mortality and was associated with additional costs.[23] Various barriers were identified among clinicians regarding the use of Cystatin C in clinical practice, such as the lack of institutional practice guidance and policy, insufficient education, and unfamiliarity with Cystatin C.[24] With some limitations, several steps and more evidence are needed when incorporating Cystatin C routine testing into clinical practice, especially related to economic assessment and health coverage policy.

Table 2 Associations between candidate biomarkers and atherosclerotic cardiovascular disease events adjusting for existing SMART2 predictors

	Sex-standardized SHR	95% CI	*P*-value
LP-a	1.05	1.04–1.07	<0.001
ApoB	1.15	1.09–1.22	<0.001
Cystatin C	1.07	1.05–1.09	<0.001
HbA1c	1.12	1.10–1.14	<0.001
GGT	1.06	1.04–1.08	<0.001
AST	1.04	1.01–1.05	<0.001
ALT	1.00	0.99–1.01	0.14
ALP	1.87	1.51–2.32	<0.001

SHR, sub-distribution hazard ratio; CI, confidence interval; ALP, alkaline phosphatase; ALT, alanine aminotransferase; ApoB, apolipoprotein B; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; LP-a, lipoprotein A.

Table 3 C-indices for predicting atherosclerotic cardiovascular disease events of baseline models and the model with additional or replaced biomarkers

	C index (95% CI)	Δ*C* (95% CI)	*P*-value
Baseline model	0.6630 (0.6581–0.6679)		
Additional biomarkers			
LP-a	0.6637 (0.6589–0.6686)	0.0007 (0.0002–0.0013)	0.01
ApoB	0.6633 (0.6589–0.6686)	0.0003 (0.0002–0.0009)	0.18
Cystatin C	0.6667 (0.6618–0.6715)	0.0037 (0.0027–0.0047)	<0.001
HbA1c	0.6694 (0.6646–0.6742)	0.0064 (0.0050–0.0078)	<0.001
GGT	0.6653 (0.6604–0.6701)	0.0023 (0.0015–0.0031)	<0.001
AST	0.6637 (0.6588–0.6685)	0.0007 (0.0002–0.0011)	0.002
ALT	0.6631 (0.6582–0.6680)	0.0001 (0.0001–0.0004)	0.30
ALP	0.6640 (0.6592–0.6689)	0.0010 (0.0005–0.0015)	<0.001
Replacing $eGFR_{Cr}$ with			
$eGFR_{CysC}$	0.6667 (0.6618–0.6715)	0.0036 (0.0026–0.0047)	<0.001
$eGFR_{Cr\text{-}CysC}$	0.6666 (0.6618–0.6715)	0.0036 (0.0026–0.0047)	<0.001

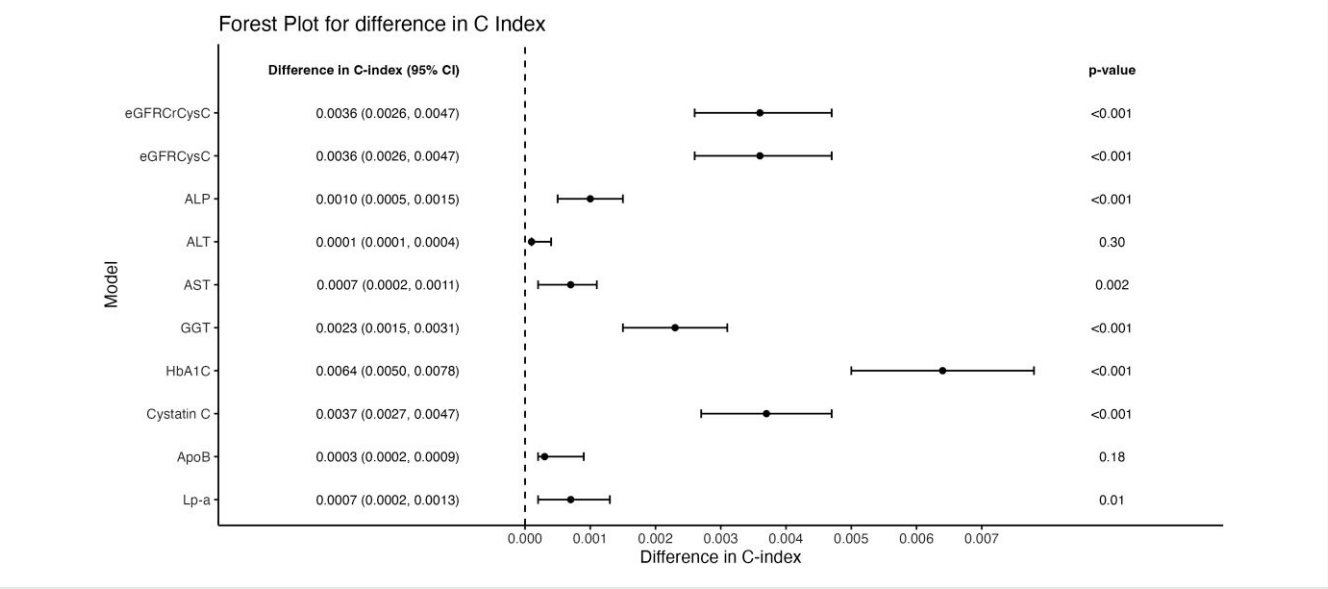


Figure 2 The comparison of the *C* index on different biomarkers used in this study.

Table 4 Continuous net reclassification index compared with the baseline model

	NRI (95% CI)	Event NRI (95% CI)	Non-event NRI (95% CI)
Adding			
LP-a	0.0124 (0.008, 0.0163)	0.0261 (0.0229, 0.0291)	−0.0137 (−0.0164, −0.011)
ApoB	<−0.001 (−0.0045, 0.003)	<0.001 (−0.0024, 0.0034)	−0.0013 (−0.004, 0.0011)
Cystatin C	0.0349 (0.0241, 0.0443)	0.0365 (0.0288, 0.0426)	−0.0015 (−0.008, 0.004)
HbA1c	0.072 (0.0602, 0.0845)	0.0544 (0.0455, 0.0642)	0.0177 (0.0092, 0.0264)
GGT	0.0303 (0.0244, 0.0368)	0.0768 (0.0726, 0.0816)	−0.0465 (−0.051, −0.0426)
AST	0.0075 (0.0043, 0.01)	0.0161 (0.0141, 0.0182)	−0.0086 (−0.0106, −0.0071)
ALT	0.0025 (<0.001, 0.0039)	0.0048 (0.0036, 0.0061)	−0.0023 (−0.0034, −0.0013)
ALP	0.0069 (0.0042, 0.0099)	0.0122 (0.01, 0.0144)	−0.0053 (−0.0068, −0.0035)
Replacing $eGFR_{Cr}$ with			
$eGFR_{CysC}$	0.0382 (0.0286, 0.0498)	0.0487 (0.0405, 0.0557)	−0.0105 (−0.0163, −0.0037)
$eGFR_{Cr\text{-}CysC}$	0.0355 (0.0276, 0.0463)	0.0362 (0.0298, 0.0434)	−0.0007 (−0.0065, 0.0057)

The relationship between glucose metabolism and the development of coronary heart disease (CHD) has been extensively investigated. Diabetes mellitus has emerged as a major contributing factor in the pathogenesis of CVD, and several studies have demonstrated a significant association between elevated concentrations of HbA1c and an increased risk of ASCVD.[25] Incorporating HbA1c into risk stratification efforts can provide a more comprehensive assessment of cardiovascular risk, particularly in individuals with established ASCVD.[25] In the general population, HbA1c levels have been linked to cardiovascular mortality, all-cause mortality, and CVD.[26] When incorporated into

Table 5 Categorical net reclassification index with 50% predicted risk threshold compared with the baseline model

	NRI (95% CI)	Event NRI (95% CI)	Non-event NRI (95% CI)
Adding			
LP-a	0.0025	−0.001	0.0036
	(−0.0015, 0.0069)	(−0.0035, 0.0015)	(<0.001, 0.0063)
ApoB	0.0043	<0.001	0.004
	(<0.001, 0.0086)	(−0.0025, 0.0029)	(0.0012, 0.0072)
Cystatin C	0.0063	0.0021	0.0042
	(<0.001, 0.0114)	(<0.001, 0.0055)	(0.001, 0.0082)
HbA1c	0.0144	<−0.001	0.015
	(0.0084, 0.0197)	(−0.0046, 0.0029)	(0.0108, 0.0193)
GGT	0.0039	<0.001	0.0033
	(−0.0014, 0.0083)	(−0.003, 0.0035)	(−0.001, 0.0066)
AST	0	<−0.001	<0.001
	(−0.0027, 0.0024)	(−0.002, 0.001)	(−0.0018, 0.0022)
ALT	0.0014	<−0.001	0.0015
	(−0.0011, 0.0038)	(−0.0015, 0.0014)	(<−0.001, 0.0033)
ALP	0.0015	0.002	<−0.001
	(−0.0024, 0.005)	(0, 0.0042)	(−0.0035, 0.0022)
Replacing $eGFR_{Cr}$ with			
$eGFR_{CysC}$	0.0072	0.0024	0.0049
	(0.0023, 0.0123)	(−0.0011, 0.006)	(<0.001, 0.0089)
$eGFR_{Cr\text{-}CysC}$	0.0058	0.0013	0.0044
	(<0.001, 0.0106)	(−0.0022, 0.0048)	(<0.001, 0.0084)

certain cardiovascular risk prediction models, such as QRISK3, ACC/AHA, and SCORE, the addition of log HbA1c modestly improved model performance as indicated by increases in *C* indices. However, there was no significant improvement in reclassification of individuals with and without outcomes, nor among those with higher HbA1c levels.[27] In patients with Type 2 diabetes mellitus (T2DM) and CHD, HbA1c was identified as a predictive risk factor for MACEs.[28] Additionally, patients with stable coronary artery disease and HbA1c levels of 7% or higher were found to have an elevated risk for ischaemic events.[29] Overall, HbA1c serves as a risk factor for both all-cause and cardiovascular mortality in individuals with and without diabetes.[30]

Gamma-glutamyl transferase is a glycoprotein which can be found in many tissues such as the liver, placenta, lung, and pancreas.[31] Gamma-glutamyl transferase plays a role in promoting the development of atherosclerotic plaque and its contribution to oxidative stress. GGT contribute in LDL-C oxidation in arterial vessel and leads to progression of atherosclerosis.[32] A prospective study reported associations between higher GGT concentrations and increased risk of both cardiovascular events and all-cause mortality.[33]

Alkaline phosphatase is an enzyme found in the hepatic, renal, and skeletal systems. Alkaline phosphatase is an acute phase reactant in the inflammatory process which shares the same pathway as CRP. Inflammatory mechanisms are known to contribute to the development of CVD and ALP concentrations have been shown to be associated with recognized risk factors for ASCVD, including non-alcoholic fatty liver disease, diabetes mellitus, and metabolic syndrome. A retrospective study in patients with no history of CVD found elevated serum ALP was associated with arterial stiffness and 10-year CVD risk in the general population.[34,35] Another retrospective study found that elevated ALP was associated with higher risk of mortality in patients with diabetes mellitus and coronary artery disease.[36,37] Alkaline phosphatase has been shown to be associated with CVD in patients with no history of renal failure[38] or liver disease.[39] While GGT and ALP have been positively correlated with CVD and cardiovascular mortality, limited studies indicate that AST is not associated with cardiovascular morbidity and mortality.[40] However, the ratio of AST/ALT is linked to an increased risk of CVD[41] in individuals without prior CVD,[42] and similar findings have been observed in patients with T2DM.[43]

Our study provided no evidence that addition of the other biomarkers we investigated—LP-a, ApoB, and ALT—improved the model based on SMART2 predictors. Our findings in relation to LP-a contrast with previous studies. HEART-UK, an organization dedicated to cardiovascular health, has recognized LP-a as an independent risk factor for CVD[44] and a meta-analysis found LP-a was associated with cardiovascular events in patients with coronary artery disease. This meta-analysis evaluates the prognostic value of elevated LP-a in CAD patients using observational studies. Cardiac events defined in this study include ACSs, cardiac death, and all-cause mortality in CAD patients. Integrating the measurement of LP-a into risk stratification for individuals with ASCVD can provide added value by enhancing risk assessment accuracy, but the prognostic utility will be improved in prospective studies in different sub-type of CAD.[45] Lipoprotein A, composed of ApoA and ApoB molecules, has emerged as a promising therapeutic target in the field of CVD. It exhibits unique characteristics, such as being a lipid-rich fraction and having structural similarities to LDL receptors.[46] Lipoprotein A has been implicated in various mechanisms that contribute to the development of atherosclerosis. However,

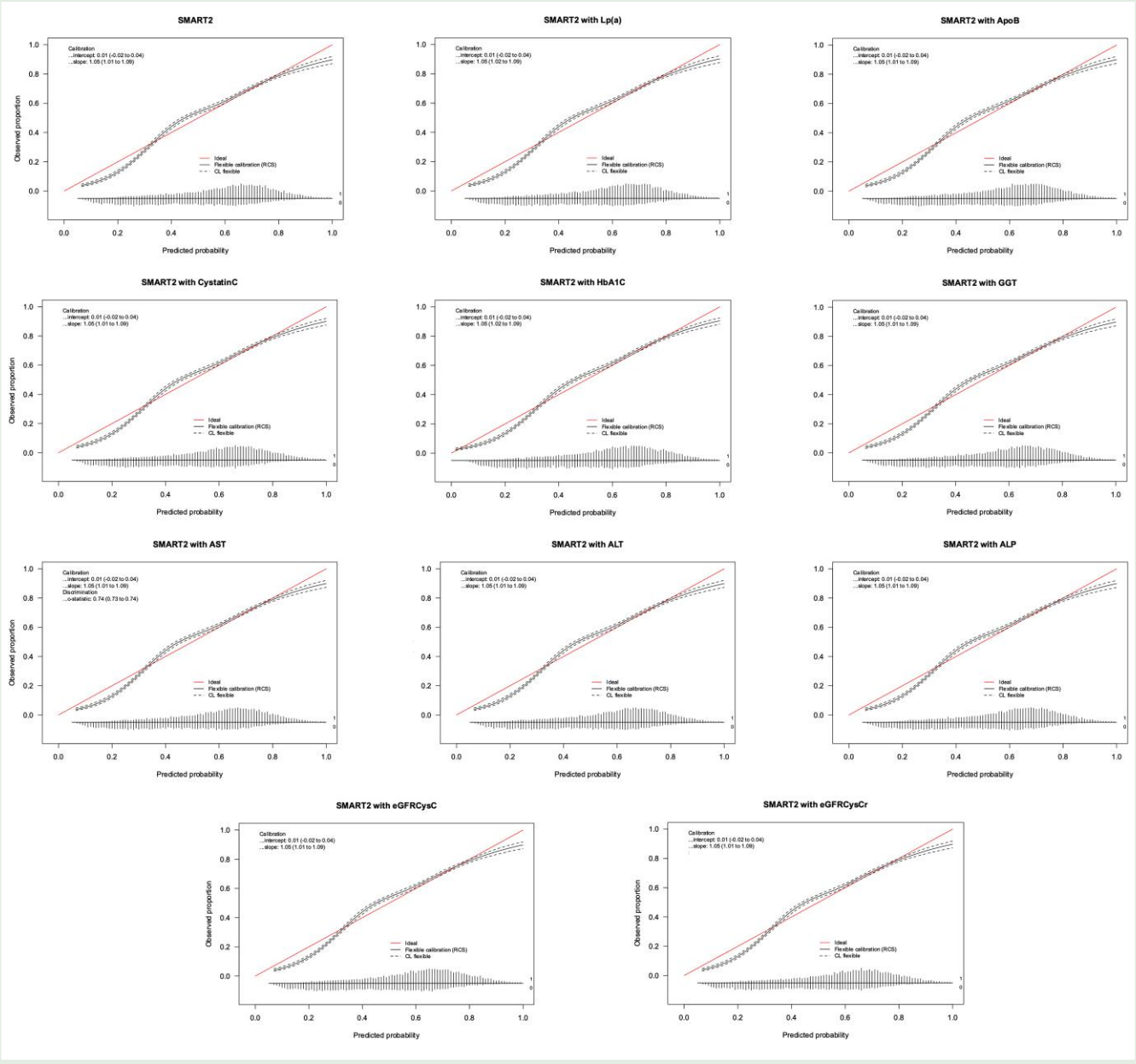


Figure 3 Calibration plots for models using SMART2 predictors with and without additional biomarkers.

it should be noted that such contrasting results from our study did not nullify the potential therapeutic importance of LP-a. The lack of predictive improvement following addition of LP-a could be due to the inclusion of non-HDL or other variables that are closely related.

Apolipoprotein B is the structural protein of atherogenic lipoproteins. Apolipoprotein B molecules present in every atherogenic particle that promotes atherosclerosis plaque development.[47] Our study did not corroborate findings that suggested ApoB might be a promising biomarker for predicting cardiovascular events used in addition to non-HDL-C, because ApoB counts atherogenic particles not cholesterol concentration.

Strengths and limitations

Our study included a reasonable number of patients who have had a medical history of ASCVD with a long 10-year follow-up for recurrent events. The unselected measurements of biomarkers in UK Biobank present a lower risk of bias by indication compared with other routine databases. First, this study systematically included a wider range of candidate biomarkers that were either routinely measured in clinical practice or have shown promise in other studies. Despite the strengths, this study has several limitations. As with all observational studies, we cannot confirm causality in this study. However, as this study focused on prediction rather than causal inference, these biases should be of minimal concern. Secondly, since UK Biobank's participants have healthier lifestyles than the general population, the findings should be validated in more representative study populations. Additional research is required to calibrate the risk prediction models, as this was not an objective of the current study. Thirdly, even though we have shown Cystatin C provided better accuracy in a model based on SMART2, it is not currently routinely used in clinical practice. The cost–benefit ratio of including

Cystatin C in clinical management warrants further investigation. Fourthly, it was reported that Cystatin C predicts risk more accurately in women than in men.[48] Whether that constitutes differential performance when added to SMART2 requires further studies. Fifthly, since the SMART2 model predicts the risk of recurrent cardiovascular events adjusted for the competing risk of non-cardiovascular death, inclusion of the COVID-19 years in follow-up might have affected model performance and the added value of predictors. Lastly, even though we included a larger set of common blood biomarkers in this study, there are further clinical (e.g. concurrent heart failure) and biomarker (NT-proBNP) variables that we did not examine and warrant future studies. Importantly, this study demonstrated predictive value in adding some biomarkers into refitted SMART2-risk prediction model. The application into clinical practice warrants more study related to its validation and practicality in another cohort.

Conclusions

Adding several biomarkers, most notably Cystatin C and HbA1c, but not LP-a, in a model using SMART2 predictors modestly improved discrimination. Future studies should externally validate the findings and examine the associated clinical utility and cost-effectiveness.

Supplementary material

Supplementary material is available at *European Journal of Preventive Cardiology*.

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Conflict of interest: none declared.

Data availability

All the individual participant data can be requested from the UK Biobank.

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